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ALGHERO 7-10 MAY

Grassland resources for extensive farming systems in marginal lands: major drivers and future scenarios

Edited by

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Foreword

Extensive grassland-based farming systems play a pivotal role in less favoured areas (LFAs), because they often support local economic growth based on the marketing of typical food products (e.g. cheese, meat), which represents the only source of income for many rural micro-economies. They are currently subjected to the contrasting threats of intensification and abandonment in a context of climate change and economic globalization. The LFAs are very weak in terms of political influence and strategies supporting the grassland-based systems often lose effectiveness due to poor coordination between agricultural and environmental policies. But extensive farming systems integrating grasslands and other forage resources offer a really good perspective for satisfying the general expectations on agriculture for an increased feed and food quality and for reducing impacts on the environment. Consumers are increasingly concerned about environmentally-friendly production and animal welfare, and there is evidence that pasture and forage-based diets can improve environmental, ethical and human health outcomes, particularly when compared with that of feed-lot systems.

The challenge we face as grassland scientists is to find effective ways of applying the general concept of sustainability, which is also an ethical and moral issue, and to give tools to the farmers, extension workers and policy makers to promote extensive grassland-based farming systems in the LFAs. The ultimate aim of these policies should encourage the development of sustainable farming systems, where farmers obtain their incomes from the added value of animal products but are also rewarded by their supply of ecosystem services.

There are great expectations for research regarding the complex interactions between livestock, grasslands and environment under marginal conditions. New holist research approaches and methodologies should be considered to analyse the multifunctionality of extensive farming systems. The 19th Symposium of the European Grassland Federation held in Alghero (Sardinia, Italy) from 7 to 10 May 2017 therefore focused on grassland resources for extensive farming systems in marginal lands. Keynote speakers from a number of different European regions have been invited to address three main topics:

- Extensive animal production systems and product quality;
- Sustainable grassland management in high nature value areas;
- Alternative and multiple-uses of grassland resources.

More than 190 scientific contributions were presented and discussed including introductory and invited papers, short oral communications and posters. The contributions were presented by specialists from 35 different countries all over Europe, but also from the other continents (Algeria, Australia, Brazil, Mexico, Morocco, Tunisia, Uruguay and USA).

We warmly thanks all authors, external reviewers and chairpersons for the scientific contribution to the Symposium. Finally, we would like to acknowledge the contribution of the members of Organizing and Scientific Committees for preparing this Symposium.

Claudio Porqueddu

General Secretary and chair of Scientific Committee

Antonello Franca

Chair of Organizing Committee

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Opening session

The role of grasslands in the less favoured areas of Mediterranean Europe

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Abstract

Mediterranean extensive grassland-based systems were in the past and are currently subjected to the contrasting threats of intensification and abandonment, especially in less favoured areas. Despite the strong socio-economic and environmental differences between and within the Mediterranean regions, they share common issues on grasslands. One of the main issues is to increase the sustainability of grasslands and the grassland-based systems. In this paper, we discuss some key factors that can strengthen the resilience and adaptability of grasslands and which could be considered also as mitigation strategies for climate change. Additionally, to enhance the results of agronomic practices, both public perception and management schemes about less favoured areas should urgently be reoriented in order to increase the attractiveness of extensive systems against intensive agriculture. For this reason, there is a rising demand for scientific knowledge and new holistic approaches aimed at (1) supporting the optimization of the economic performance of extensive livestock systems by increasing the added value of products, and (2) encouraging policies for the development of sustainable farming systems, where farmers are also rewarded for their supply of ecosystem services.

Keywords: extensive farming systems, marginal areas, ecosystem services, product quality

Introduction

In the European Union, less favoured areas (LFAs) is a definition applied to mountainous or hilly areas or areas with natural handicaps for cropping (lack of water, harsh climate, short cropping season), or that are remote, with difficulties in rural mobility or that are at risk due to depopulation. LFAs are widespread in the Mediterranean region (EEA, 2004). Despite their biophysical and structural limitations, LFAs have greatly contributed to sustain the rural economy in the northern Mediterranean basin. In these areas low-intensity and site-specific agricultural practices, mainly based on grassland resources, were developed to limit the risks associated with inter- and intra-annual climatic fluctuations and ensure more regular production (Caballero *et al.*, 2009; Jouven *et al.*, 2010; Hadjigeorgiou, 2011). Moreover, an integrated land use pattern was developed, which promoted multifunctionality at field, farm and landscape levels (Henkin *et al.*, 2011; Sternberg *et al.*, 2015). As a consequence, the agrarian landscape in the European Mediterranean regions appears as a complex mosaic of feed resources, animal species and local breeds as an effect of the local socio-cultural traditions. The practices carried out in these areas are often considered environment-friendly and landscape-preserving, and farmlands evaluated are of High Nature Value (Opperman *et al.*, 2012). In fact, Mediterranean grassland-based systems are usually extensive, with a low use of pesticides, fertilizers, concentrates and irrigation, where small ruminants predominate due to their high efficiency in the use of available feed resources (Cosentino *et al.*, 2014; Porqueddu, 2008). These farming systems have been shown to be resilient to frequent but moderate disturbance factors (i.e. deforestation, periodic fires and grazing) by developing strategies to maximize production of multiple goods and ecosystem services over several millennia (Plieninger *et al.*, 2010; Zapata and Robledano, 2014). This background gives room for optimism on the perspective that new grassland-based farming systems could be developed also in response to changing climate scenarios. Unfortunately, such systems are currently subjected to the contrasting threats of intensification and abandonment (Moreira *et al.*, 2011; Kyriazopoulos *et al.*, 2013). Moreover, as pointed out by Porqueddu *et al.* (2016), the decreasing public sector support for grassland research requires a greater level of international scientific cooperation among the few institutions operating in the different Mediterranean-climate areas of the World.

The aim of this paper is to describe the current state and role of grassland-based extensive farming systems in the LFAs of the Euro-Mediterranean regions. In the first part, an overview of the evolution of the area of grasslands and of the related farming systems of the main Mediterranean countries will be given. In the second part, the productive aspects and the main key agronomic tools for the rehabilitation and adaptation to climate change will be listed, with the awareness that research outputs in this field are just a part of an holistic approach to grassland evaluation, involving also market and policy initiatives aimed at fully recognizing the environmental and cultural role of grassland-based systems.

Grassland areas and LFAs in the main Euro-Mediterranean countries

LFAs cover 25% of the European surface, and permanent grasslands around 61 million hectares across the EU-28 (Eurostat, 2012), representing 16% of the area of EU ecosystems (MAES, 2016), while accounting for 35% of the total EU Utilised Agricultural Area (UAA). In the main Euro-Mediterranean countries (Greece, Italy, Portugal and Spain), there are over 15.2 million ha of permanent grasslands in total. According to EUROSTAT (2012), these four countries show a considerably lower proportion of natural or agriculturally improved grasslands compared to the states of northern and central Europe (Table 1). Nonetheless, grasslands constitute a large share of the UAA in the South European countries.

Mediterranean grasslands, by the definition of Peeters *et al.*(2014), are represented by mesic and sparsely wooded grasslands (EUNIS habitat type), but traditionally, wooded grasslands with up to 10-40% tree and shrub cover are used to support livestock production in these regions (Suttie *et al.*, 2005). In fact, within the European context, Southern countries are particularly rich in forest and wooded land and Mediterranean garrigue or other shrubby vegetation cover (Huyghe *et al.*, 2014; INFC, 2015). This is the reason why the available data referring to 'pastures' (e.g. FAOSTAT data) are sensibly higher than the statistics for grassland area in EUROSTAT reports. The importance and economic role as feed resource of wooded pastures and shrublands increase in LFAs. Some farming systems, diffused in these countries, make large use of another feed resource, the temporary grasslands, especially in Italy.

Portugal and Spain

In the Iberian Peninsula, the most abundant pasture types in dry mountain areas are oak and pine forests, alpine pastures, permanent meadows and xerotrophic grasslands. In the inland regions shrublands and natural pastures are predominant, with steppic vegetation in the arid areas (Caballero *et al.*, 2009). Finally, in the *dehesas* and *montados* of the SW region, open forests of evergreen holm oak are the dominant landscape, providing grass, browse and acorns for cattle, sheep and ranging pigs. The relative proportion of the different pasture types has changed in the recent past. In Portugal, permanent grasslands increased by +30% between 2000 and 2013, as a consequence of the +50% rise of the rough grazing area (pastures grazed at low stocking rates), while the *montado* has an estimated annual regression rate of -0.14% per

Table 1. Land covered by different pasture types in the main Euro-Mediterranean countries (EUROSTAT, 2012; INFC, 2015).

	Portugal	Spain	Italy	Greece	France[1]
Natural or agricultural improved grassland (% total area)	16	16	17	14	18
Grasslands (% utilised agricultural area)	50	34	27	43	68
Forest and wooded land (% total area)	37	30	33	30	40
Mediterranean garrigue or other shrubby vegetation (% total area)	18	17	7	26	17

[1] Only Mediterranean regions: Provence-Alpes-Côte d'Azur, Languedoc-Roussillon and Corsica.

year (Godinho *et al.*, 2016). On the opposite, in Spain grasslands have decreased by close to -15% in the 2000s, namely because of the increase in shrub and forest areas. These have been caused by the changes in extensive livestock censuses, but also because of the different management patterns applied to the grazed lands. A similar trend has been observed in dry mountain areas, where the intensification of management in sheep farms was negatively related to the use of natural grazing resources (Riedel *et al.*, 2007), and lack of succession compromised the continuity of the most extensive farms. But the consequences of these land use changes can be diverse and sometimes positive. For example, in the *dehesa*, a certain degree of shrub encroachment by given species has proven to facilitate tree regeneration and maintain or even enhance pasture, browse and acorn productivity (López-Díaz *et al.*, 2015).

Italy

LFAs are mainly lands with natural handicaps, where the severity of slopes is the main determinant of the vegetation types. Seeding is carried out on slopes up to 25-30%. The steeper slopes (gradient up to 35-40%) are covered by woods and permanent grasslands and the more severe slopes (gradient >40%) are overgrown by forests and pastures. The pasture types than can be found are very variable and characterized by several tree associations. In central Italy, Mediterranean shrubland associated with evergreen schlerophyll trees and shrubs (*Quercus ilex*, *Quercus suber*, *Quercus coccifera*) can be found along the Thyrrenian coast; forests of deciduous trees (e.g. *Castanea sativa*) prevail in mid hills and forests of *Fagus sylvatica* in mid mountains. Finally, natural meadows are associated with various types of vegetative association (*Brachipodietum*, *Brometum*, *Arrhenatheretum*, *Festucetum*, *Lolietocynosuretum*) in high mountains. In Southern Italy, grasslands are associated to thermoxerophytic shrubs along the coasts, together with forest and evergreen sclerophylls. Forests of holm oak are common in mid mountains, where they form mixed woods with the deciduous species *Quercus pubescens* at higher altitudes. Permanent grasslands are found mainly in the Apennine zone above 500 m a.s.l., while more recent agriculturally improved pastures are present in the lower zones. Permanent grasslands underwent a gradual reduction from 1990 to 2000, when more than 340,000 ha were lost, while from 2000 to 2013 their area remained stable (EUROSTAT). The largest decrease involved pastures and meadows. Currently, permanent grasslands and pastures are widely diffused in the main islands (Sardinia and Sicily, 40.7% of their UAA), then in the regions of North West (32.5%), North East (24%), Southern Italy (20.6% of their UAA) and Central Italy (18.5% of UAA) (ISTAT, 2016).

Greece

About 50% of the country's grazing lands are found in mountainous areas (>600 m a.s.l.) and a further 30% in hilly areas (300>x>600 m a.s.l.) and the majority are covered by woody vegetation. The CORINE 2000 data describe the land covered at 10% by grassland and phrygana, 17.7% by sclerophyllus vegetation, 9.4% by transitional shrublands, while a further 18% is covered by forest. Grazing lands are predominantly (75%) State- or community-owned lands which are used as Common land within each municipality (Hadjigeorgiou *et al.*, 2002). Common land in Greece accounts for 49% of the UAA and represents mostly rough grazing areas used to pasture cattle, sheep and goats. However, a much larger area is grazed to a varying degree, which includes a large part of forests, permanent crop (e.g. olive groves) and annual crop fields (e.g. cereal stubbles etc.) thus increasing the total area affected by pastoralism to about 65% of the whole country. Therefore, landscapes and biodiversity of the Greek land, which have been shaped through the ages by livestock husbandry (Hadjigeorgiou, 2011), are still maintained by pastoral activity. The grassland habitat type with the highest share of plant associations is *Thero-Brachypodietea*, which includes the annual plant communities (Mucina, 1997). Furthermore, 17 grassland habitat types covering 137 plant associations have been recorded (Dafis *et al.*, 2001). The most common grassland phytosociological classes are *Junceteatrifidi, Festuco-Brometea* and the mesophile and wet heaths of the class *Molinio-Arrhenatheretea* (Fotiadis *et al.*, 2006). However, the plant communities closely associated with sheep and goat grazing are those of the high calcareous mountains dominated by dwarf shrubs

(*Daphno-Festucetea*), usually with many endemic species (Alados *et al.*, 2004; Bergmeier, 2002) and those of the xerothermic lowland areas with dwarf, dry-tolerant shrubs (phrygana of *Cisto-Micromerietea*), where the plant diversity is very high (almost 100-120 species per 200 m^2) (Bergmeier, 2002; Phitos *et al.*, 1995; Tan and Iatrou, 2001).

On the Greek mountains woody vegetation consists of a variety of tree (*Quercus frainetto, Ostrya carpinifolia, Tilia tomentosa, C. sativa, F. sylvatica, Carpinus orientalis* and coniferous forests of *Pinus nigra, Abies* spp., etc) and shrub species (*Juniperus foetidissima, Vaccinium myrtillus, Rubus* sp. and *Rosa* sp.). Major vegetation types associated with pastoral activity are the phryganic vegetation (*Phlomis fruticosa, Sarcopoterium spinosum, Coridothymus capitatus, Cistus* spp.) and the garrigues (*Q. coccifera*, *Pistacia lentiscus*, *Arbutus unedo*, *Juniperus phoenicea*) (Caballero *et al.*, 2009).

Mediterranean France

About 72% of the surface of Mediterranean France (comprising Provence-Alpes-Côte d'Azur, Languedoc-Roussillon and Corsica) is considered as mountainous or disadvantaged areas (98% only in Corsica). In these three regions, the percentage of forage crops and permanent grassland on the UAA is on average 68%, with a very high percentage in Corsica (more than 90%). Non-productive permanent grasslands characterize large hilly and mountain areas of all three regions. Woodlands represent a very important trait of the mountain landscape covering around 40% of the total surface, protecting the soil against erosion and preserving biodiversity. These regions benefit from agri-environmental measures dedicated to pastoral systems, and grazing animals are directly involved in the overall system of forest fire prevention and utilized for fuel biomass-reduction purposes within firebreak-management plans (Pastomed, 2007).

Livestock population and farming systems in the main Euro-Mediterranean countries

The livestock in the four main Mediterranean countries of Europe accounts for around 96.7 million heads, corresponding to 29% of the total amount for EU-28 (EUROSTAT, 2016). They host 17% of the cattle, 28% of the pigs and up to 39 and 67% of the EU-28 sheep and goat census, respectively (Table 2). In particular, sheep and goats represent most of the grazing livestock units in Greece (73%) and Spain (32%). Moreover, and unlike most European countries, in Spain and Portugal there are more suckler cows used for meat production than there are dairy cows.

Portugal and Spain

In Portugal ruminants are predominant (59% of the census, in terms of livestock units, LSU), while in Spain pigs and poultry represent a larger share (57%) (EUROSTAT, 2016). This is the result of intense changes in the number of farms and their orientation in the previous decade (2000-2010) in both countries. In Portugal the number of livestock holdings decreased by -36% and LSU by 13%, with the

Table 2. Livestock census by species in the main Euro-Mediterranean countries (EUROSTAT, 2016), thousand heads (% of EU-28 census). Data are referred to 2015.

	Cattle		Pigs		Sheep		Goats	
EU-28	89,152		148,724		85,524		12,502	
Portugal	1,606	(2%)	2,247	(2%)	2,043	(2%)	373	(3%)
Spain	6,183	(7%)	28,367	(19%)	16,523	(19%)	3,010	(24%)
Italy	6,156	(7%)	8,683	(6%)	7,149	(8%)	962	(8%)
Greece	582	(1%)	877	(1%)	8,852	(10%)	4,017	(32%)
France[1]	343	(0.4%)	587	(0.6%)	1,190	(1.4%)	104	(0.8%)

[1] Only Mediterranean regions: Provence-Alpes-Côte d'Azur, Languedoc-Roussillon and Corsica.

sheep census decreasing by -24%. In Spain, farm numbers decreased by -41% (especially the smallest ones, while mega-farms larger than 500 LSU increased by +32%), but the total LSU only changed by -1%. This masks heterogeneous patterns across animal species, with increments of poultry and pigs (+11 and +7%, respectively) and reductions in other species (-7% in cattle, -20% in sheep and goats). Pig numbers have increased both in intensive and extensive farms, and it is remarkable that the number of extensively managed Iberian sows have more than doubled in the recent past (+146% from 1995 to 2015) (MAPAMA, 2015). The attractiveness of the high prices paid for Iberian ham has sometimes altered the equilibrium between the supply and demand, with an effect on market prices. In the case of ruminants, trends have been very different considering the orientation of livestock production. Cattle censuses have decreased significantly since 1995 in the dairy sector (-37%), mostly because of the reduced competitiveness of small holdings, but the number of suckler cows in extensive farms has increased (+17%). On the opposite, the number of sheep in extensive meat-oriented production systems has decreased sharply (-38%), but the higher added value of dairy products has buffered the impact for milk-oriented sheep farms (-4%) (MAPAMA, 2015). In Spain, all the extensive Iberian pig farms, and most of the sheep (93%) and suckler cattle farms (74%) are concentrated in Mediterranean regions. In these areas ruminant production is based on extensive farming systems where beef cows, sheep and their lactating offspring graze throughout most of the year on the aforementioned pastures, but after weaning, lambs and calves are frequently fattened off-pasture, on high-concentrate diets. Transhumance is still utilized to overcome the seasonality of forage production by moving herds short or long distances between summer (highlands or Northern areas) and winter pastures (lowlands, Southerly latitudes). Another common practice is to associate ruminant farms to cereal crops, enabling them to increase their self-sufficiency by combining the use of natural pastures with stubble and fallow in croplands (Olaizola *et al.*, 2015). In the *dehesas* and *montados*, systems commonly include a mixture of beef cattle, sheep and pigs raised on these sylvopastoral areas for extensive meat production (Gaspar *et al*, 2007). Extensive pig production using the autochthonous Iberian breed is unique to this ecosystem, where animals use herbaceous sources during the spring, with supplements in the summer and are kept on pasture and acorns (*montanera*) in the autumn and early winter. Depending on birth season and forage availability, pigs can be finished indoors on concentrates or ranging on the *montanera,* resulting in a particular meat quality (Timón *et al.*, 2002) that confers a high added value to this product.

Italy

Updated data referred to 2015 on consistencies of the main grazing livestock animal categories are reported in Table 2. In relative terms, cattle heads recorded a decrease of about 3.6% in the last ten years, pigs a reduction of 5.6%, while the numbers of sheep and goats have remained stable (+0.02 and +0.05%, respectively). More than 60% of animal heads are raised in the northern regions with intensive systems. Nearly 60% of national livestock holdings are located in the central-south regions and on the main islands (EUROSTAT, 2012), where the larger part of the labour force in national agriculture is working. In the last ten years, the number of livestock holdings has decreased drastically (-65%), but at a higher rate than livestock heads: -0.6% at national level, with higher peaks in Central Italy (e.g. -25% in Tuscany). The smallest size-class farms in the mountainous regions in central Italy were the most affected by reduction (e.g. -83% in Marche region) while the larger size holdings (>500 LSU) showed an increase in number (+9%), indicating a deep restructuring of the livestock system in Italy (EUROSTAT, 2012). Due to the uneven distribution of livestock among species, cow milk is the main animal production, except for Tuscany and Sardinia, where sheep milk is produced. Sardinia, in particular, is the Italian leader in sheep rearing: 43.8% of total heads of the country are found in the island that is the centre of the Pecorino Romano PDO cheese industry. In LFAs of Central and Southern Italy and Isles, the traditional farming system is based on both temporary and permanent grasslands and, occasionally, on woody pastures that are grazed by locally-adapted breeds during summer, sometimes in mixed farms (cattle and sheep). Animals are then fattened on farm in the richer plains. Pastures are also used in the higher altitudes of the Apennines in the summer period by sheep and cows. Transhumance is currently practised in rare cases. Goat farms are mainly located in the most difficult areas. The forage systems are quite variable, depending on the farming systems. On the coastal plains and in the dry low hills where mixed crop-livestock systems are present, the base of the feeding systems is a combination of annual forages and cereal stubbles. In hills where there are possibilities for mechanization, feeding systems based on permanent grasslands and the use of hay storage and pastures are diffused. The agro-pastoral systems, widespread in interior hilly areas with little mechanization, are based on diversified resources, and semi-natural grasslands and improved pastures coexist in the better areas. Finally, silvopastoral systems are based on woody pastures, but grazing-animal breeding is associated with other agricultural activities to improve the income of farmers (e.g. cork production in Sardinia).

Greece

The grazing livestock population is composed by cattle, sheep and goats which comprise by far the largest part of the raised herds, while other herbivorous animals (horses, asses, mules and pigs) are minor (3% of LSU) additional potential grazers. Livestock production represents about 27% of the total Greek Agricultural production value. Within this value, sheep and goat products contribute 60%, of which milk is the largest part (38 and 22% for milk and meat, respectively) (HMA, 2011). Similarly to other Mediterranean countries, these animal figures have undergone substantial changes over the last two decades (1994-2014). Most notable changes were registered for the numbers of goats (-26%) and cattle (+12%) while the numbers of sheep were relatively stable (+4%). However, a strong shift was experienced in the direction of production within each species, since dairy cattle were reduced and suckler cows increased, while sheep and goats shifted towards specialization of milk production and the rejection of the old mixed production system. Moreover, the respective farms were reduced sharply (about -30% for cattle, -45% for sheep and -70% for goats), due to the intense evolution towards specialization and reorganization of this sector (Hadjigeorgiou, 2011, 2014). This trend involved mostly small units, whereas the medium and large sized units became larger and more specialized, and based on production intensification. Moreover, there was a movement from higher altitudes (mountain and semi-mountain areas) to the lowlands and closer to the population centres (Dover *et al.*, 2011). Therefore the decrease in farms coupled with an increase in headage suggest that holdings are intensifying livestock production by feeding in barns rather than by shepherding or transhumance, especially as much of the income of a sheep and goat farm derives from milk production requiring high hygiene standards and easy access to dairies (Galanopoulos *et al.*, 2011). These changes have tremendous effects on the status of rural societies and on the use of grasslands. On the other hand, feeding systems changed towards higher use of concentrate feeds while the forage systems that sustain grazing animal production in Greece are very limited. Forages are cultivated in some 400,000 ha, of which warm-season crops like lucerne (*Medicago sativa* L.) or forage maize (*Zea mays* L.) hold a large share (30 and 14% of the area, respectively) being cultivated mostly using irrigation, while the cool-season forage crop are barley and oats for hay (48%) accompanied by vetch for hay (6%). All other forage plants are cultivated very marginally.

Mediterranean France

In the three Mediterranean regions of France, grazing livestock comprise cattle, sheep and goats, with some peculiarities for each region. In the sheep sector, Languedoc- Roussillon (LR) has two distinct chains, sheep milk and meat. The first one is an intensive production system with highly priced milk sold to the industry for the manufacture of DOC Roquefort and other products. There is a reduction of sheep meat livestock, but with valorization of local breeds through collective certifications. Provence-Alpes-Côte d'Azur (PACA) is specialized in sheep meat production with three emblematic farming types: one is outdoors (plein air), with the use of the steppe areas of Crau and large transhumance, one in the foothills and arid plateaux (sometimes in combination with grain or hay productions) and a third on mountains, with possible dual activities. In Corsica, the dairy sheep sector is strongly related to the production of the

PDO cheese Brocciu. Goats are relevant for the livestock sector in LR and Corsica, with systems ranging from pastoral to almost intensive. For the cattle sector, LR and PACA still maintain interesting extensive dairy systems in pastoral lands (*estives* and *alpage*), even if this is within a general trend of reduction in the number of heads.

Primary production of grasslands in Mediterranean environments

A severe limitation for grassland productivity in the LFAs of the Mediterranean basin is represented by physical constraints, which complicate the mechanization of soil tillage, and climate characteristics, namely summer drought coupled with high solar radiation levels, cool winter temperatures during the growing season, and highly erratic and variable rainfall. For these reasons, annual species prevail in semi-natural Mediterranean grasslands. Their growing season ranges from 4 to 10 months, depending on rainfall amount and timing and plant tolerance to water deficit (300-1000 mm). It is characterized by two growing peaks, in spring and autumn. Dry matter accumulation ranges between 110 kg ha^{-1} day^{-1} in the most favourable season (spring) and 20-40 kg ha^{-1} day^{-1} in autumn (Snaydon, 1981; Caredda *et al.*, 1992). Annual and inter-annual forage productions under rainfed conditions are usually extremely variable, but generally limited, and depend on the grassland management and soil fertility. Typically, average dry matter yields range from 0.5-1.0 t ha^{-1} $year^{-1}$ in semi-natural grasslands, which prevail in marginal soils, to 6.0-7.0 t ha^{-1} $year^{-1}$ in agriculturally improved grasslands (Huyghe *et al.*, 2014). In grasslands subjected to shrub encroachment, herbage production and its nutritional value both decline with the increasing of shrub cover (Zarovali *et al.*, 2007). In the latter case, an appropriate agronomic or grazing management aimed at controlling shrubs should be introduced to promote grassland renovation and conservation (Bagella and Caria, 2011). In semi-natural grasslands, forage usually has a low quality, often worsened by a relative high rate of unpalatable species. A better forage quality can be attained by applying P-fertilizers once a year to boost production of annual pasture legumes, but when their natural seed bank is not sufficient, the re-sowing with annual self-reseeding pasture legumes is appropriate (Porqueddu and Gonzales, 2006). The most used mixtures include 3-4 species and are based on subterranean clovers (*Trifolium subterraneum* L. *sensu lato*) and annual medics (*Medicago* species). More recently, complex seed mixtures (10-20 components) have been utilized with contrasting results (Porqueddu *et al.*, 2010).

To complement the insufficient pasture production in Mediterranean regions, annual temporary grasslands are widely exploited because of their high growth rates in winter and flexible use. Traditionally, mixtures of annual forage legumes and winter cereals (oats, barley and triticale) or grasses (especially Italian ryegrass, *Lolium multiflorum* Lam. ssp. *italicum* and ssp. *westerwoldicum*) are used for short-term forage crops on arable lands. The most used legume species are common vetch (*Vicia sativa* L.), woolly pod vetch (*Vicia villosa* ssp. *dasycarpa* (Ten). Cav.), Persian clover (*Trifolium resupinatum* L.), crimson clover (*Trifolium incarnatum* L.) and berseem clover (*Trifolium alexandrinum* L.). These temporary grasslands are exclusively cut for hay production or mowed after the winter grazing (one or more grazings per season). Often farmers harvest forage with a delay which has negative consequences on quality. Recently, farmers have introduced some mixtures based on annual self-reseeding pasture legume and winter cereal to extend the duration of temporary grasslands to two or three years. Among perennials, lucerne represents the primary temporary grassland species for neutral and alkaline soils. Very frequently, the seed of local ecotypes is utilized in pure stands as green forage, hay or dehydrated forage (3-4 cuts between June and October). In the LFAs, lucerne stands typically persist for 3-4 years under rainfed conditions or occasional irrigations, before a rotational crop is grown. Despite their widespread natural distribution in hilly areas, the perennial legumes red clover (*Trifolium pratense* L.) and birdsfoot trefoil (*Lotus corniculatus* L.), which are adapted to moderately acidic soils, have been little sown. The same is true for sulla (*Sulla coronaria* (L.) Medik.) and sainfoin (*Onobrychis* spp.), although there is renewed interest in these perennial legumes (Re *et al.*, 2014). A few varieties of perennial grasses, particularly cocksfoot (*Dactylis glomerata* L.), tall fescue (*Festuca arundinacea* Schreb.) and bulbous canary grass

(*Phalaris aquatica* L.), are sown in higher rainfall areas with deeper soils and they are generally included in seed mixtures with annual or perennial legumes.

An alternative use of net primary production of grasslands is represented by the conversion of biomass feedstock into a range of fractions and products for bioenergy and green biorefinery (Bullitta *et al.*, in this volume). The use of grasslands for energy production has been advocated also for Mediterranean marginal environments, but some authors have expressed warnings about this final destination, highlighting that in low-productive grasslands, biomass yield could be insufficient for also satisfying the requirements of forage for livestock production (Peeters, 2009). In South Europe, the interest of grasslands for energy purposes relies on their tolerance to drought, while conventional perennial grasses studied for energy purposes (switchgrass, miscanthus and reed canary grass) have shown strong limitation when grown under rainfed conditions (Scordia *et al.*, 2013). Giant reed seems the only alternative to conventional grasses thanks to a higher drought tolerance, but it shows the great disadvantage being a rhizomatous plant, difficult to eliminate after crop cessation. To this regard, some native perennial grasses widespread in southern Europe were evaluated in the FP7 project OPTIMA for their physiological and productive responses and, when possible, for their ability to be sown by seed (www.optimafp7.eu). *Piptatherum miliaceum* (L.) Coss. seemed the most promising species in Sardinia, thanks to its long growing season and its ability to survive the summer drought. Moreover, smilo grass showed a great potential for the double utilization for winter forage production and summer use for bioenergy (Melis *et al.*, 2016a; Porqueddu *et al.*, 2014).

Key aspects for adaptation to climate change and rehabilitation of grasslands

Climate change, as predicted by a range of climate models, is forecast to have a great impact on agricultural production systems in Mediterranean climates. Several negative effects are expected on grasslands: increased failures at establishment, decreased grassland productivity and long-term persistence, and shortening of the grazing season unless the grassland is irrigated (Del Prado *et al.*, 2014); reductions in desirable grassland species are likely to occur, in favour of species with low palatability and broad ecological niches, due to reduced competition for water and nutrients (Ouled Belgacem and Louhaichi, 2013); nodulation and N-fixation in legumes may become limited by low nutrient supply (especially P and K) and high temperature (Irigoyen *et al.*, 2014). The quality of forbs, grasses and legumes in Mediterranean areas may not be influenced by elevated CO_2, warming and drought under Mediterranean conditions (Dumont *et al.*, 2015). Evidence indicates that changes in climate have occurred historically and Mediterranean ecosystems have shown considerable resilience to these changes (Hopkins, 2012; Seddaiu *et al.*, 2013). In any case, to prevent possible negative effects caused by climate change, increasing the resilience of grasslands and improving forage production and rehabilitating permanent grasslands are now compulsory. In the following sections we discuss the main key-factors that can increase resilience and adaptability and could be considered also as mitigation strategies from climate change.

Sowing annual and perennial species with high summer drought survival

The predicted changes in rainfall distribution, consisting of relatively lower and more variable autumn rainfall and a shorter spring, mean that some or all of the following traits are needed in annual legumes: (1) earlier maturity for reliable seed set in shorter growing seasons; (2) more delayed softening of hard seeds to reduce seedling losses from more prevalent false breaks; (3) greater hardseededness to compensate grassland survival for more frequent seasons of little or no seed set; and (4) a less determinate flowering habit to take advantage of longer growing seasons when they occur (Revell *et al.*, 2012).

In perennial species, desired characteristics include dormancy or low growth during the drought period (Volaire *et al.*, 2013), survival across drought periods (Annicchiarico *et al.*, 2011), and high water use efficiency during the growing season. The concurrent use of plants with different strategies to overcome drought is one of the adaptation approaches proposed by Kreyling *et al.* (2012) to establish permanent and multi-specific grasslands with greater ecosystem stability (Volaire *et al.*, 2013).

Increasing legume utilization

The biological N-fixing activity of legumes contributes to the soil N-enrichment, and this feature could contribute to land rehabilitation of degraded soils, increasing the potential for the re-establishment of native species (Perez-Fernandez *et al.*, 2004). The different species have different efficiencies in fixing atmospheric nitrogen, being 70% in subterranean clover, 90% in lucerne and up to 92% in field beans (Testa and Cosentino, 2009). The amount of fixed nitrogen reaches 184 kg N ha^{-1} year^{-1} in sulla and lucerne (Cosentino *et al.*, 2003; Sulas *et al.*, 2009). In the past, the traditional annual legumes used for grassland rehabilitation were *Trifolium* spp. and *Medicago* spp. Nowadays, many other species belonging to the genera *Ornithopus*, *Vicia*, *Lathyrus*, *Melilotus*, *Biserrula* and *Astragalus* are available on the seed market (Melis *et al.*, 2016b). Most cultivars of these species have been developed from germplasm collected in the Mediterranean basin by Australian scientists (Loi *et al.*, 2008; Nichols *et al.*, 2013). Nonetheless, they showed a poor adaptation to the variable climatic conditions and management systems of southern Europe (Porqueddu *et al.*, 2010; Salis *et al.*, 2012). Native genotypes of these species have also been selected in Mediterranean Europe, but efforts to promote their multiplication in Mediterranean areas have been unsuccessful.

Among perennial legumes, lucerne is the most appreciated species in many farming systems but some limitations to its use arise under rainfed conditions, where lucerne shows a low forage production, limited persistence and scarce tolerance to grazing, requiring the selection of suitable cultivars (Annicchiarico *et al.*, 2011). Currently, other investigations focus on new deep-rooted, drought-tolerant and slow-declining quality perennial legumes, including tallish clover (Hall *et al.* 2013), Caucasian clover (*Trifolium caucasicum* Tausch), stoloniferous cultivars of red clover and more drought-tolerant genotypes of birdsfoot trefoil (Nichols *et al.*, 2012a). Recent research also indicates that *Bituminaria bituminosa* (L.) C.H. Stirt has potential as a perennial forage legume for dry Mediterranean areas (Martínez-Fernández *et al.*, 2012; Porqueddu *et al.*, 2011; Reaside *et al.*, 2013). Other perennial legumes such as sulla (Molle *et al.*, 2003) and sainfoin (Lobón *et al.*, 2015; Theodoridou *et al.*, 2011), are summer-dormant and are already used for their contribution in stabilizing grassland production and forage quality (Re *et al.*, 2014) and for their content of condensed tannins, which can promote amino-acid absorption in the intestine (thereby decreasing nitrogen excretion and reducing greenhouse gas emissions to the atmosphere) and also reduce the load of gastro-intestinal parasites (Piluzza *et al.*, 2014).

Promoting the use of grassland mixtures

The potential agronomic, environmental and economic advantages of sowing mixtures of forage species and cultivars are widely recognised (Finn *et al.*, 2013), especially when mixtures are based on well-adapted genotypes (Dear and Roggero, 2003). Porqueddu and Maltoni (2007) and Maltoni *et al.* (2007) showed that grass-legume mixtures belonging to different functional groups, achieved higher dry matter yields, better seasonal forage distribution, better weed control and higher forage quality than pure stands of each species. More persistent grasslands in LFAs could be also obtained using mixtures of summer-dormant and summer-active perennial species and varieties able to exploit available soil moisture throughout the year (Norton *et al.*, 2012). Mixed swards are also expected to deal better with climatic variability and to show higher resilience (Lüscher *et al.*, 2014). Some problems arise when mixtures are needed to improve the herbaceous layer under a tree canopy, as happens in many woody pastures in marginal regions of the Mediterranean area, where herbaceous species cope with shade and resource competition with trees. In fact, legume abundance generally decreases beneath tree canopies (Marañon *et al.*, 2009). At the moment, specific mixtures for silvopastoral purposes are not available. Some recent experiences in Portuguese *montado* and in Sardinian oak woodlands are being carried out in the framework of the FP7 project AGFORWARD aimed at identifying legume species capable of persistence, and to fix adequately atmospheric nitrogen and to tolerate tree shading and grazing pressures in different Mediterranean pedoclimatic conditions. Preliminary results obtained by the sowing of biodiverse permanent mixtures rich in legumes in Portugal (Aguiar *et al.*, 2011) and mixtures of autochthonous pasture grasses and legumes in Sardinia are available (Franca *et al.*, 2016).

Benchmarking grassland typologies to improve the management of pastoral resources

Within an overall context of climate change, adaptation responses in the annual growth cycle of grasslands species are prompting a range of management-practice adaptations (Ergon *et al.*, 2016). Thus, knowledge of grassland typology is needed to adopt the best management practices; in fact, the differences in vegetation and phytosociological associations are still relevant in LFAs. Agronomic typologies based on the forage value of dominant or reference species, or synthetic indexes were designed in different countries, and recently, a first attempt to synthesize and homogenise grassland typologies at plot, farm and regional level in the different EU states was done by Peeters (2015). With regards to grazing, the extent and intensity of grazing differ among vegetation types and geographical locations (Casasús *et al.*, 2013). Among methods utilized by technicians and extension services for grassland typology assessment, the pasture-type approach, based on the determination of the Pastoral Value (PV) of grasslands, has been applied in several Mediterranean, Alpine and Apennine areas, with the main goal of characterizing pasture vegetation and its potential carrying capacity (Argenti and Lombardi, 2012; Re *et al.*, 2014). This approach may play an important role in defining the management of LFAs, particularly in territories affected by land abandonment and strong grazing pressure, where multiple-benefits of Mediterranean grasslands might be seriously jeopardized. It is based on the concept of pasture type, which could be defined as a semi-natural vegetation (mainly exploited by grazing animals), rather homogeneous in terms of botanical composition and influenced by environmental factors and agro-pastoral management. However, these methods show some limits, due to the fact that they are either too general or too specific and do not combine ecological and pastoral dimensions. A new promising method (Mil'Ouv) was recently developed for the environmental conditions of southern France (Garnier *et al.*, 2016). This diagnosis method is based on a multiscale analysis that considers farm, management unit and topo-facies, highlighting the fundamental interest in integrating local practitioner knowledge into comprehensive and collaborative ecological-pastoral strategies.

Extending forage availability

The traditional transhumance from the plain to the hills and mountains was the most common practice to overcome forage shortages in summer or winter, but in many areas it has almost disappeared (Azcárate *et al.*, 2013). Nevertheless, it is still core to the sustainability of many farms in the Iberian peninsula (Oteros-Rozas *et al.*, 2012). According to Velado Alonso and Gómez Sal (2016), the new transhumance model of short movements has demonstrated flexibility and adequacy to the current condition, and indeed it presents an opportunity to develop a transition towards more sustainable systems. Short distance transhumance has potential to be integrated into multifunctional grassland-based systems in LFAs, e.g. by reducing biomass to prevent summer wildfires. Other alternative solutions to transhumance have been proposed. They include the intensification of agriculture, but in LFAs this model weakens the sustainability of farms (Darnhofer *et al.*, 2010). Thénard *et al.* (2016) identified four self-sufficiency patterns and a set of 20 indicators for dairy sheep farms in southern France, which addressed agronomical and environmental features. These authors found that the use of wide diversity of meadow and species increases the concentrate sufficiency. The exploitation of alternative forage resources could also be a strategy to cope with seasonality of forage production. Shrubs, foliage and acorns from trees are traditionally used in oak-based woodland grasslands as forage supplements or for browsing in some parts of the Mediterranean basin (Papanastasis *et al.*, 2009). Alley cropping based on leguminous shrubs (*Medicago arborea*, *Chamaecytisus palmensis*, *Acacia* sp.) and other species (i.e. *Atriplex* spp.) is a promising way to overcome seasonal shortage of forage in the more semi-arid areas (Norman *et al.*, 2010) but the integration of shrub species into existing farming systems is not straightforward due to the long period of establishment and high implantation costs of shrubs, so nearly no implementation at farm level has yet occurred in southern Europe. In other areas with Mediterranean climate (Western Australia), summer-active C4 sub-tropical perennial grasses (e.g. *Pennisetum clandestinum*, *Chloris gayana, Megathyrsus maximus*) have been successfully introduced. However, their potential use appears very limited in Europe (i.e. risk of introduction of alien species).

Can ecosystem services provided by grasslands represent a tool for supporting pastoral systems in LFAs?

Greenhouse Gas (GHG) emissions and extensive grassland-based systems

Grassland-based farming systems produce not only the roughage, but also a large part of the animal's feed. Most rely on some purchased inputs, such as fertilisers and supplementary feed, and they always use direct energy derived from fossil fuels (Soussana *et al.*, 2010). From a global change perspective, managed grasslands contribute to anthropogenic GHG emissions due to the effects of the livestock raised (Gerber *et al.*, 2013) but they also have the ability to sequester carbon (C) due to plant activity, thereby partly offsetting C emissions (Fornara *et al.*, 2016). The effects on GHG emissions of the intensification/extensification level of livestock farming systems are again being discussed by scientists (Sintori, 2014). The low input techniques related to grassland, requiring less fertilization and field operations than arable land, have lower environmental impacts from eutrophication, acidification, greenhouse gas emissions and non-renewable energy use on grassland-based farms (Rotz *et al.*, 2010). Batalla *et al.* (2015) estimated average values for carbon footprint of sheep milk production systems in Northern Spain as ranging from 2.0 to 5.2 kg CO_2eq kg^{-1} Fat Protein Corrected Milk (FPCM), depending on the level of extensification. Life cycle analysis (LCA) is the standard method to measure the carbon footprint of a product. However, its application to extensive grassland-based systems has a number of shortcomings (EIP-AGRI Focus Group on Profitability of Permanent Grasslands, 2016). Recently, a LCA approach has been utilized to evaluate the environmental performances of sheep farms (Ripoll-Bosch *et al.*, 2013; Vagnoni *et al.*, 2015). Among others, the LCA demonstrated that the substitution of crops such as irrigated maize and wheat with grasslands improved the overall environmental performances of the farm, but only to a minor extent, because of the predominant effect of enteric fermentation with respect to other impact factors (Vagnoni and Franca, pers. comm.).

Environmental policy tools and grasslands

Different environmental policy tools have been implemented to preserve Mediterranean grasslands in a sustainable way. Among them, Natura 2000 is a pan-European initiative started in 1992 with the aim of ensuring the long-term survival of highly valuable and threatened species (fauna and flora) and habitats, listed under both the Birds Directive and the Habitats Directive. In 2015, 18% of the EU28 land area was protected either as Special Areas of Conservation or Special Protection Areas. This proportion rose to 37% in the case of predominantly Mediterranean countries (Cyprus, Croatia, Greece, Italy, Spain, and Portugal). This region harbours 146 different habitat types (more than half of those listed in the Habitats Directive), 37 of which are endemic (Sundseth, 2009), from dry forests (oaks in *dehesas/montados*, black pine forests), scrublands (*maquis, garrigue*) to a large variety of grasslands (dry, calcareous, steppe or mountain grasslands). This programme involves active management of Natura 2000 sites, where the compatibility of agriculture and livestock farming with nature conservation has been achieved through the implementation of agri-environmental schemes. To provide a sound basis for policy making, the LIFE programme has financed a large number of projects aiming to establish the best farming practices to maintain or enhance the natural value of sites (Silva *et al.*, 2008). However, LFAs are very weak in terms of political influence and grassland-based systems are victims of the lack of coordination of agricultural policies with environmental ones. For this reason, problems among farmers and park managers of damage to grasslands and livestock from the wild animals in protected areas and in their surroundings are not uncommon (Fernández-Gil *et al.*, 2016). The ultimate aim of these policies is to encourage the development of sustainable farming systems, where farmers obtain their incomes from animal production but are also rewarded by their supply of public goods that do not have a market price (Bernués *et al.*, 2011), the so-called non-provisioning ecosystem services.

The provision of ecosystem services by European farming systems have been analysed both in permanent grasslands (Huyghe *et al.*, 2014) and wood pastures (Plieninger *et al.*, 2015), highlighting their multifunctionality. It has been proposed that conserving what is left is more effective than getting back to what has been lost, and consequently biodiversity conservation is more likely to be effective on farmlands that are already managed at low intensity and that retain a certain amount of semi-natural vegetation (Kleijn *et al.*, 2011). Their role in regulatory processes with global impact is also substantial, given that the sequestration of soil carbon in grasslands holds a potential for greenhouse gas mitigation (Soussana *et al.*, 2010), and can be reinforced by specific practices such as reducing farm C losses or fire risk (Bullitta *et al.*, in this volume), particularly important in the Mediterranean context. The sociocultural values of these systems are associated with cultural identity, because they are a reservoir of traditional knowledge and result in products that can be differentiated according to their origin or quality, and also with landscape aesthetic values, which can result in opportunities for recreation and tourism. The relative importance of these ecosystem services may be perceived differently by different members of society: interestingly, farmers tend to have a deeper instinctive knowledge about them, especially of regulation and supporting services associated with their farming practices, whereas non-farmers show more global concerns related to mostly to provision and cultural services (Bernués *et al.*, 2016).

Euro-Mediterranean regions are applying different strategies for supporting grassland-based livestock systems. In particular, in relation to the use of agro-environmental measures of the Rural Development Programme (the second pillar of the CAP) in the previous decade (2000-2010) a different positioning of the pastoral farms in relation to the rural development was shown: i.e. relatively aggressive positioning by French Mediterranean regions for the sheep sector, but more timid or almost non-existent by others. This is related, of course, with the ability for mobilization of the profession, with the influence of the various 'lobby' (agricultural, environmental, etc.), with the role and expectations of public authorities and with the willingness of farmers themselves to engage in certain innovative forms of contract (PASTOMED, 2007).

Branding high quality products of grassland-based systems

The optimization of the economic performance of extensive livestock systems can also be achieved by increasing the added value of products. Consumers are increasingly concerned about environmentally-friendly and ethical livestock (i.e. animal welfare) production and product nutritional quality (Bernués *et al.*, 2012; Moreno *et al.,* 2014), and there is evidence that pasture or forage-based diets can improve environmental, ethical and human health outcomes, especially when compared with that from feed-lot systems (Entz *et al.*, 2002; Tsiplakou *et al.,* 2008). Available results suggest that there is an opportunity for products of a superior quality from systems that include a high proportion of forage (Acciaro *et al.*, 2016; Blanco *et al.*, 2012; Zervas and Tsiplakou, 2011). In countries such as France and Italy, the specific natural qualities of individual 'terroirs' define not only the agronomic conditions of production but also the distinctive taste and consumption experience associated with the product. Moreover, grassland-based Mediterranean farming systems located in LFAs can easily evolve to organic (Zoiopoulos and Hadjigeorgiou, 2013), thus leading to a higher valorisation of the products and having a greater capacity to meet the requirements of 'organic' consumers.

Management practices have influence on different quality traits and the main challenge for the farmers is to ensure a constant product quality (Coppa *et al.*, in this volume). In fact, there are strong seasonal changes in the grasslands both in terms of phenology and botanical composition during the growing season (pasture shortage during summer), fluctuations between years and also the need to switch to a diet based on conserved forage or even on concentrates in winter. However, especially in small-scale enterprises, the lack of a reproducible standard in seasonal production could be considered a positive characteristic of uniqueness.

Even if grasslands are highly appreciated for their production of local high-valued animal products (Bernués *et al.*, 2015), at present the market-share of branded grassland-based products is not fully exploited. A validated method to assess and certificate the permanent grassland-based origin of milk and meat products, i.e. through the identification of biomarkers and their traceability from pasture to the final product, is compulsory (Danezis *et al.*, 2016). These biomarkers might represent a proof of the geographical identity of milk, cheese and meat and can be used for developing value chain valorisation, through labels such as Traditional Speciality Guaranteed (TSG), Protected Geographical Indication (PGI) or Protected Designation of Origin (PDO). Currently available authentication methods to distinguish, discriminate or authenticate milk, cheese or meat according to the management practice and constraints to their implementation in routine authentication were summarised by Coppa *et al.* (in this volume). In the end, consumer's willingness-to-pay for the products is necessary to ensure an adequate remuneration to the farmers.

Conclusions and future directions

Grasslands, often with different degrees of tree or shrub cover, still have an important economic and social role in Mediterranean LFAs. Currently, they face several constraints that require a holistic approach to be overcome, the farming systems being quite complex and not specialized. Unfortunately, most studies have focused on the effects of only one or a few influential factors on the grasslands without considering others that may play a relevant role on the system. Currently, some agronomic objectives need to be satisfied. The development of new varieties of grassland species for dry Mediterranean areas and multi-site investigations are needed to identify the best adapted grassland species along with the most appropriate grazing management. However, the successful development of a forage and pasture seed industry in the Euro-Mediterranean countries is critical to guarantee seed supplies of the best-adapted cultivars to the region.

In order to be able to compete with products coming from intensive agriculture, both public perception and the management schemes about LFAs should urgently be reoriented. Management and rehabilitation should be conducted in a participatory manner involving all stakeholders using a territory, and institutional and policy support is needed, in addition to technical aspects, for the sustainability of grassland resources. For this reason there is a rising demand for scientific knowledge and new approaches in scientific research aimed at:

- developing farming systems based on the use of local breed and forage resources, where the most adequate management techniques are implemented for optimal technical and environmental performance and the delivery of products with high added value;
- promoting the involvement of farmers in the assessment of experimental field trials to communicate scientific results and obtain a qualified feedback for future research needs;
- strengthening the connections among research and practice, by means of efficient brokering systems and facilitator agents, whose role is nearly unknown in Mediterranean areas;
- developing innovative management tools that may constitute an integrated system of support for decision (DSS) making in extensive grassland-based farming systems.

The training of young specialized pastoral agents for rural land planning (i.e. synergies among farms and regions located in the more fertile areas and the marginal areas) and new immigrated workers (technical graduates, experienced people specialized in all aspects of grazing: social, land, environment, animals, etc.) could also be a way to help the management of LFAs and they should be involved in the technical boards of protected areas.

Long-term multidisciplinary experiments are also needed to continuously check the pastoral resources, environmental outputs and ecosystem services associated with Mediterranean grasslands, in order to provide a better understanding of the complexity of grassland ecosystems and to define specific indicators for better management decisions and mitigation of climate change. Also, the integration of data sets at local level is necessary, aimed at implementing the LCA tools for interconnecting extension services to academic and research centres and, thus, obtaining a more timely and accurate dynamic picture of the territorial context. Such an approach might facilitate the creation of territorial management permanent committees of stakeholders, where farmers should participate directly in such committees and be effective in identifying and maximizing all the opportunities for multifunctional benefits.

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Changes in Euro-Mediterranean pastoralism: which opportunities for rural development and generational renewal?

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Abstract

Mediterranean rural regions represent rich and fragile settings in agro-ecological and socio-economic terms. The region is increasingly beset by growing human presence and climate change dynamics. Agro-pastoral systems are still important activities in terms of employment and income, but also for ecosystem functioning and landscape management. Traditional agro-pastoral systems have gone through important reshaping in recent decades and they have to confront today the dynamics challenging their future. This paper examines how problems and issues in the generational renewal of EU-Mediterranean farms affect their profile and their future roles in the development of the area, in particular in Italy and Greece. Current dynamics seem to indicate that the younger members of livestock farm families often seek alternatives to pastoralism, thus favouring the depopulation of mountain areas and exposing grasslands to problems of abandonment and socio-economic desertification. This context witnesses a growing presence of immigrant shepherds, who reach southern Europe from other pastoral areas in the Mediterranean region, coming to provide skilled labour at a relatively low cost. Their presence enables the pastures of mountainous areas to be maintained and kept productive, reproducing the patterns of generational renewal associated with an ethnic substitution that has characterized Euro-Mediterranean pastoralism in the last century. Women also seem to gain an active role in the family farms once again, as in the past their role had been neglected.

Keywords: farm labour, migrations, small ruminants, multifunctionality, resilience, rural development, sustainability

Agro-pastoralism in Mediterranean rural regions, a key factor for a sustainable development

After the end of the Second World War, many rural Mediterranean areas were described as underdeveloped and not suitable for agricultural modernization. European policies discouraged investments in these areas, where it was difficult to organize forms of intensification of agricultural production; the traditional, peasant agriculture, oriented to self-consumption and livelihood subsistence, was considered inefficient, uncompetitive and backward. Therefore, these regions steadily experienced land abandonment, depopulation and economic decline. The Mediterranean rural areas, in contrast to urban areas, remained trapped by a negative perception.

This situation has become even worse over the past thirty years. Market deregulation and liberalization, and changes in the production systems and policies aimed at rationalizing public expenditure have all contributed to increasing precariousness in these areas and to a growing social vulnerability, which is general to many local groups and various sectors of the local economy (Ascoli and Pavolini, 2015; Kvist, 2013; Matsanganis, 2011; Ranci, 2010). Nonetheless, according to recent studies (Espon, 2014; Crescenzi *et al.*, 2016; Milio *et al.*, 2014a,b) rural areas have demonstrated an unexpected resilience to the recent economic crisis that started in 2008, putting into doubt the 'slogan' that 'rural is linked to backwardness'. Moreover, despite a partial degradation of the ecological quality of the rural landscape due to the abandonment of agricultural activities, employment crisis and depopulation phenomena

(Bertolini *et al.*, 2008; SERA, 2006), the countryside of internal rural areas is less affected by the process of trivialization and environmental standardization related to agricultural modernization techniques (Agnoletti and Emanueli, 2016).

Within the emerging rural development paradigm (Ploeg *et al.*, 2000), the development trajectories of Mediterranean rural regions are heterogeneous and localized (OECD, 2006). Ongoing research in Greece demonstrates that urban dwellers are more affected by the crisis, while young people tend to 'rediscover' rural areas and farm labour as alternatives to the generalized lack of employment opportunities in other sectors of the economy (Ragkos *et al.*, 2016b). In addition, the Mediterranean countryside is recognized as an expression of the 'historical rural landscape' (Agnoletti, 2013; Antrop, 1997, 2005). It is now evident that Mediterranean rural areas nowadays are invited to play a new role – away from the erroneous perceptions of the past. In comparison with the lowland and coastal areas, these regions represent a differentiated setting with specific agro-ecological characteristics that express intertwined relationships between the environment, the economy and society.

Mediterranean marginal and internal areas are actually in search of a sustainable pattern of development (Mahoney, 2000), where existing activities and know-how could trigger a territorial development process. Within this context, agro-pastoral systems have historically been of central importance in the development of these areas; especially local agriculture and transhumant pastoralism have traditionally been complementary activities (Campbell, 1964; Le Lannou, 1979; Mattone and Simbula, 2011; Meloni, 1984; Pernet and Lenclud, 1977; Ravis-Giordani, 1983). Nowadays, pastoralism – i.e. extensive livestock rearing based on natural grazing – remains a key economic activity for Mediterranean marginal territories; it is a way to sustainably manage resources, and is suitable for preserving and reproducing the various agro-ecological characteristics of the Mediterranean (Meloni and Farinella, 2015). Pastoralism is multifunctional as it produces a wide range of goods and services (cultural features, ecosystem services, landscapes, etc.) jointly with food (milk, meat, dairy products, etc.). It demonstrates high resilience and adaptive capacities through time as well as important potential for further development in terms of income and employment for areas where alternatives and competition for land uses are limited. In this sense, agro-pastoral products – especially dairy – represent strategic resources for the well-being of these areas. Also, the issue of provision of ecosystem services by Mediterranean grasslands (see for instance Varela and Robles-Cruz, 2016) is constantly gaining attention and proposes novel dynamics for the development of agro-pastoral areas. This whole bundle of alternative resources (ecosystem services and unique products) needs to be well embedded in scientific research and governance in order to achieve benefits for Mediterranean territories.

Nowadays, a development pattern based on agro-pastoralism could be pursued in the form of 'social innovation' (Mulgan *et al.*, 2007). This type of innovation stems from actors themselves, as a response to specific socially acknowledged needs; it is not limited to a particular group but rather it can be developed by the vast majority of members of a rural society – the original and localized mixture of human and natural settings expressed the embeddedness of agro-pastoralism in Mediterranean rural communities. Natural biodiversity, landscapes and environment management, historical cultivars, specific foods, craft and tacit knowledge, informal regulation and local codes, local economies based on the self-production, self-consumption, trust and reciprocity's network and the general way of living and building the countryside are part of the 'territorial capital', immaterial patterns able to produce local collective goods (Ostrom, 1990). Recent studies (Barca, 2009; Barca *et al.*, 2014; Mantino and Lucarelli, 2016; Milone *et al.*, 2015) show the efforts of rural areas to activate their intangible and often unrecognized resources and to claim their participatory governance strategies, in order to integrate production of local collective goods. Other research has highlighted the emergence of multifunctional agriculture models to cope with the economic crisis (Ploeg and Roep, 2003; Wilson, 2007), reacting pro-actively to the lack of services

(Cersosimo, 2012; Connor, 2009; Di Iacovo) through combining tourist hospitality to agricultural production (Cawley and Gillmor, 2008; Kinsella *et al.*, 2000).

Under the light of novel approaches of the development of rural areas, this paper examines the prospects of agro-pastoralism in one of the most typical Mediterranean settings – the inland areas of islands. In these areas, the main constraints have to be addressed at local as well as global levels in order to support the development of agro-pastoralism and its potential. This paper provides a general framework on the characteristics of Mediterranean agro-pastoralism, using case studies in Greece and Italy (in particular Sardinia), which are typical of the situation prevailing in rural areas in the Mediterranean. The paper goes on to analyse the main constraints affecting contemporary pastoralism in the region, including generational renewal, and the new, diverse actors that hold a stake in the sector – especially the role of women, the subaltern role of immigrant workers and the limitations set by European policy makers. Finally, through a SWOT analysis, the various aspects and factors influencing Mediterranean agro-pastoralism will be assessed with a view to contributing to the design of efficient policies.

Changes in contemporaneous pastoralism in the Euro-Mediterranean rural region of Italy and Greece

In the Italian case, sheep farming is concentrated mainly in the areas of Central and Southern Italy. In particular, this type of farming is typical of Sardinia, where 43.8% of animals reared in Italy are kept; in 2016 there were 3,153,580 ewes and 11,213 dairy sheep farms in Sardinia (source BZN – date Bank Registry National Animal Husbandry). For this reason the Italian analysis is focused on the Sardinia Region.

Sardinia is the main producer of sheep milk in Italy (65% of Italian production in 2015). Sheep farming in Sardinia was traditionally organized in the form of long and short transhumance from inland to coastal areas with milder climates. At the core of the farm's management was the family. Today, pastoralism has become sedentary along the old ways of transhumance, where the shepherds have acquired the land abandoned by farmers in the fifties, following the crisis of more traditional cereal farming. Sheep farming is based on a semi-extensive system and, in some areas, on an extensive one, totally based on natural pasture grazing. Pasture grazing yields good quality milk – which is the main product of agro-pastoral flocks – due to the variety and richness of natural pastures. In Sardinia, the permanent grassland is the 60% of the arable land (694,760 ha) compared with only 26.9% in Italy (ISTAT – Census of Agriculture, 2010). Generally, the farms maintain this multifunctional character. In 2013, only 39% of farms were specialized only in the ovine sector, while the remaining 61% associated sheep with other types of breeding, in particular pigs, cattle and goats (Elaboration on data from Anagrafe Zootecnica Nazionale, LAORE, 2013). The size of farms has tended to increase in the last 30 years, but farms remain of medium size: only 4% have more than 750 sheep and only 2% have more than 1000. There is a large presence of farms with not more than 100 sheep (70% of the total) (LAORE, 2013). Farms of larger size are situated in the lowlands; they are of entrepreneurial organizations and are more organized in terms of operation and innovation adoption. These breeders are specialized in milk production. However, the main source of weakness is its dependence on a single product of milk that is sold to local processing industries that decide the price. The smaller farms are in the mountainous or hilly areas more suited to extensive grazing; in these areas sheep farming provides complementary incomes to farm families and is combined with other activities to ensure decent living conditions. Despite its extensive organization, the Sardinian dairy sheep system is being increasingly pushed towards an intensification and standardization of production, which is likely to impair its quality and overall resilience. During the last 30 years Sardinia was characterized by a process of concentration and modernization of farms. The number of farms diminished, but the livestock increased and so did the average farm size. Farmers used more grazing land and invested in sheds and milking parlours and other agricultural machinery, with EU funds. Actually, breeders are very dependent on the industries and the low milk profitability is a key problem of the local dairy chain. The majority do not process milk into cheese, thus providing an important shift from the traditional shepherd labour.

In Greece, the livestock sector contributes 28.3% to the total added value of its primary production and almost 1% to national GDP (ELSTAT, 2015; PASEGES, 2013). Sheep and goat farming is the most important livestock production activity, in a variety of systems, from extensive and semi-extensive – based on grazing and little adoption of innovation – to intensive, which have undertaken large investments in animal capital, buildings and machinery and are based on the provision of feedstuff (Chatziminaoglou, 2001). Most flocks are dual purpose, with milk being the main product and lamb meat being of importance especially during peaks in demand (Easter and Christmas). The actual contribution of livestock systems is very important socially and economically, especially for particular rural areas of the country. Family labour has been the only source of labour for Greek livestock farms for centuries. The unpaid labour of family members allowed family farms to operate as, in difficult times, they resorted to their own resources, and reduced their standards of living and survived until external conditions were better (Holzner, 2008). In this context, which also included the massive migratory movements of the 20th century, small and medium-sized livestock farms prevailed in Greece (see e.g. Kasimis and Papadopoulos, 2013).

Despite the extensive characterisation of pastoralism, pastoral operations have intensified as a result of agricultural restructuring (Meuret, 2010; Nori, 2017), and livestock owners have developed managerial skills in order to comply with a growing administrative, bureaucratic and technical demands and tasks. Shepherds' living and working conditions have hardly improved; most of the time is spent in harsh settings, with limited access to public services, scarce connectivity and few opportunities for leisure and alternative activities. The growing presence of predators and climatic vagaries add further hardening factors. The prices of small ruminants' milk and meat have fluctuated, while production costs have increased constantly (ISMEA, 2010); dependence of Common Agricultural Policy (CAP) schemes and subsidies have grown accordingly, and represent today about 40% of the sector revenue (data from ongoing research projects). Such restructuring has thus contributed to creating unattractive conditions for the new generations, who have often decided not to follow their fathers' footsteps, and to avoid engaging in a profession with uncertain prospects. Through this lens one can understand the crisis of pastoral 'vocation' and the relative problems of generational renewal which is affecting this sector.

Another big constraint for agro-pastoral farmers in Mediterranean rural regions is compliance with regulatory requirements. Numerous policies at various levels (EU/International (CAP), national, regional) affect the operation of these systems. Scientific debate has analysed the governance practices related to local and rural development policies (Barca, 2009; Meloni and Farinella, 2016; OECD, 2006; Ray, 2000, 2006; Shortall, 2008; Storti and Zumparo, 2009), introducing more recently the rural welfare dimension (Bertolini *et al.*, 2008; Di Iacovo and Scarpellini, 2012; Farmer *et al.*, 2010; Fazzi, 2011; Halloran and Calderón, 2005; Tulla *et al.*, 2014; WHO Regional Office for Europe 2010a,b).

The treatment of the specific problems and needs of agro-pastoralists through the CAP (Reg. EC/1307/2013 and Reg. EC/1305/2013) is superficial and farmers fall within the same criteria with conventional intensive or semi-extensive systems. Local/regional legislation regarding quality standards also poses restrictions to agro-pastoral products (e.g. cheese from raw milk) and hinders the expansion of informal marketing networks. Nonetheless, using the 'correct' mixture of policy incentives, the combination of these policies could prove to be a useful tool for territorial development, if the biases against agro-pastoral farmers are efficiently revised.

Generational renewal, female work and the role of immigrants

Women in livestock farming: a 'rediscovery'?

The Mediterranean agro-pastoral farm is based on family work, with a gender division of labour. Although, at least for the case of Sardinia, it is not correct to speak of 'matriarcato', until the 1970s the role of women in the management of household was central, because of the long absence of men engaged in transhumance (Cois, 2015; Da Re, 1982; Meloni, 1984; Murru Corriga, 1990; Paulis, 2015).

This model change during the post-war period, when women were ruled out of the operation of family farms, as the productivist model led to a 'masculinisation' of farming (Saugeres, 2002). Combined with an abundance of hired labour, women were assigned an auxiliary role and looked for employment in non-farm sectors (Bharadwaj *et al.*, 2013) or remained within their household duties. With women away from the production process, however, it was difficult to continue performing some of the tasks in the same efficient way, as women's labour is endowed with particular emotional elements ... 'which is crucial for the sustainability of rural people and places' (Herron and Skinner, 2012). In this aspect, Trauger (2004) underlined the fact that women are up to three times more likely to be the operators of a farm following a sustainable production pattern, demonstrating their persistence to quality and to the performance of multiple functions – other than production of food and fibre – through their engagement in the primary sector.

Today, a partial return of women in livestock farms is observed. For example, in the case of Sardinia, in the year 2013 around 10% of farms were owned by women (source: elaboration of LAORE on BZN data – Laore, 2013). An important similarity between the Sardinian and the Greek case lies in the – often fallacious – increase of female heads-of-farms. It has been very usual that husbands have passed the farm or part of it to their wives, sisters or mothers for several reasons, including tax alleviation or to access CAP incentives or to increase the income support they get (e.g. for the LFA payments in Greece). Also, some heads-of-farms have passed the farm to other family members in order to be eligible for early retirement; however, they have still a very active role in the farm – or even continue to be heads of farms. In Greece, a survey of collocated farms revealed that the true number of transhumant farms was 22% lower than the officially registered farms; of course, it was found that the average size of the 'true' farms was significantly higher, indicating that these farms were of a more viable size.

An example where women maintain very active roles in livestock production, comes from the extensive production system of Pomaks in the Greek Muslim minority (Ragkos *et al.*, 2016a). Their livestock farms are pastoralist – being heavily dependent on grazing – and small-sized; they rear autochthonous breeds and a significant part of the production is used within the farm household. The wife is involved in farm operation, providing manual labour or becoming the manager of the farm when the husband migrates to work as a sailor or worker. It is the members of the family that run the farm and women have maintained their essential role, demonstrating that the character of the Pomak livestock production system has not been abolished after the crisis.

Not always so for women who actually work on farms. A survey by Ragkos *et al.* (2016b) in North-Eastern Greece (Evros) showed that under the economic crisis, farmers substituted hired labour in order to reduce labour costs. However, intensive farms have high human labour requirements and only males and heads-of-farms were not sufficient. As a result, while in 2010 26% of labour was offered by hired workers, in 2014 this percentage did not exceed 7% and a female member of the family would work regularly or part-time in 36 of the 41 farms. It was found, however, that this strategy increased the marginal product of labour by more than 40 per cent from 2010 (2.27 €/h) to 2014 (3.21€/h). However, during empirical research in Sardinia (Farinella, 2016), cases of female shepherds were not scarce. Moreover, when women help in administrative issues and are present in the management of the farm, this is most dynamic and innovative in terms of products and markets. This is particularly the case in small farms.

Young farmers in the Mediterranean

The presence of young people in the livestock sector is very important because they demonstrate a big capacity to innovate. Young farmers have been the initiators of collective actions in rural areas; their networks often play important roles to achieve common goals, such as better access to knowledge, diffusion of information and economic performance. Young farmers are expected to play an essential role in the formulation and implementation of modern patterns of rural development – strongly sought by the CAP – which provides incentives to counter-balance the decline of rural youth and the negative perception of agricultural work that affect rural areas.

Today, the low and decreasing percentage of young farmers in EU countries is considered a major problem for the future of agriculture (Zagata and Sutherland, 2015) and is especially of concern for marginal areas. In 2007 in agro-pastoral areas of southern EU it was reported 'the very high rate of over-55 compared with those under 35 years of age ... and in many areas, the presence of elderly people 10 times more than young ones!' (Pastomed, 2007:18). In 2013 the European Union farmers under 35 years accounted for 6% of all farmers, while in Greece this was 5.2%, while farmers over 65 years accounted for 31.1 and 31.3%, respectively (Eurostat 2015). Sardinia is indeed one of the regions with the highest ageing index and the lowest birth rate in the world. In 2013, out of a total of 30,260 sheep farmers, over a third of farmers were aged above 60 and over 50% were older than 50, while only 5% were aged less than 30 years. The low (relative) proportion of young farmers over older farmers is considered problematic and is expected to determine to a significant extent the future of European agriculture. Structural consequences are expected including land abandonment, depopulation, and lack of services, which will reduce the attractiveness of rural areas. This presents a serious crisis for the peasant family and its ability to ensure survival. In many cases there is not a son to ensure continuity of activity.

In recent decades different programmes have been applied by EU and national institutions to attract young people to farming, under the overall policy of farm-heads age-renewal. In particular, the CAP has set the generational renewal of agricultural populations as a target. In Italy, the programme is operated by regional governments and provides a grant (not a loan – up to 40,000 Euros) for newly establishing farmers. This project has been in force since the 1990s in Greece – but is operated at the central government level. The results of this policy are controversial, with cases where the funding supports new multifunctional sheep farms, while in other cases it is a way to obtain money by formally sharing the family farm among siblings. A typology of Greek young farmers (Koutsou *et al.*, 2011) revealed that only very few young farmers were actually involved in the farm family business or even lived in the rural community. The majority were only auxiliary workers in the farms or were employed in other sectors and just joined the 'Young Farmers Installation' scheme for the funding.

According to a Greek study in 2016 (Koutsou *et al.*, 2016) there are significant differences between the profile of young farmers among Greek prefectures (differences in gender, mean age and education). In remote areas, young people preferred to enrol in the programme as a solution to unemployment, while in a more dynamic lowland area it was found that beneficiaries created farms with high labour requirements. A closer observation of the data shows that the production patterns of the new farms did not change significantly than the general local pattern, but in lowland areas a trend of diversification towards more dynamic sectors was found. Also, according to the results of a typology, there are three distinct types of young farmers: the first includes young people who have lived in the rural community their whole lives and chose to become farmers, the second those who were 'forced' to follow the family farm, and the third those who enrolled to the programme just for funding. The first type is the most innovative and prone to cooperation, while the third type is not interested in innovation – in fact, this type involves mainly women.

Migrant labour in EU-Mediterranean agro-pastoralism

The growth of migrant labour in agriculture is associated with the lack of young people in the countryside, and the depressive demographic dynamic, together with the difficulty to obtain a right, fair remuneration and income. As a result, agricultural activities in Europe are increasingly carried out by foreigners, often involved in low-skilled activities. Today more than a third of the officially employed agricultural workforce in Italy, Spain and Greece is of foreign origin (Caruso and Corrado, 2015; Collantes *et al.*, 2014; Kasimis *et al.*, 2010; Nori, 2017) (see also Table 1). Immigrant communities play though a relevant role also in specialized agricultural sectors; this is the case in livestock farming, where the presence of the foreign workforce is increasing, in both quantitative and qualitative terms. However, in Greece the reduced profitability of farms during the economic crisis brought about a decrease in migrant labour: from 2009 to 2013 a 4.6% reduction has been witnessed in the number of permanent hired workers and a 13.6% reduction in seasonal hired labour in the Greek primary sector, although opposite patterns are being reported for other Southern European countries, e.g. Italy (Caruso and Corrado, 2015).

No matter the entrepreneurial trajectory pursued to cope with and adapt to the sector restructuring, immigrant shepherds have provided a quite skilled labour force at a relatively low cost. Without foreign workers, many pastoral farms would face today great difficulty in pursuing their activities. Immigrants in rural areas not only participate in productive agro-silvo-pastoral activities, but represent as well an overall strategic resource for the sustainability of mountain societies, providing a critical contribution to repopulate remote villages and most marginal communities – such as the cases where local shepherds married foreign women, so that people mobility enhances family and community demography and dynamics (INEA, 2009; Kasimis, 2010; Osti and Ventura, 2012). However, several studies have analysed the exploitation of migrants in the intensive agricultural system as a negative effect of global market price competition in the agri-food chain (Corrado *et al.*, 2016; Ortiz-Miranda *et al.*, 2013; Pugliese, 2011). The salary and the quality of life are not very good and the immigrants do not have great opportunities to improve their conditions and they thus leave the sector as soon as better options arise. An on-site survey by Nori and Ragkos (2017) discerned two types of migrant workers in agro-pastoral farms of Central Greece; the first includes migrants aged over 40 who had grown up in their countries of origin, while the second involves younger workers who have lived most of their lives in Greece. In-depth discussions with these workers showed that the former mainly undertake this job to earn their living without considering professional changes while most of the latter find themselves temporarily working in farms due to lack of other opportunities.

The typical profile of the immigrant who works as a salaried shepherd is that of a man between 25 and 40, native of a country of the Mediterranean region (predominantly from Romania, Morocco, FYROM or Albania) but recently also from Asia (e.g. Pakistan, India) and sub-Saharan Africa (e.g. Ghana, Senegal), often with previous, direct exposure to animal breeding, although at different scales. Workers with

Table 1. Recent demographic trends in Euro-Mediterranean countryside (Caruso and Corrado, 2015; Eurostat, 2008).

Spain	France	Italy	Greece	
9.8	4.8	7.9	20.8	% rural/active population in 2008
56.4	41.9	62.2	57.2	% older >55 years 2008
19.1	–	19.4	17	% immigrants in labour force in 2008
24	–	37	>50	% immigrants in labour force in 2013

previous experience and specific skills are highly welcomed, but a significant part of migrants come from urban settings with little previous exposure to livestock production. Immigrant shepherds are appreciated for their endurance, flexibility and adaptability (Nori and Ragkos, 2017); concern has been raised by some breeders concerning socio-cultural attitudes and technical gaps on certain aspects related to the adequate management of forestry resources, wildlife presence and relationships with farming as well as with protected areas.

In Greece, the massive influx of labour migrants in Greek rural areas in the 1990s was a factor that helped ensuring the reproduction of Greek family farms (Papadopoulos and Roumpakis, 2009), leading to the formation of large intensive livestock farms (Karanikolas and Martinos, 2012). The dairy cattle sector is an example of this sort. According to a survey in Northern Greece (Ragkos *et al.*, 2015) the large specialized dairy farms that appeared after 2003 resorted to hired labour, because family labour was not enough for such intensive production patterns. The labour requirements of the 39 sampled dairy cattle farms were covered almost equally by family members and hired workers. The employment of even more immigrant workers did not seriously affect the cost structure of the farms, as the labour costs do not exceed 8.4% of the total costs. However, these farms have maintained their predominantly family character. A recent example was pointed out by Ragkos *et al.* (2016b) in Northern Evros, Greece, where large, modern, entrepreneurial, innovative farms emerged as a result of CAP changes in 2006. They relied on hired labour, mainly migrants, as spouses remained in charge of their farm household duties and younger family members looked for off-farm jobs or left their homes to study in other parts of the country. Concerning agro-pastoral transhumant farms in Central Greece (Thessaly), here the farms operate under a rather traditional pattern and labour expenses account for more than 25% for the average farm. Despite the undeniable family character of the farms, almost 25% of the total labour requirements are still covered by hired migrants. The analysis of the economic performance of these farms demonstrated that large farms use hired labour more efficiently (Ragkos *et al.*, 2014).

In Italy, the presence of migrants covers a large part of the salaried shepherd workforce (Nori, 2015). Apart from the better-known case of the Parmesan (Lum, 2011), immigrants play as well a strategic contribution in the value chains of Fontina and Pecorino cheeses, which are issues from pastoral settings (Nori and de Marchi, 2015). In Sardinia (Italy), the use of a foreign cheap workforce (in particular Romanians) reflects the structural problems of the Sardinian sheep dairy system (low milk profitability and dependence on Pecorino Romano, a low-cost cheese, subject to price volatility), as well as the difficulty of recruiting local people willing to live and work in the countryside. Romanians work in medium-sized sheep farms (more than 500 sheep and with intensive milk production), they get an accommodation on the farm and accept working conditions and salaries usually rejected by the local people. Their aim is to earn money and return to Romania, with a clearly temporary migrant project (Farinella and Mannia, 2016, 2017). The vast majority, in fact, are not thinking about remaining in this sector, neither in the country of destination, specifically mentioning limitations in accessing land and/or credit. However, especially among Romanians, some invest in the purchase of family land and livestock in their home communities – so that this migratory phenomenon contributes in some way to pastoralism within the framework of the EU (Nori, 2017). Overall, the impact in terms of generational renewal is very limited; the transition from manual labour to entrepreneurship and livestock ownership in this sector shows very low rates for migrants, and this undermines the ability of the incoming population to contribute to the future of this sector. Cases through cases exist where immigrant shepherds look into opportunities to set up their own flocks, and/or cooperate amongst themselves or with local ones in sharing land, subsidy or credit assets.

Assessing strengths and weaknesses of Euro-Mediterranean pastoralism

In order to better comprehend the dynamics of Euro-Mediterranean agro-pastoralism a SWOT analysis was undertaken. This method enables the detection of advantages and disadvantage, as well as opportunities and barriers in the internal and external environment respectively. Empirical evidence, published data and survey results from different case studies in the Mediterranean regions (Cois, 2015; Farinella *et al.*, 2013; Galanopoulos *et al.*, 2011; Hadjigeorgiou, 2011; Mannia, 2013; 2014; Meloni and Farinella, 2015a,b; Pastomed, 2007; Piteris *et al.*,, 2015; Ragkos *et al.*, 2014) were combined in order to produce Table 2. This schematic presentation enables us to detect specific development resources and to pinpoint policy priorities, as the information included here depicts the situation in the agro-pastoral sector of rural areas of the EU Mediterranean.

The SWOT analysis (Table 2) demonstrated that the strengths of the system are connected to its multifunctional character. The provision of ecosystem services is very important; in addition marketing prospects for local dairy products are vast, as they are of very high quality, especially considering the 'story behind' them: intangible cultural heritage (ICH), tacit knowledge, folklore festivals and an alternative way of life in general. The main weakness of Mediterranean agro-pastoralism involves the low productivity of animals, due to the rearing of autochthonous breeds, slow adoption of new technologies, traditional practices and limited access to feedstuff. A particular disadvantage involves the lack of skilled labour; the sector is sometimes unattractive for new generations, who often decide to avoid a profession with uncertain prospects. Immigrant labour has contributed in many cases to revert this depopulation trend, which can be considered as an opportunity rather than a weakness.

In the external environment, numerous opportunities are detected mainly in tourism-related activities. Actually, low-fare tourist packages call for industrial products, which are cheaper but of inferior quality compared with agro-pastoral ones; hence the integration of agro-pastoralism could ensure mutual benefits. Also, the various virtues of agro-pastoral products pertain highly to niche markets of consumers who are particularly aware of food quality and safety. Regarding the key threats, agro-pastoral systems

Table 2. SWOT analysis of Euro Mediterranean agro-pastoralism in rural areas.

Strengths
• High resilience and high farmer commitment
• High quality dairy products often certificated as PDO/IGP (i.e. Halloumi in Cyprus, Graviera in Crete, Fiore Sardo dei Pastori in Sardinia, etc.)
• Provision of ecosystem services from the use of grasslands
• Intangible cultural heritage (festivals, music, tacit knowledge, habits, architecture)
Weaknesses
• Low productivity (rearing of autochthonous breeds, high prices of purchased inputs)
• Slow adoption of technological innovation
• Lack of skilled labour (migrant workers?)
• Poor generational renewal and concentration of production
Opportunities
• Tourism and other related activities
• Changing trends in food consumption and distribution patterns (Mediterranean diets animal welfare, sustainable use of natural resources)
• Pluriactivity (e.g. agro-pastoralism and silvopastoralism combined with olive production and agrotourism)
• Migrant labour force
• Specific capabilities of female family members
• Return of rural youth due to unemployment in other sectors
Threats
• Economic crisis (reduced liquidity and profitability; high production costs)
• Climate change and desertification
• Competition with other activities (intensive systems, alternative energy sources, crop production, tourism, industry, etc.)
• Exodus of rural youth due to unemployment

are exposed to climate change, which directly influences the productivity of flocks and the quality of their products. The primary sector suffers also from increasing competition from numerous activities but also from the effects of the economic crisis, which heavily intensifies existing problems related to efficiency and profitability. The economic crisis, and especially its effects on employment, constitutes an opportunity – as farm employment attracts young people unable to find jobs in other sectors – but also a threat, where farm labour is under rewarded and family members are unable to fill in the gap in generational renewal.

Conclusions

Modern pastoralism in the Mediterranean faces various degrees of unpredictability and risks that relate not only to ecological and climatic factors but also (more and more) to those originating in the political, commercial and administrative spheres. Paradoxically, modern society is increasingly appreciating the products and services of pastoralism (quality proteins, organic production, biodiversity, ecosystem services, landscape and culture, etc.), but flocks and shepherds are decreasing all over the countryside (Nori, 2017). In order to guarantee the sustainability and the development of pastoralism, it is nevertheless necessary to ensure decent living and working conditions for extensive breeders and shepherds (foreign and local) alike, and to provide a perspective of upgrading in social as well as economic terms (Eychenne, 2011).

Drawing on the SWOT analysis, important policy guidelines can be proposed. First and foremost, agro-pastoral farmers should be targeted as a specific group with particular needs, which are often different than the ones of other livestock farmers. Strategic design is essential to integrate agro-pastoral products – and possibly also ecosystem services and ICH – to the tourism industry of coastal areas in order to revert asymmetric development. Policy measures should be especially designed to provide motives and funding to agro-pastoral-related entrepreneurial skills and activities. In particular, opportunities for young people and newcomers could be pursued accordingly to tackle the problems of generational renewal of inland areas. Specific promotion activities should be supported for short supply chains, including brand names, visitable farms, ICT applications, etc. Such improvements could be achieved through the support of collective actions and networks which bring together the different islands' development actors – producers, manufacturers, public services, local authorities, associations, traders, tourism agencies and operators, etc. Last but not least, specific measures should be targeted to the protection of local biodiversity.

The large presence of foreigners in pastoralism is a clear indicator of the importance of the migrant workforce for a sector that is strategic for keeping mountain territories alive and productive, as well as for managing natural resources and protecting the population against natural risks. Immigrants only represent though one of the options to revive the sector and buffer its declining trends. Opportunities to enhance the attractiveness of this sector amongst local youth should also be pursued – as experience of 'neo-ruralism' in certain areas seems to attest. Another strategic asset for this sector is the sophisticated knowledge that is critical to manage such rich but fragile territories in the face of the important socio-political and ecological changes affecting the region; a number of schools exist in France and Spain accordingly, while similar opportunities are discussed as well in Italy.

Sustainable pastoralism will therefore be the result not only of a system of aid and subsidies, but rather it requires the articulation of an enabling political framework, including a review of agriculture, professional and migration policies, together with ad-hoc initiatives and investments, all of which will support efforts aimed at recognizing and appreciating this profession. A more effective policy framework that properly translates EU-CAP policy principles into effective actions in support of the pastoral economy and society could consider, amongst others, regulating pastoral products value chains, efforts aimed at recognising and appreciating this profession, implementation of schemes aimed at attracting rural youth and integrating the migrant workforce through CAP subsidy schemes, land banks and credit facilities.

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Theme 1.
Extensive animal production systems and product

Grassland-based products: quality and authentication

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Abstract

There is increased consumer demand for food products with a perceived positive image. Product quality can be assessed in terms of food safety, nutritional composition, technological and sensory characteristics and ethics. In order to summarise the current knowledge linking quality traits and management practices, a literature review was performed in relation to seven types of management practice: four concerning animal feeding, one concerning breeds and two concerning ethical aspects of the production, and information is presented as seven tables. Literature on use of authentication and traceability for marketed grassland-based products is summarised in a further five tables. Scientific evidence of the differentiation of grassland-based products is currently consolidating, but some practices lead to uncertain results and deserve further research. Authentication is possible but is used to a relatively small extent because of high costs or the lack of analytical techniques suitable for routine use. Marketing appears to be successful without authentication, but this may lead to product counterfeiting. Research and development should be targeted to find viable low-cost solutions to introduce authentication practices to safeguard the added value of grassland-based products and ensure fair remuneration for producers.

Keywords: quality traits, safety, dairy products, meat, management practice

Introduction

In the last decades, consumers have increased their demand for food products with a perceived positive image concerning food safety, nutritional value, healthiness, high sensory quality, production practices and the environment in which the production takes place. Grassland-based animal products, especially if pasture-based and of regional origin, are currently perceived positively by consumers (Bernués *et al.*, 2015). These products, therefore, carry a potential added value, which should be exploited in terms of 'premium products', i.e. products reaching a higher price needed to sustain the cost of the product specifications. This can in turn contribute to sustain the income of farmers and/or counterbalance the higher production costs due to management practices which aim to guarantee the sustainability of the production or to compensate for constraints (e.g. climatic or topographic) in less favoured areas. Farmers can get higher prices for these products, but this need not necessarily result in higher prices for consumers if products are sold via short marketing chains. In order to successfully establish 'premium products' on the market, there are several elements that require attention.

First, a sound product quality must be provided. This can be assessed from different point of view:

1. Hygiene and food safety: absence of chemical product residues, of pathogen germs, of dangerous prions and of all substances that have a negative effect on human health (i.e. *Listeria* in milk and cheese); therefore respecting the precautionary principle.
2. Nutritional composition: content of substances known to be relevant for human health, which is largely (but not exclusively) depending on management and diet of the animals; i.e. protein (biopeptides), fat content and composition (unsaturated fatty acids (FA), omega 3 FA, etc.), soluble carbohydrate, vitamins, minerals, antioxidants (polyphenols, carotenoids, vitamin A and E, etc.).
3. Technological characteristics: these traits relate to the aspect of microbiological and chemical composition, which have direct implication on transformation process of animal products, ripening or on the shelf life of the product (i.e. casein content and composition in milk, *Clostridium* and gram-bacteria in cheese, antioxidants content, fat composition, SEUROP classification of meat carcass).
4. Sensory characteristics (i.e. flavour, odour, taste, texture, juiciness, tenderness, milk or cheese colour, fat colour or proportion in meat), which are a pivotal issue for the gastronomic valorisation of the products.
5. Ethical dimension: by buying the product, the consumers support specific values, including improvement of the agricultural landscape or of the image of farming practices (i.e. by grazing animals), traditional management (maintenance of the cultural landscape), animal welfare and health, grassland biodiversity. Products responding to principles of responsible consumption can be promoted by means of specific labels. These include: (1) 'green': pasture-feeding, animal welfare, environmental sustainability (i.e. short distance travelled from production to consumption, no feedstuffs from faraway countries requiring high non-renewable energy consumption), biodiversity; (2) 'social': fair remuneration of producers, especially from economically disadvantaged areas; (3) 'origin': support to regional economy, geographical and quality specifications including permanent grassland-based feeding.

Besides the knowledge on obtaining quality through suitable management practices, authentication and traceability of the products must be ensured, in order to guarantee the product's quality and image. Authentication and traceability along the market chain may also prevent false advertising resulting in unfair market advantages for producers that do not comply with the quality standards or the production practices.

Product authentication is the process by which a food product is verified as complying with its label description (Dennis, 1998), thus verifying that the product really possesses certain characteristics ensuring its authenticity. However, the analytical methods do not always perfectly discriminate products ('grey zones') according to management practices. Thus, depending on the accuracy of the analytical method used, decisions are required about the compliance with the specifications for the products not perfectly discriminated.

Product traceability is defined as the ability to follow the movement of a food product from of the production site, via processing and distribution to the consumers (WHO/FAO, 2007). This is particularly important if the quality of the product cannot be directly or easily assessed on the product itself. For instance, grassland biodiversity may not be reflected by the nutritional composition or sensory traits. Traceability controls are often performed by external certification bodies (i.e. for some Protected Denomination of Origin (PDO) production chains or for organic products). Both authentication and traceability require affordable monitoring tools in order to be implemented in practice.

The biggest challenge faced by farmers involved in agro-industrial marketing chains is to ensure uniform product quality over time (i.e. producing milk with a standard quality over the whole year using a grassland-based diet). On the other hand, especially in small-scale enterprises, and particularly those marketing their products in short and local chains, the existence of seasonal changes in production quality could be perceived by the consumers as a positive characteristic of uniqueness and authenticity.

Furthermore, for regional products, the total offer of products with added value has to be kept balanced with the market demand for these products at a regional scale.

In the end, consumers' willingness-to-pay a special price for these products is necessary to ensure an adequate remuneration to farmers. For this reason, there is a need for further developing specific markets for these products.

Linking quality traits and management practices

An analysis of the state of the art concerning the assessment of the quality traits and the authentication methods of the products is pivotal to the identification of the constraints preventing a successful establishment of the products on the market. In order to summarise the current knowledge providing a link between quality traits and the management practices, a review of the relevant literature was performed in relation to seven main types of management practices: four concerning animal feeding, one concerning breeds and two concerning ethical aspects of the production (Tables 1 to 7).

Feeding animals with fresh herbage instead of conserved forages and/or concentrates induces a general improvement of nutritional properties of animal products (healthier FA composition, higher antioxidant concentration), a difference in sensory properties (yellower and softer products, with richer sensory profile) and a potential increase in product shelf life (Tables 1 and 2). However, the seasonal availability of fresh herbage implies the use of conserved forages in animal feeding, at least in winter in upland areas or under continental climate, and during summer and autumn under Mediterranean environment. During this period, the use of hay or grass silage instead of maize silage and concentrates in animal diet induces a general increase in nutritional properties of animal products, a differentiation in sensory properties and a potential increase in product shelf life, although this occurs to a lower extent in comparison with feeding fresh herbage (Table 3).

A ban on silages, which occurs in the specifications of several cheeses and of the so-called 'haymilk' (German: 'Heumilch') (European Commission, 2016), has less-definite effects on the quality traits (Table 4), except on the technological aspects (silages, if not correctly conserved or consumed, can induce late swelling of ripened cheese); however, it surely implies significantly higher feeding costs (Borreani *et al.*, 2013).

The occurrence of a relevant amount of species that are rich in plant secondary metabolites (PSM) in the herbage positively affects the nutritional and sensory properties of dairy products, but the effect concerns only a small number of quality traits (Table 5). PSM could have benefits in animal production when they are fed in the correct form and dose (Buccioni *et al.*, 2012; Cabiddu *et al.*, 2013). Tannins have also shown antimethanogenic properties, with different tannin sources showing variable results on palatability with low adverse effects on animal performance and organic matter digestibility (Waghorn, 2008).

The use of local breeds is also a possible farm management strategy to increase the added value of a product (Table 6). The effects of using local breeds on quality traits have been demonstrated in controlled conditions for dairy products (i.e. specific sensory traits of milk/cheese composition), but they are often not clearly perceivable when operating under on-farm conditions. This leads to the choice of rearing local breeds mainly based on a prevalent ethical dimension (biodiversity, landscape, cultural heritage preservation). The same consideration can be done for the low input strategies (low management intensity

Table 1. Effect of a fresh herbage-based diet on different quality traits of dairy products, challenges to the farmers to ensure a constant product quality and methods to assess the quality trait. Superscript numbers refer to literature in Appendix 1.

Management practice	Quality trait	Quality aspects involved	Product the trait is relevant to	Effect on product	Main challenges for the farmers to keep a standard product quality	Available methods of analysis	Costs of analysis methods
Fresh herbage-based diet (grazed, indoor-fed) vs diet based on conserved forage and/or concentrates	Oleic acids, PUFA, CLA, Omega-3 PUFA, odd and BCFA, vaccenic acid	Nutritional/technological	Milk, cheese, butter and all fat-containing dairy products	Increase [1,4, 5, 6, 7, 8, 9, 69, 70, 80, 81, 82]	Need to switch to a diet based on conserved forage or even on concentrates in winter, pasture shortage during summer, changes in the botanical composition (i.e. plant families) of pastures due to site conditions, grazing management, seasonal changes (phenology) during the growing season and fluctuations within and between years depending on weather conditions	GC	Expensive
	Omega-6/omega-3 PUFA ratio	Nutritional		Decrease [1,4, 5, 7, 8, 69, 70, 80, 81, 82]		Prediction model of some FA on milk and cheese by IR	Cheap but reliable only for some FA at the moment
	Oleic/palmitic fatty acid ratio (spreadability index)	Sensory/ technological		Increase [1,10,11, 12, 13, 69, 70, 80, 81, 82]			
	Vitamin A and E	Nutritional/technological		Increase [14, 15, 16, 17, 18, 19, 21] Increase of shelf life		HPLC/UPLC	Expensive
	Carotenoids	Sensory/nutritional/ technological		Increase [14, 20, 15, 17, 18, 21] Increase of shelf life			
	Texture	Sensory	Butter and cheese	Increased softness and spreadability [22, 23, 12, 13]		Sensory panel or dynamometers	Expensive
	Colour	Sensory	Milk, cheese and butter	More yellow [14, 23, 12, 13, 69]		IR Visible reflectance spectrum	Cheap
						Sensory panel	Expensive
	Flavour/Odour/Taste	Sensory	Milk, cheese and butter	More grassy and flowery aroma, more intense notes [14, 23, 13, 69]		Sensory panel	Expensive
	Volatiles compounds	Sensory	Milk and cheese	Increase in odour active compounds [3, 24, 25, 26, 27, 28, 83]		VOCs extraction (steam distillation, SPME, DHSME, Purge and trap, etc.) coupled with GC-MS	Expensive

Table 2. Effect of a fresh herbage-based diet on different quality traits of meat, challenges to the farmers to ensure a constant product quality and methods to assess the quality trait. Superscript numbers refer to literature in Appendix 1.

Management practice	Quality trait	Quality aspects involved	Product the trait is relevant to	Effect on product	Main challenges for the farmers to keep a standard product quality	Available methods of analysis	Costs of analysis methods
Fresh herbage-based diet (grazed, indoor-fed) vs diet based on conserved forage and/or concentrates	PUFA, PUFA/SFA, Omega-3 PUFA, CLA; Omega-6/omega-3 PUFA ratio	Nutritional/sensory/technological	Meat and adipose tissue	Increase [3, 29, 72] Decrease[72]	Need to switch to a diet based on conserved forage or even on concentrates in winter, pasture shortage during summer, changes in the botanical composition (i.e. plant families) of pastures due to site conditions, grazing management, seasonal changes (phenology) during the growing season and fluctuations within and between years depending on weather conditions, parasitism affecting growing rate and therefore age at slaughter, lower growth (and therefore higher age at slaughter) when animals are pasture-fed than when they are indoor-fed	GC	Expensive
						Prediction model only of some FA in the meat by IR [78, 79]	Cheap
	Carotenoids; vitamin E	Sensory/nutritional/technological		Increase [20, 30, 31, 32, 59, 72, 74] increase of shelf life		HPLC/UPLC	Expensive
						Reflectance spectrum of adipose tissue in the area of light absorption by carotenoids	Cheap
	Skatole, indole	Sensory		Increase [20, 34, 35, 36, 33]		VOCs coupled with GC-MS	Expensive
						HPLC	Expensive
	Colour	Sensory		More yellow, lower brightness [33, 72]		Sensory panel	Expensive
	Flavour/odour/taste	Sensory		More animal, more grassy, more intense [20, 35, 36, 72]		Sensory panel	Expensive

Table 3. Effect of the increase of conserved grass proportion, reduction or renunciation to feeding maize silage or concentrate on different quality traits of dairy products, challenges to the farmers to ensure a constant product quality and methods to assess the quality trait. Superscript numbers refer to literature in Appendix 1.

Management practice	Quality trait	Quality aspects involved	Product the trait is relevant to	Effect on product	Main challenges for the farmers to keep a standard product quality	Available methods of analysis	Costs of analysis methods
Increase of conserved grass proportion in the diet, reduction or renunciation to feeding maize silage and concentrate, including winter periods of pasture-based systems	PUFA, CLA, Omega-3 PUFA, odd and BCFA, vaccenic acid	Nutritional/ technological	Milk, cheese, butter and all fat-containing dairy products	Small increase [4, 5, 7, 8, 37, 38, 39]	Changes in the botanical composition (i.e. plant families) of grassland due to site conditions, seasonal changes (phenology) during the growing season and fluctuations within and between years depending on weather conditions	GC	Expensive
	Omega-6 PUFA, Omega-6/ omega-3 PUFA ratio	Nutritional		Decrease [4, 5, 7, 8, 37, 39]			
	C18:1t10/C18:1t11	Nutritional/animal welfare		Decrease [37, 40, 71]		Prediction model of some FA on milk and cheese by IR	Cheap but reliable only for some FA at the moment
	BCFA	Nutritional/animal welfare		Increase [68, 71]			
	Oleic/palmitic fatty acid ratio (spreadability index)	Sensory/technological		Increase [37, 39]			
	Vitamin A and E	Nutritional/technological		Increase [17, 18, 19, 21] increase of shelf life		HPLC/UPLC	Expensive
	Carotenoids	Sensory/nutritional/ technological		Increase [17, 18, 19, 21] increase of shelf life		HPLC/UPLC	Expensive
						Reflectance spectrum of adipose tissue in the area of light absorption by carotenoids	Cheap
	Texture	Sensory	Butter and cheese	Less firm [14, 23]		Sensory panel or dynamometers	Expensive
	Volatiles compounds (including terpenes)	Sensory	Milk and cheese	Higher terpene content [3, 27, 41]		VOCs extraction coupled with GC-MS	Expensive

Table 4. Effect of feeding only hay and of renunciation to the use of grass silage on the quality traits of dairy products, challenges to the farmers to ensure a constant product quality and methods to assess the quality trait. Superscript numbers refer to literature in Appendix 1.

Management practice	Quality trait	Quality aspects involved	Product the trait is relevant to	Effect on product	Main challenges for the farmers to keep a standard product quality	Available methods of analysis	Costs of analysis methods
Feeding only hay, renunciation to the use of silage in systems based on conserved forage or in pasture-based systems during winter period	Oleic acids, PUFA, CLA, Omega-3 PUFA, Odd and Branched chain FA, vaccenic acid	Nutritional/ technological	Milk, cheese, butter and all fat-containing dairy products	Increase [3, 4, 5, 7, 8, 37, 38, 39]	Changes in the botanical composition (i.e. plant families) of grassland due to site conditions, grazing management, seasonal changes (phenology) during the growing season and fluctuations within and between years depending on weather conditions	GC	Expensive
	Omega-6/omega-3 PUFA ratio	Nutritional		Decrease [3, 4, 5, 7, 8, 37, 39]		Prediction model of some FA on milk and cheese by IR [64, 65, 66]	Cheap but reliable only for some FA at the moment
	Oleic/palmitic fatty acid ratio (spreadability index)	Sensory/technological		Increase [37, 39]			
	Vitamin A and E	Nutritional/ technological		Decrease [15, 17, 42, 18, 43, 19, 21] decrease of shelf life		HPLC-UPLC	Expensive
	Carotenoids	Sensory/Nutritional/ technological		Decrease [15, 17, 42, 18, 43, 21] decrease of shelf life			
	Texture	Sensory	Butter and cheese	More elastic [44, 14, 23, 45]		Sensory panel or dynamometers	Expensive
	Colour	Sensory	Milk, cheese and butter	Less yellow [44, 14, 23, 43]		Sensory panel	Expensive
						IR or visible reflectance spectrum	Cheap
	Flavour/Odour/Taste	Sensory	Milk, cheese and butter	Less intense, acid, bitter, pungent, persistent, yogurt, fermented cream [44, 14, 23, 45]		Sensory panel	Expensive
	Volatiles compounds (including terpenes)	Sensory/none	Milk and cheese	Increase [43]		VOCs extraction (steam distillation, SPME, DHSME, Purge & trap, etc.) coupled with GC-MS	Expensive
	Clostridium	Technological	Milk and cheese	Decrease of late swelling of ripened cheese (increase of safety) in comparison to diets including silage not correctly conserved or consumed		PCR and DNA microbial analyses	Expensive (in some cases not available)

Table 5. Effect of the occurrence of species rich in plant secondary metabolites in the forage on different quality traits of dairy products, challenges to the farmers to ensure a constant product quality and methods to assess the quality trait. Superscript numbers refer to literature in Appendix 1.

Management practice	Quality trait	Quality aspects involved	Product the trait is relevant to	Effect on product	Main challenges for the farmers to keep a standard product quality	Available methods of analysis	Costs of analysis methods
Occurrence of a certain amount of plant secondary metabolites (PSM)-rich plant species in the forage	Omega-3 PUFA	Nutritional	Milk and cheese	Increase [7, 8, 46, 84, 85]	Changes in the abundance of PSM-rich plants over time (within and between seasons)	GC	Expensive
						Prediction model of some FA on milk and cheese by IR	Cheap but reliable only for some FA at the moment
	Flavour/Odour/Taste	Sensory	Milk, cheese and butter	More intense and increase in aroma richness [23, 13, 84, 85, 86]		Sensory panel	Expensive
	Volatiles compounds (including terpenes)	Sensory/none	Milk and cheese	Increase [3, 24, 25, 26, 27, 28, 47, 20, 41, 43, 84, 85]		VOCs extraction (steam distillation, SPME, DHSME, Purge and trap, etc.) coupled with GC-MS	Expensive

Table 6. Effect of the use of local breeds on different quality traits of dairy products and meat, challenges to the farmers to ensure a constant product quality and methods to assess the quality trait. Superscript numbers refer to literature in Appendix 1.

Management practice	Quality trait	Quality aspects involved	Product the trait is relevant to	Effect on product	Main challenges for the farmers to keep a standard product quality	Available methods of analysis	Costs of analysis methods
Use of local breeds vs use of cosmopolite breeds	Texture, taste	Sensory	Meat, cheese	Specific sensory attributes of local breeds products [48, 49, 50]	Maintain high performance or balancing production costs with the lower performances of local breeds	Sensory panel	Expensive
	Milk fat content	Technological	Milk, cheese	Decrease [49, 50]		IR	Cheap
	Fatty acid composition	Nutritional/ sensory	Milk and cheese	Specific fatty acids composition of local breeds [19, 21, 51]		GC	Expensive
	Casein content and composition	Nutritional/technological	Milk and cheese	Increased casein content, different casein profile [39]		IR for total caseins	Cheap
						HPLC for casein profile description	Expensive
	Animal biodiversity	Ethical	All	-		-	-

Table 7. Effect of low management intensity of grassland and local origin of products on quality issues of dairy products and meat, challenges to the farmers to ensure a constant product quality and methods to assess the quality trait.

Management practice	Quality trait	Quality aspects involved	Product the trait is relevant to	Effect on product	Main challenges for the farmers to keep a standard product quality	Available methods of analysis	Costs of analysis methods
Low management intensity of grassland, low stocking rate, use of adapted breeds flock type, see also PSM-rich grassland[1]	Biodiversity	Ethical	All	No general effect can be defined	Define minimum management intensity to conciliate production and conservation aims	Presence/absence of bioindicators	Costs depend on the implementation and on logistic aspects, as expert persons and *in situ* assessments are required
Local origin of products	Geographical origin (incl. zero kilometre-origin, mountain origin, *terroir* origin), preservation of local agriculture and thus of the cultural landscape	Ethical	All	No general effect can be defined	-	-	-
Implementation of practices improving animal welfare	Animal welfare	Ethical	All	No general effect can be defined	Define minimum management practices to conciliate production and animal welfare	Presence/absence of indicators of animal welfare	Costs depend on the implementation and on logistic aspects, as expert persons and *in situ* assessments are required
Implementation of grazing[2]	Animal welfare	Ethical	All	See Table 1 and Table 2	See Table 1 and Table 2	-	-

[1] PSM-rich plants may occur in high amount also in intensively managed or sown temporary grasslands.

[2] The achievement of a high standard of animal welfare requires an optimal pasture management and adequate animal nutrition. Animal welfare may be sometimes higher in the consumers' perception than it really is.

of grassland: low stocking rate, low nitrogen input, etc.) on farm management, which positively affect biodiversity and landscape conservation (Table 7). Organising local market chains for animal products reinforces their linkage with the territory of origin (*terroir*), which leads to the acceptance of higher prices by consumers. This again supports the preservation of local agricultural and cultural landscape (including local breeds) under an ethical dimension. A quality trait emerging among the consumers' demands is that of the welfare of animals during their productive life. For dairy products, only a few traits directly measurable in the product are known (i.e. some specific FA ratio or sums can be indicators of metabolic diseases that can be avoided by specific feeding strategies). However, there are several traits related to animal welfare directly measurable on meat (muscle glycogen and free radicals content, meat pH). These parameters may negatively affect meat sensory properties (increase of peroxidase products, decrease in meat and fat colour) and technological characteristics (shorter shelf life).

In general, the effect of feeding-management practices can vary significantly in their extent according to the quality of the available fresh herbage and conserved forages. The measurement of the quality traits is often precise, but implies the use of analytical techniques that are expensive and time consuming and thus rarely applicable in routine use. This problem is also mirrored in the authentication of farming practices. Tables 8 to 12 provide an overview of the recent literature reporting the use of quality traits or of other methods to authenticate milk, cheese or meat according to the management practices. Several authentication methods are available for animal feeding-management practices, based on the analyses of the content of specific components or on the infrared spectra of the animal product. Such techniques show high performance in distinguishing between contrasting diets (i.e. high proportion of fresh herbage vs conserved forages), but can have large overlap when diets are mixed or less contrasted. This means that an effective discrimination often requires coupling several methods of analysis and/or the analysis of several tissue (for meat) consequently increasing authentication costs (Prache *et al.*, 2003, 2009). Recently, some cheap and rapid methods, based on infrared spectroscopy on animal tissue or dairy products are being developed. The results are promising, but further research is required to highlight their effectiveness and limits.

Use of authentication and traceability for marketed grassland-based products

In order to assess the use of authentication and traceability in marketing strategies of grass-based products in practice, at the end of 2014 a survey was conducted on 15 marketing enterprises from six countries. The enterprises were as follows: Arktisk Lammekjøtt (NO), PDO cheeses of Massif Central (F), AOC Prés Salés du Mont Saint Michel (F), Burgundy pasture beef (USA), Omega Beef Direct (UK), Pastureland (USA), ARGE Heumilch (A), Biobeef (I), Kovieh (I), Gourmet pasture beef (USA), QRCheese (I), Laugenrind (I), Green Grass Dairy (I), Weidemelk (NL), Slowfood (I). Further details on the collected data are given in Peratoner *et al.* (2015). Information was obtained by interviewing informed persons directly involved in the enterprises or acting as scientific consultants. For three enterprises, however, no answer could be obtained and the data rely on information available on the Internet only. The set of enterprises was relatively well balanced concerning the product type (eight enterprises marketing meat products, six marketing dairy products, one encompassing a broad range of food products). Nutritional (health) and ethical issues (maintenance of cultural landscape, animal welfare, historical heritage, local origin of the product) were equally used as part of the marketing strategies (36%), while sensory aspects were used slightly less frequently (29%). Eight enterprises made use of more than one issue for their own marketing strategy. It is remarkable, however, that less than half of the enterprises (42%), for which direct information through interviews was obtained, had a system for monitoring the product quality. Quality monitoring was ensured in three out of four cases by sensory panel tests. Much more frequent was the use of a traceability system, with only two enterprises being devoid of it. This suggests that tracing back to the origin of the product was more relevant in the marketing than the quality issues. Indeed, traceability is a requirement in the case of certified production systems and a relevant issue for plausibility checks within

Table 8. Methods to authenticate milk and dairy products obtained with a fresh herbage-based diet and constraints to their implementation in routine authentication. Superscript numbers refer to literature in Appendix 1.

Management practice	Quality trait	Product the trait is relevant to	Authentication methods	Methods of analysis	Costs of authentication	Tested in controlled conditions or validated on farm	Limits of the methods
Fresh herbage-based diet (grazed, indoor-fed) vs diet based on conserved forage and concentrates	Fatty acid composition	Milk, cheese, butter and all fat-containing dairy products	Discrimination model using milk fatty acid composition [15, 53, 52, 10] Prediction model of proportions of various feeds based on fatty acid profile [54, 2]	GC	Expensive	On farm	Mixed diets (presence of low proportion of fresh herbage and high proportion of others feedstuffs); precision of the models
	Vitamin A and E	Milk	Discrimination model using vitamins content [15, 52]	HPLC/UPLC	Expensive	On farm (small area and numbers)	Vitamin supplements in the diet
	Carotenoids	Milk	Discrimination model using carotenoids content [15, 52]				Mixed diets. Light exposure (photosensitive pigments)
	Phenolic compounds	Milk	Discrimination model using phenolic compound content [55]				Mixed diets
	Colour	Milk, cheese, butter	Discrimination model based using sensory attributes [52, 12, 13]	Sensory panel	Expensive	On farm (small area and numbers)	Mixed diets
			Index based using reflectance spectrum in the area of light absorption by carotenoids [15, 52, 17, 31, 32]	Visible or IR spectrum	Cheap		Mixed diets
	Flavour/Odour/Taste/ Texture	Milk, cheese and butter	Discrimination model based on sensory attributes [23, 13]	Sensory Panel	Expensive	On farm (small area and numbers)	Mixed diets; results depends on cheese type + ripening time
	Stable isotopes (δ^nC, δ^nN, δ^nS, δ^nO)	Milk, cheese, butter, meat	Discrimination model based on stable isotopes ratios [52, 56]	MS coupled to a C, N, S, O analyser	Expensive	On farm (small area and numbers)	Mixed diets
	Volatiles compounds (including terpenes)	Milk and cheese	Discrimination model based on VOC composition [28, 52, 33]	VOCs coupled with GC-MS	Expensive	Controlled conditions; terpenes on farm	Mixed diets, low analytical repeatability
	Infrared Spectroscopy	Milk and cheese	Discrimination models based on IR spectra [58, 61, 9, 59]	IR	Cheap	On farm	Mixed diets

Table 9. Methods to authenticate meat obtained with a fresh herbage-based diet and constraints to their implementation in routine authentication. Superscript numbers refer to literature in Appendix 1.

Management practice	Quality trait	Product the trait is relevant to	Available authentication methods	Available methods of analysis	Costs of authentication methods	Tested in controlled conditions or validated on farm	Limits of the methods
Fresh herbage-based diet (grazed, indoor-fed) vs diet based on conserved forage and concentrates	Omega-3 PUFA	Meat	Discrimination model of muscle using fatty acid composition [3, 29], Functional genomics? [3]	GC	Expensive	Controlled conditions	Mixed diets, changes in feeding regimes, differences between individuals
	CLA		Discrimination model of muscle using fatty acid composition [3, 29], Functional genomics? [3]	GC	Expensive	Controlled conditions	Mixed diets, changes in feeding regimes, differences between individuals
	Carotenoids		Index based on reflectance spectrum of adipose tissue in the area of light absorption by carotenoids [20, 59, 60, 31, 32, 74, 75]	HPLC/UPLC	Expensive	Controlled conditions	Mixed diets (presence of low proportion of fresh herbage and high proportion of others feedstuffs)
				Spectrocolorimetry	Cheap	On farm + on-line in the abattoir	Change in feeding regimes; differences between breeds and individuals
	Colour		Index based on reflectance spectrum of milk, cheese in the area of light absorption by carotenoids [15, 52, 17, 31, 32]	Visible or IR spectrum	Cheap	On farm (small area and numbers)	Mixed diets, changes in feeding regimes, differences between individuals
	Stable isotopes ($\delta^{n}C$, $\delta^{n}N$)		Discrimination model using stable isotopes ratios [57]	MS coupled to a C, N, S, O analyser	Expensive	Controlled conditions + on farm	Mixed diets, differences between mixed diets, changes in feeding regimes, differences between individuals
	Volatiles compounds (including terpenes)		Discrimination model using VOC composition[33]	VOCs coupled with GC-MS	Expensive	Controlled conditions	Mixed diets, changes in feeding regimes, differences between individuals
	Infrared Spectroscopy		Discrimination models using visible and infrared spectroscopy[30, 60, 73]	IR	Cheap	On farm	Mixed diets, changes in feeding regimes

Table 10. Methods to authenticate milk and dairy products obtained with a diet with an increase of conserved grass proportion and a reduction or renunciation to feeding maize silage and concentrate, and constraints to their implementation in routine authentication. Superscript numbers refer to literature in Appendix 1.

Management practice	Quality trait	Product the trait is relevant to	Available authentication methods	Available methods of analysis	Costs of authentication methods	Tested in controlled conditions or validated on farm	Limits of the methods
Increase of conserved grass proportion in the diet, reduction or renunciation to feeding maize silage and concentrate, including winter periods of pasture-based systems	Fatty acid composition	Milk, cheese, butter and all fat-containing dairy products	Discrimination model using milk fatty acid compositions [15, 53, 52, 10]; Prediction model of proportions of various feeds based on fatty acid composition [54]	GC	Expensive	On farm	Mixed diets; precision of the models
	Vitamin A and E	Milk	Discrimination model using vitamins content [52]	HPLC/UPLC	Expensive	On farm (small area and numbers)	Vitamin supplements in the diet
	Carotenoids	Milk	Discrimination model using carotenoids contents [52]	HPLC/UPLC	Cheap	On farm (small area and numbers)	Mixed diets. Light exposure
	Colour	Milk, cheese and butter	Index based on reflectance spectrum of milk or cheese in the area of light absorption by carotenoids [52]	Visible or IR spectrum	Cheap	On farm (small area and numbers)	Mixed diets
	Volatiles compounds (including terpenes)	Milk and cheese	Discrimination model based on terpene composition [52]	VOCs coupled with GC-MS	Expensive	On farm (small area and numbers)	Mixed diets, low analytical repeatability
	Stable isotopes ($\delta^{n}C$, $\delta^{n}N$, $\delta^{n}S$, $\delta^{n}O$)	Milk, cheese and butter	Discrimination model based on stable isotopes ratios [52, 56]	MS coupled to a C, N, S, O analyser	Expensive	Controlled conditions	Mixed diets
	Infrared Spectroscopy	Milk and cheese	Discrimination models based on IR spectra [58, 61, 9]	IR	Cheap	On farm	Mixed diets

Table 11. Methods to authenticate milk and dairy products obtained with a diet with an increase of conserved grass proportion and a reduction or renunciation to feeding maize silage and concentrate, and constraints to their implementation in routine authentication. Superscript numbers refer to literature in Appendix 1.

Management practice	Quality trait	Product the trait is relevant to	Available authentication methods	Available methods of analysis	Costs of authentication methods	Tested in controlled conditions or validated on farm	Limits of the methods
Feeding only hay, renunciation to the use of silage in systems based on conserved forage or in pasture-based systems during winter period	Fatty acid composition	Milk, cheese, butter and all fat-containing dairy products	Discrimination model using milk fatty acid composition [15, 53, 52, 10]; Prediction model of proportions of various feeds based on fatty acid composition [54]	GC	Expensive	On farm	Mixed diets; precision of the models
	Vitamin A and E	Milk	Discrimination model using carotenoids vitamins content [15]	HPLC/UPLC	Expensive	On farm (small area and numbers)	Vitamin supplements in the diet
	Carotenoids	Milk	Discrimination model on carotenoids contents [15]	HPLC/UPLC	Expensive	On farm (small area and numbers)	Mixed diets. Light exposure
	Colour	Milk, cheese and butter	Index based on reflectance spectrum of milk or cheese in the area of light absorption by carotenoids [15]	Visible or IR spectrum	Cheap	On farm (small area and numbers)	Mixed diets
	Stable isotopes ($\delta^{n}C$, $\delta^{n}N$, $\delta^{n}S$, $\delta^{n}O$)	Milk, cheese and butter	Discrimination model based on stable isotopes ratios [56]	MS coupled to a C, N, S, O analyser	Expensive	Controlled conditions	Mixed diets
	Infrared Spectroscopy	Milk and cheese	Discrimination models based on IR spectra [58, 61, 9]	IR	Cheap	On farm	Mixed diets

Table 12. Methods to authenticate milk and dairy products obtained with a diet containing a certain amount of plants rich in secondary metabolites or of local origin, and constraints to their implementation in routine authentication. Superscript numbers refer to literature in Appendix 1.

Management practice	Quality trait	Product the trait is relevant to	Available authentication methods	Available methods of analysis	Costs of authentication methods	Tested in controlled conditions or validated on farm	Limits of the methods
Occurrence of Plant Secondary Metabolites (PSM)-rich plant species in the forage	Volatiles compounds (including terpenes)	Milk	Discrimination model based on VOCS composition [28]	VOCs coupled with GC-MS	Expensive	Controlled conditions	Mixed diets, Low analytical repeatability
Local origin of the product	Stable isotopes ($\delta^{n}C$, $\delta^{n}N$, $\delta^{n}S$, $\delta^{n}O$)	Milk	Discrimination model based on stable isotopes ratios [62, 56, 67, 63]	MS coupled to a C, N, S, O analyser	Expensive	On farm (small area and numbers)	Product originating from regions close one to each other, interaction with diet effects

the production and distribution chain, as well as for hygiene and food safety controls. This trend can easily lead to food counterfeiting. This is a risk, especially after a product has achieved commercial success.

Prospects

Some open research questions emerged from the literature review. Some of the main challenges concerning the standardisation or interpretation of the results of quality traits in relation to forage quality or characteristics have not yet been fully tackled and accomplished. Furthermore, strategies and management practices to ensure a standard product quality over time have to be further developed. The main issue of future research would be to develop affordable and rapid analytical methods for routine authentication, including a large-scale, on farm validation in order to take into account the between-animal variability (genetic component), the variability of grassland composition, herbage quality, grazing and animal management and their interaction with diet composition. According to this development, a set of markers able to ensure a good discrimination of the products based on management practices and/or origin (multiple markers, also in more than in one tissue) should be identified and validated on a large scale (on farm). This process would lead to the optimisation of authentication and traceability protocols minimising the bureaucratic effort of farmers and is a determinant for the identification of label strategies to clearly and synthetically illustrate the quality traits which determine the added value of a 'premium product'.

The increase in production costs (or reduced production) should be quantified to identify the economic threshold under which the specifications make the production no longer economically sustainable at the achievable market price or to identify the price needed to cover the increased production costs. Finally, all knowledge with respect to grassland-based products should be shared in participatory approaches involving all the stakeholders playing a role in the production and marketing chain (from farmers through to consumers) in order to ensure successful implementation of these products in practice.

Conclusions

Scientific evidence of the differentiation of grassland-based products is currently consolidating. Different qualitative issues can be used successfully for marketing and some of these are already used in practice. The authentication is possible in several cases, but it is used for commercial enterprises only to a relatively small extent because of its high costs or the lack of analytical techniques suitable for routine use. Marketing appears to be successful also without authentication, but this may easily lead to product counterfeiting. Research and development should thus be targeted to find viable low-cost solutions to introduce authentication practices behind the marketing strategies, to safeguard the added value of grassland-based products and ensure a fair remuneration for the producers.

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Adding value to sheep production in the marginal areas of Northern Europe

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Abstract

Sheep production is falling in Northern Europe due to poor financial viability. Adding value to multifunctional grassland services from sheep farms offers a way to address this issue which if unresolved has significant social, environmental and food security downsides. Examples of adding value are described. Change is thwarted by traditional practices and market access. With attention to biosecurity issues smaller hill breeds could address problems with the current stratified breed structure in the UK sheep industry. This system adds value to output from marginal farms but may be a victim of its own success. On farms where grass is conserved, added value from improved quality silage offers feed and transport cost savings and greater carbon efficiency. Examples of small-scale niche marketing of products shows how individuals can add value but is not helped by regulatory issues and scaling problems. The best option for adding value is to concentrate on the least marginal farms using modern rotational grazing systems to enhance grassland utilisation.

Keywords: less favoured areas, adding value, sheep production

Introduction

For the purposes of this paper, marginal areas have been defined as less favoured areas (LFAs) which cover 25% of Europe. In the European Union, LFA is a term used to describe an area with natural handicaps (lack of water, climate, short crop season and tendencies of depopulation), or that is mountainous or hilly, as defined by its altitude and slope. These areas benefit from specific area and headage payments, and additional interest rate subsidies to support investment. Based on the severity of environmental constraint (designated as A, B or C in descending order of severity) technical and financial hotspots are described and case studies where innovation has shown potential for adding value to products are demonstrated. The potential for these strategies to be implemented on a wider scale, the constraints to this and the pros and cons at a social and environmental level are discussed and conclusions drawn.

Sheep systems and their hotspots

Sheep meat production on the most marginal areas severely constrained by environmental factors

The most marginal areas include extensive grassland and heathland regions of high altitude and northern latitudes – in these areas, unsuited to direct human-edible food production, grazing sheep convert grass and herbs into meat and wool. These areas possess high natural beauty and wildlife diversity. They provide high recreational value, water provisions and other public goods. Therefore agricultural production, which maintains the landscape, ecology and rural populations, has historically received significant financial support keeping farms viable, some of which produce relatively little food. The challenge in these marginal areas is to add value to sheep production whilst maintaining or enhancing all of the services grasslands provide.

The necessary dependence on financial support can be understood in the context of the biology behind production of lamb from LFAs. Grazing animals need to harvest sufficient food to reproduce. Surpluses to flock requirements for replacements are sold for meat along with wool. Ewes producing a single lamb every year are only marginally financially viable. In temperate marginal areas, sheep have to cope with increasingly wet winter weather due to climate change, which increases their maintenance costs and, as natural herbage is spatially variable and scarce, their grazing area requirement ranges from 0.4 to 4 ha ewe^{-1}. In order to harvest this large environmentally challenging area, ewes adapt by being genetically small. They are termed hill ewes; examples include Shetland, Welsh Mountain or Herdwick at circa 35 kg liveweight and Scottish Blackface or Hill Cheviot at circa 55 kg liveweight. They produce lower value lambs owing to low carcass yield and poor conformation. Wool yield at <2 kg ewe^{-1} year^{-1} is generally of carpet-making quality and costs more to produce and remove than it receives from sale.

Without supplementary forage in winter, ewes kept in the most marginal areas of North West Scotland produce around 0.75 lambs ewe^{-1} year^{-1} (Vipond and Gunn, 1985). This reduces the scope to cull poorer animals, therefore genetic progress is, at best, slow and can be negative. With low lamb prices, poor ewe performance and increasingly wetter winters, coupled with delinkage of the Single Farm Payment (SFP) to the number of animals kept, ewe numbers in the UK have gradually fallen by 35-60% since the late 1990s (SAC, 2008). In the absence of viable farmed livestock alternatives, such areas are now grazed by wild deer. Many hill and mountain areas in Europe have similar issues.

Matching feed supply with demand is a difficult challenge for sheep farmers in marginal areas. Winter feed supply sets the maximum carrying capacity of the farm; when overstocked, ewes lose body condition affecting lamb survival. In contrast, summer herbage production exceeds requirement, it is underutilised, leading to low feed quality and the need for periodic controlled burning of excess vegetation. This is disruptive to wildlife but prevents serious wildfires, which increase in occurrence and intensity with the loss of grazing sheep.

Meat production systems on moderately constrained LFAs

On less marginal farms there is the opportunity to improve 5-10% of the pasture with re-seeding and fertiliser. In this way the nutritional needs at critical times of mating and lactation can be met, ewe fecundity and lamb survival increases and financial viability improves. Pure bred ewes are brought down from the native pastures (unimproved land) after three to four lambing seasons for a further one to two seasons on the improved pastures. On the improved pasture, the ewes are crossed to Longwool sires. The Mule ewe lamb progeny are sold at a premium to lowland farmers. Alternatively, farmers lacking less marginal land for pasture improvement, may sell the older ewes off the native pasture to less marginal farms for crossing – thus the marginal and the less marginal areas are interconnected.

Meat production systems in least constrained areas of LFAs

On the least marginal farms, a proportion of the available land is likely to be arable, grass is conserved for winter feed and much of the grassland is sown pasture. Farmers may opt to purchase crossbred Mule ewes sourced from B areas to mate to terminal sires for meat production. In addition, unfinished (store) lambs are bought from A and B areas in the autumn and finished over winter. This stratified system of breeding sheep (Figure 1) integrates marginal land, characterised by a short growing season, and lowland areas, with a long growing season, to effectively extend the seasonal supply of lamb and provide healthy, hardy replacements for lowland farmers.

Continental European sheep meat production

The further north one goes in Northern Europe from the UK, e.g. Denmark, Norway, Sweden, Iceland and inland from mid-Jutland, the greater the snow cover in winter and need for housing of ewes, some being housed for up to seven months. In areas with good quality summer grazing and conserved forages for winter, nutritional constraints are minimised. Ewes are heavier than average, at circa 80 kg. This is due to better nutrition and genetic selection where environmental constraints are low. For the same reasons, fecundity rate can be high (e.g. >200% is typical of the Finnish Landrace breed). Constraints to stocking

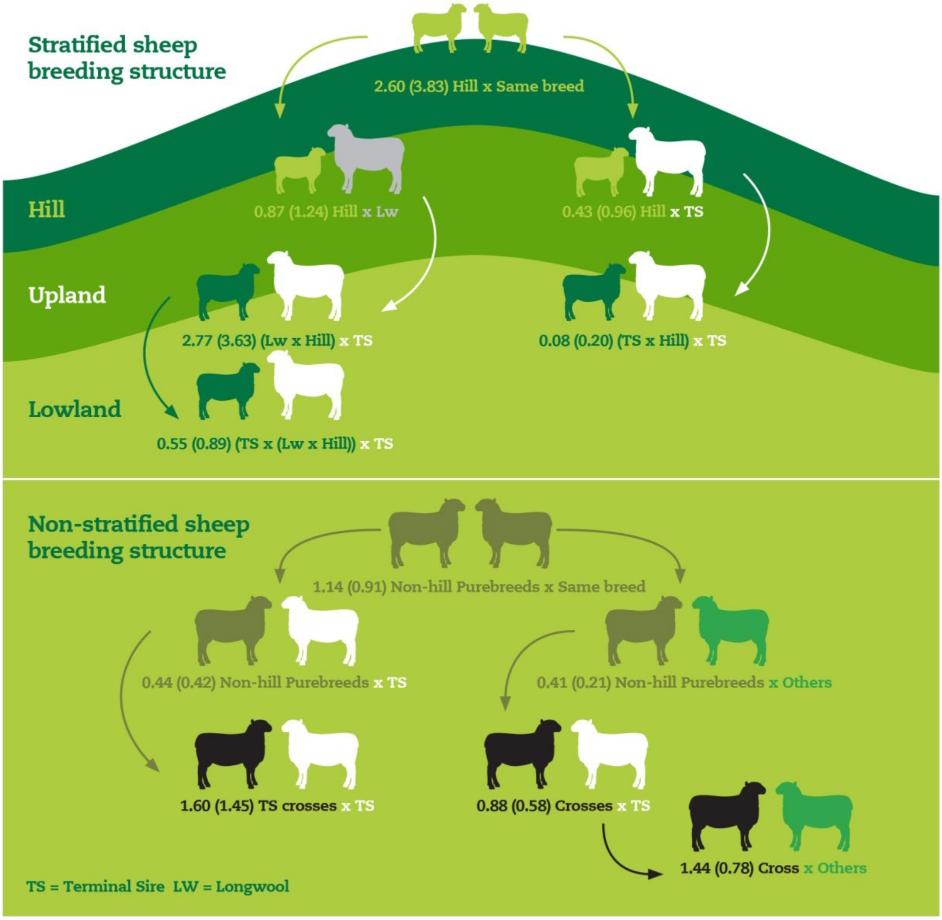

Figure 1. Depiction of the stratified and non-stratified breeding structure of the UK. Numbers (millions) of mating type frequency as estimated in the 2012 Sheep Breed Survey (2003 results in brackets) (AHDB Beef and Lamb, 2014)

rate of housed ewes are: the area of land available for conservation of forages, housing capacity and, in extensive areas, the labour resources to gather ewes off the hill at the end of summer.

Taking production in Denmark as an example of Continental European systems it is worthwhile highlighting some of the differences a continental environment brings. Land competition from intensive arable, dairy and pig production has resulted in a relatively small national flock in Denmark, of around 70,000 ewes. These utilise spare grass associated with grass seed production, dairy pastures in winter and areas of special conservation value in summer. Average flock size is 14 ewes – most enterprises are hobby farms. Flocks exceeding 500 ewes are often grazed off farm during the autumn/early winter providing services such as preventing build-up of grass fungal diseases.

The return of nutrients and organic matter from the dung of grazing animals on arable farms benefits the soil. Specific constraints are short term access to land, variable grass availability between years and high transport costs associated with high animal welfare systems. Breeds used include the Dute, which are a native Swedish breed adapted to low input systems, but increasingly ewes are Texel crosses out of Gotland ewes.

Farmers are not allowed to castrate lambs, only vets can do this operation. Therefore fewer are castrated and when put onto late autumn grass sexual activity results in smaller lambs being bullied, with growth and conformation being reduced. This restriction constrains the range of genetics that could be used to improve the industry – farmers are interested in crossbreeding but use of UK Longwool breeds would create problems of finishing by-product ram lambs due to poor conformation.

Extensive low input systems are threatened by the reappearance of wolves since the fall of the Berlin Wall, which acted as a barrier to wildlife migration from Eastern Europe. Some farmers with access to the specialist restaurant trade have carved out niche markets for meat and dairy sheep with cheese production associated with tourism, and this can pay well, but with a small home market for meat, targeting the Muslim population, margins are lower than in similar areas of low constraint in the UK.

The financial output of UK sheep production systems categorised by levels of environmental constraint are shown in Table 1.

From the data in Table 1, it is clear that the financial viability of sheep businesses is a problem. Throughout the remainder of this paper, examples are provided of how individuals and communities have overcome these financial challenges by adding value or cutting production costs. The potential for wider uptake of these innovative strategies along with potential environmental and social consequences are discussed.

Case studies: strategies to add value on most severely constrained hill farms

Farms in the most marginal areas are not profitable, even after subsidy, and many are maintained from non-farm sources. The problem is complex. Output is very low and of low value; small carcasses may be below supermarket specification (16-21 kg) and the store lamb market is sensitive to business confidence and feed supply. Market failure can occur in some years.

Variable costs are high relative to output. Transport costs, especially in island communities, are substantial. Most UK hill farmers target a 16 kg carcass weight to meet UK supermarket pack sizes and use medium size 55-60 kg liveweight breed (e.g. Swaledale, Blackface or Cheviot). These are High Nature Value (HNV) farming systems with low intensity, low input systems that utilise semi-natural vegetation with grazing livestock.

Exploiting niche market opportunities

The light-lamb market is a niche alternative to the standard Northern European 16-21 kg carcase weight product. Southern European consumers prefer light carcasses produced as a by-product of dairy sheep.

Table 1. Physical data and profitability of UK farming systems typically associated with degree of environmental constraint[1] (SRUC, 2016).

	A. Severely constrained	B. Moderate constraint	C. Low constraint
Fecundity rate (% lambs born per ewe mated)	80	150	170
Lamb output (€)	2,327	10,315	11,481
Output per ewe less replacement cost (€ ewe^{-1})	26.38	80.53	114.81
Variable costs (€ ewe^{-1})	22.70	36.94	53.78
Gross margin (€ ewe^{-1})	4.11	43.60	61.03
Typical Farm business income, including subsidies (€ ewe^{-1})	-14.10	29.94	37.87

[1] Sheep production systems associated with environmental constraints: (A) hill breeding ewes bred pure producing unfinished lambs from the most marginal and severely constrained areas for further finishing; (B) hill breeding ewes producing crossbred Mule ewe lambs for lowland breeding; (C) Mule or purebred lowland types on upland farms, mainly unsuited to cropping and producing finished lambs.

Typically these are used for feast activity and weigh 8-12 kg. The UK hill lamb product when weaned early from small ewes is the right specification by weight, but has a more intense flavour.

Starting from a flock of 350 ewes and 100 replacements of small (35 kg liveweight), hardy Herdwick and Welsh Mountain ewes, a young farmer built up a flock of 3300 ewes and replacements in South West Scotland (335-460 m a.s.l. and at 55.5° N) over eight years. Missing the Single Farm Payment entry date, everything had to be profitable without support, so variable costs were cut to essential vaccines, two lamb treatments for worms and trace element boluses. Reasonable output (ultrasound pregnancy scanning 115% lambs per ewe and rearing 90% lambs per ewe) was achieved by selling entire lambs at carcass weights of 8-12 kg, € 53, to the light-lamb market in Southern Europe.

Welsh Mountain twin lambs on the improved land were weaned in late July, followed by the Herdwick ewes on the hill in August after 12 weeks lactation. This early weaning strategy allowed ewes to regain body condition before hill pasture quality deteriorated leading to a high lambing percentage and increased ewe body fat reserves for winter. This, combined with a lower bodyweight and using sheep bred in the heaviest rainfall areas of the UK, reduced the need for winter feed supplementation, restoring profitability.

In this low labour input system mature ewes with singles spend all their time on the hill. The flock is gathered up to six times a year for essential tasks and 800 replacements managed on improved areas. Time freed up was used to develop a fencing contractor business.

The farming system proved profitable and, from an initial farm of 190 ha, a further 1,200 ha were purchased over a series of years. With fencing (28.5 km, grant-aided), the grazing pressure could be controlled and productivity of pasture improved. With strong emphasis on biosecurity, disease problems have been limited and ewe losses average 3.5% year^{-1}. With minimal supervision, the system suits the UK as no wild predators are capable of killing ewes. An estimated 10,000 ewes would make this system a full time occupation for one highly skilled farmer. The system shows highly innovative thinking outside traditional paths delivering profitability (net margin in good marketing years could be up to € 35 ewe^{-1}). The business is now developing new markets for breeding ewes suitable for further crossing after removal from the hill environment.

Reviewing feed inputs to reduce transport costs

In Shetland, at 60 °N, winters are long and weather is extreme so many ewes are housed in winter on slats and fed grass silage plus concentrates. A recent revision of the AFRC (1993) protein requirements of sheep (Robinson, 2008), shows that protein recommendations are 25% below the needs of modern genotypes and digestible undegradable protein (DUP) is needed to top up rumen microbial supply in late pregnancy. Targeted DUP feeding can replace high quantities of concentrate feed; 25-30 kg of concentrate feed/ewe has been replaced with 6 kg of soybean meal/ewe during pregnancy on many sheep farms with no detrimental effects on lambing performance. This is particularly relevant to marginal farming areas such as the Shetland Isles, where transport costs for feed add € 37 tonne^{-1}.

To reduce variable costs in 2016, a Shetland farmer produced high quality grass silage, with over 11 megajoules (MJ) metabolisable energy (ME)/kg dry matter (DM) and fed this as a sole feed (with added minerals and vitamins) to meet energy needs of multiple bearing ewes in the last month of pregnancy. In addition 100 g of soybean meal was fed per day for each lamb scanned to provide DUP. Through this dietary change only 20% of the normal amount of compound was needed. This resulted in a € 14 ewe^{-1} saving in winter feed and associated transport costs.

Marketing innovation for diminutive Native Shetland Lambs

The native Shetland lamb carcass, at 8-12 kg, leaves insufficient margin for the abattoir, processor and retailer to achieve sustainable margins. Shetland farms are a long way from urban centres where affluent consumers and restaurateurs are looking for something special and can afford to pay a premium. One Shetland farmer sells direct to UK consumers. He uses a local meat processing cooperative and, with innovative packaging, sends product by the Royal Mail's next day special delivery service. Boxes of meat are also delivered from September to December around the Aberdeen to Edinburgh road network using refrigerated transport. Lamb sold is of the EU recognised Protected Designation of Origin (PDO) and qualifies only when born, raised and slaughtered in Shetland.

Consumers and restaurants pay a considerable price premium, cuts are differentiated by being smaller and celebrity chefs and food writers have described Shetland lamb as very tender and sweet. Speciality 'seaweed lamb' is also sold from lambs that have evolved to harvest seaweed as part of their diet and have an enhanced flavour from this. No concentrates are fed to maximise the image of naturally fed lamb from herb rich native pastures. The farmer uses modern media to promote a unique product and overcome local marketing difficulties for over 100 carcasses year^{-1}.

Case studies: strategies to add value in moderately constrained in upland farms

The sale of quality breeding stock to other sheep farmers is a great opportunity for moderately constrained upland farms to add value to their outputs. The sheep on these farms are naturally hardy and adapted to cope with low input systems with fewer disease issues due to extensive stocking.

Group marketing activity to add value to breeding stock

Crossbred lambs out of hardy hill ewes achieve ewe lamb premiums where size, appearance and sound constitution criteria are met for live auction. Typical of this market is the North of England Mule bred mainly out of Swaledale ewes using a Bluefaced Leicester crossing ram. Well-fed ewe lambs that reach around 55% of mature body size at sale (five to six months old) make a premium of 30% above finished lamb price. This premium incurs costs for face dressing and fleece preparation for sale. Concentrate feeding at circa. 5 kg lamb^{-1} (fed over three to four weeks) is typical to improve appearance. Rams used as crossing sires are more expensive by € 250-350 ram^{-1} and have shorter working lifespans than terminal breeds. Producers support a marketing organisation called the North of England Mule Sheep Association (NEMSA) to promote sales at shows, organise auctions and advertise stock – facilitating the sales of approximately a quarter of a million Mule replacements.

Lambs and two tooths (one- to two-year-old sheep) are sold on the reputation of the farmer for consistency; repeat purchasers are valued by both seller and buyer, the latter hoping to avoid buying in sheep diseases from unknown sources.

Live auctions keep farmers motivated in production and have great social benefits. However, the emphasis on appearance at livestock shows and sales adds unnecessarily to production costs without genetic improvement. Top prices based on size encourage breeding from hill type ewes kept on lowland farms at the sacrifice of hardiness and biosecurity and excessive concentrate feeding.

Adding value by health declarations for breeding stock

In the Highlands of Scotland where sheep are run extensively and few farms buy in female stock (a source of disease), Enzootic Abortion of Ewes (EAE) is less common than the rest of the UK. Monitored flocks in the SRUC Premium Health Scheme sell lambs as 'Highland Mules' with a premium of up to € 12 head^{-1}. Purchasers on bio-secure farms can save on vaccination costs.

Historically this has been a good model for disease control; however, sustainability is challenged by the emergence of many so called iceberg diseases – diseases of which there are few diagnosed cases, higher prevalence is suspected, yet unknown (Table 2; Lovatt, 2016). Currently there are no health declarations for these diseases when selling UK replacement ewes or rams.

A study to understand the gap in knowledge of the prevalence of disease was conducted using the Fallen Stock centres in the UK (Lovatt and Strugnell, 2013). By law, all fallen stock (animals which die on-farm) from UK farms must be collected and disposed of by a licenced collector. The authors performed post-mortems on this material and recorded cause of death. Amongst the better known health issues on farms, such as fascioliasis, pasteurella and pneumonia, two of the iceberg diseases – Johne's and Jaagsieke were surprisingly high in the rankings.

Breeders may have concealed the extent of the issue; acknowledging a problem impacts breeding sales. Hence prevalence data is sparse and thus research and eradication efforts have been limited.

Case studies: strategies to add value in least constrained marginal areas

Farm size is often a major constraint to profitability in LFA areas of least environmental constraint. Many farms are family-owned or tenanted and there is an increasing trend to negate the need for permanent staff by using contractors. Business development must come from the same labour and land resource. The key aspiration: sell more kilos of lambs per hectare without incurring extra costs and get a premium.

Organic production can add to lamb value (premiums can be 10%) and reduces many variable costs, e.g. fertiliser and sprays. However, organic feed is expensive (often around 30% higher price than conventional feed). Organic farms that rely more on grass and clover swards are well placed to add value.

Sheep farmers are generally unaware of how much grass they grow in tonnes of dry matter (DM)/ha, or how much is utilised by livestock (average 50%, potential 80%). A 30% increase in grass utilisation is effectively equivalent to a 30% increase in farm size. This order of improvement in utilisation can be achieved by rotational grazing.

Rotational grazing – an opportunity to reduce cost and increase production

A 250 ha organic family farm in the Scottish borders (altitude 150-275 m a.s.l., lat 55.8 °N) utilised rotational grazing to reduce costs and add value. In 2004 the farm was set stocked and ran 800 ewes and replacements and 60 beef cows plus progeny to 18 months. Two hundred hectares were improved land and permanent pasture, the remainder seasonally available under a rural stewardship agreement. The

Table 2. The Five Iceberg diseases and prevalence investigation studies, adapted from Lovatt (2016).[1]

Iceberg Disease	Estimates of prevalence of Iceberg diseases		Reference
	Percentage of fallen stock diagnosed	Percentage of farms testing positive using blood sampling	
Maedi Visna (MV)	n.d.	2.8%	Ritchie (2012)
Ovine Johne's Disease (OJD)	6%	n.d.	Lovatt and Strugnell (2013)
Jaagsiekte (OPA)	6%	n.d.	Lovatt and Strugnell (2013)
Caseous lymphadenitis (CLA)	n.d.	18% of terminal flocks	Baird *et al.* (2004)
Border disease (BD)	n.d.	n.d.	No published data. Estimated to be rare but on the increase

[1] n.d. = not determined.

farmer's endeavour was to integrate livestock management and rural stewardship for the benefit of both farmed and wild species.

With outdoor lambing in late April /May, the farmer's initial target was to better match feed supply with demand while reducing labour cost. Ewes were bred and culled to avoid the need for labour interventions at lambing and other times and the flock was closed. Initially ewes were the NZ Romney breed and more recently the UK Easycare, a white-faced, hornless wool shedding composite of medium live weight (70 kg) derived from the wool shedding Wiltshire Horn and hardy white faced hill breeds. Wool shedding breeds incur less labour for shearing, crutching and tail docking, require no chemicals to prevent blowfly strike and need less supervision.

The farm lies mainly on stony, free draining soils and, as such, lends itself to out-wintering stock and extending the grazing season. In 2011, the farm undertook a change of policy to measure grass growth and allocate it to stock through rotational grazing.

The first form of rotational grazing the farmer operated was All Grass Wintering: daily allocation of grass to meet the winter ewe flock requirements on grazed grass alone. In late-autumn, the farmer quantified grass supply and determined whether there was enough to winter the flock. Then, using temporary electric fencing he allocated grass DM by area. Ewes in mid-pregnancy with low feed requirements and good body reserves can cope, as the farmer validates the budgeting calculations with practise – if the calculations under-estimate ewe requirement, body reserves can meet the shortfall, providing ewe body condition is monitored and managed. Infrastructure costs are limited to mechanised temporary electric fencing systems costing around € 1-2.90 ewe^{-1} depending on flock size.

The feed cost saving from all grass wintering justified higher infrastructure investment for summer rotational grazing: water and permanent electric fencing costing around € 88 ha^{-1}. This led to 95% of the lambs finished or allocated to the breeding flock by November and an average of 1.35 kg daily live weight gain in yearling cattle from early June to September. Silage production increased 150% and the farm in 2017 carried 30% more cattle and 5% more sheep than in 2013. An extra 20 ha of red clover aftermath was brought into the system and replacements grazed away from late September until Christmas. Emphasis on body condition scoring ewes and management made the farming more focussed and intensive.

After three years refining the system and knowledge exchange with a group of farmers (Quality Meat Scotland Grazing Group), the farmer believes they are approaching optimum paddock area, grazing mob size and shift frequency. The farmer now reports, 'it has been exhilarating putting it all into practice and being able to learn from, and share with like-minded farmers and consultants'. Now the focus is on ewe lamb growth to increase the number that can be mated in their first year.

Implications of uptake of added value strategies on a wider scale

Two themes emerge from these case studies: added product value and reduced cost of production; both serve to buffer financial pressures and improve economic viability. It is pertinent to discuss the wider social and economic issues of these examples, identify constraints and link with industry direction.

Targeting the light-lamb market in Southern Europe

The light-lamb production system for very marginal farms, whilst highly commendable on an individual basis, cannot sustain a great number of suppliers. The light-lamb market is sensitive to global disruption. If Romania loses Middle East export markets due to war, their export lamb is diverted to the Southern European market, in competition with the UK export lamb. The result is that the UK light lamb price falls from € 53-64 lamb^{-1} in export value to around € 18 lamb^{-1} for the only alternative market – the domestic store lamb trade. Future sustainability is dependent on unimpeded European trade which is highly problematical.

This light lamb model could be used to reintroduce sheep to areas of abandonment. Done carefully to protect bio-security and with the right marketing effort, it could reinvigorate the stratified sheep system and provide medium-size, hardy, disease-free replacements for lowland farms. It would serve to reduce wildfire risk and maintain the upland landscape, but this is at odds with pressures from organisations that seek to reintroduce large carnivores such as the lynx and wolf.

Marketing challenges and potential solutions

Marketing of meat depends on consumer faith in the product. Farm assurance in the UK, shown by the Red Tractor logo or, specific to Scotland, the Specially Selected Scotch lamb label, are a must-have attribute ensuring traceability, safety, welfare and best practice from farm to plate. Farmers and the industry have embraced these fully. There is a role for further food labelling and certification schemes for protection of products in marginal areas so returning customers can seek out the qualities they value.

The low-input, low-output systems of marginal areas clearly deliver high external positivity, but are challenged with limited networking and supply chain collaboration due to the small-scale business size. Current agricultural policy does not favour production from marginal areas and ideas such as a 'mountain food' label for Europe are beset with definition and compliance problems and are probably unworkable (McMorran, 2015). Better solutions may be with the Slow Food Movement.

Targeting premiums for lambs off hill grazing for eating-quality attributes relies heavily on access to local abattoirs and marketing skills; it is difficult to extrapolate the success of the talented individual to mass-market supply. A huge problem is EU hygiene law which favours large scale abattoirs that can afford dedicated staff for compliance. Small local abattoirs such as the one on Shetland are supported by local authority funding derived from oil revenues. Lamb eating quality is highest from lambs killed straight off their mothers from a herb rich diet, e.g. grass and clover (Vipond *et al.*, 1995) but it is not conducive to carcass quality, welfare or carcass yield to truck such immature lambs hundreds of miles to where current abattoirs are located (Miranda-de la Lama, 2012).

There is evidence that meat produced from botanically diverse pastures has higher concentrations of omega-3 polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA) relative to ryegrass-based pastures (Moloney *et al.*, 2008). CLA in the diet is linked to lower incidence of cancer and coronary heart disease. Compared to grain feeding, pasture feeding has been shown to increase omega-3 fatty acids, CLA, vitamin E concentration, colour lipid stability and yellow fat. However, there are variable reports of the effects on fresh meat colour and meat tenderness (Scollan *et al.*, 2005).

In one study, grazing lambs fed a range of forage types, including saltmarsh, heather and moorland, yielded meat with a stronger lamb flavour and lower abnormal flavour/odour values compared those on a perennial ryegrass based system (Whittington *et al.*, 2006). Shetland lamb (n=20) from a range of habitats contained 15-30% higher levels of poly- and mono-unsaturated fatty acids than Suffolk and Welsh Mountain pasture-fed lambs (Fisher *et al.*, 2000). Speculatively this could be due to the diversity of pasture types, rumen fermentation effects or the evolution of mechanisms to protect animals in extreme cold environments against frostbite (Adalsteinsson, 2000).

Health attributes of lamb from botanically diverse feed sources are probably of less interest to consumers driven by taste, but could possibly add value. Although potential beneficial health effects of foods can be used in marketing there is a paucity of long term studies on humans showing genuine effects and the animal models widely used may not be appropriate. Thus, significant changes to health recommendations are released by the medical profession regularly. Today's health food is tomorrow's health warning – a poor basis for a food production/processing business plan.

A need to review the UK stratified sheep system

Competition from breeding companies offering composites or pure bred ewe lambs with Estimated Breeding Values (EBVs) threatens the premium for crossbred breeding sheep, particularly if they address growing bio-security risks. Over recent years, the concentration on size and appearance of breeding stock has resulted in an increase in live weight of Mule ewes from 70 to 80 kg.

Smaller hill breeds are better placed to exploit sexual dimorphism from the size and improved growth genetics of modern terminal sires. Small ewes at 64 kg produced 25% more lamb/ha using the same resources as 78 kg ewes (Vipond *et al.*, 1987). With abattoirs now imposing maximum payment restrictions to carcasses over 21 kg, there is need to review Mule ewe live weight. The future of the stratified system to add value to lambs will require marketing effort and changes to meet lower labour input systems.

Balancing productivity and environmental goods from upland marginal areas with least constraint

The final example of adding value on the moderately constrained farms by increased efficiency of grassland use is the most likely to feature in the future. It addresses the key profitability issues relevant to the lamb production areas of Northern Europe. The technical details of rotational grazing systems are established and sources of suitable breeds and crosses widely available.

The difference between the meat output that is technically possible from a given land area and what is actually achieved can be attributed to the effects of nature. This can range from effects of low yielding herbs and flowers of low digestibility (yet beneficial to pollinators) to effects of competing grazing herbivores, such as rabbits and hares, and predators.

Given the preponderance of marginal land used by sheep in Europe, farmers have an outstanding opportunity to deliver environmental goods. In the UK they are not perceived as particularly driven to do this, extreme populist commentators saying the country is 'sheepwrecked' (Monbiot, 2013), countered by the less extreme views of the National Sheep Association (NSA, 2016).

If environmental issues are not seen as a high priority by most farmers it could be a reflection that they are relatively uninvolved in the decision-making processes relating to prescriptive environmental schemes. This is not due to disinterest in the environment – completely the opposite is more likely the case – it is just that the approach used so far has failed to enthuse them. There are also great difficulties identifying sustainable environmental benefits from reductions in grazing intensity/access that feature in most environmental protection schemes (Holland *et al.*, 2008).

If rotational grazing gives 30% better utilisation, farmers in marginal areas effectively gain a 30% increase in land area. They can then afford to take at least 1% of the area out of production entirely as sites specifically for wildlife with access by visitors, where appropriate, unimpeded by electric fences. Another 10% can be set aside on a temporary basis in summer, when grass production exceeds consumption, for environmental protection and flood prevention using specially bred grass varieties, e.g. cv. Aberniche, an Italian ryegrass-type Festulolium. This is undergoing National Recommended List Trials in the UK and is from the IBERS SUREROOT project which is hoped will shortly supply a perennial ryegrass Festulolium more suitable for sheep pastures.

As most upland farmers are more motivated by profitability than environmental protection, a political tool to develop multifunctional grasslands would be to encourage rotational grazing, using environmental payments, with the proviso that a small percentage of the land is maintained solely for wildlife. Impractical field corners and winding watercourse margins, difficult to subdivide for grazing, could be allowed to grow tall for invertebrate nest sites and watercourse buffers and with some areas (2-10%) grazed leniently and not topped, to provide habitat for invertebrates which are food for birds (DEFRA, 2013). Another potential 'win:win' is the use of Festuloliums in grazing seeds mixtures. These have the ability to both extend the grazing season and retain more carbon and water in root systems to enhance flood protection.

To reverse declines of pollinators, other invertebrates and birds, and enable them to adapt to climate change, improved grassland needs to be more permeable to wildlife (Peel, 2014). To achieve this, farmers on improved grassland should correct low pH and phosphate status of soils to encourage clover and reduce their use of nitrogen fertiliser. This improves the nutritive value of swards (Lüscher *et al.*, 2014) and increases lamb production and eating quality of lamb (Vipond *et al.*, 1997) as well as supporting pollinators. There could also be a role for separate areas of red clover/plantain chicory mixes to extend the seasonal supply of food for lambs and pollinators.

Conclusions

Marginal grassland areas of northern Europe that are managed by sheep farmers have many options to add value. The most constrained areas have few alternative enterprise options to sheep and have suffered greatest declines in sheep numbers. Whilst some individuals have shown how to add value to products by extensification there is limited scope for more widespread application. There are also negative social effects of very low input systems. Sustainability is dependent on access to EU markets and absence of large predators. The moderately constrained areas can add value by exploiting hardiness and health of ewes derived from the environment they are kept in. Crossbreeding these ewes to supply lowland farms is viable but faces competition from new breeds and emerging diseases. The social benefits of community marketing schemes could be lost if these issues are not addressed. The least constrained areas offer the most potential for enhanced added value and provision of multiple grassland services through changes in grazing management.

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Relationships between contents of fatty acids and forage quality compounds in forbs

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Abstract

Diversity in botanical composition of pastures may enhance animal-derived product quality. Objectives were to analyse relationships between concentrations of fatty acids (FA) and herbage yield, digestibility, protein, fibre and vitamin contents in herbage species as affected by harvest date. We hypothesized that relationships between FA and other compounds would be similar across investigated species and that total FA and *α*-linolenic acid (ALA) contents would be positively related to digestibility and concentrations of vitamins and protein. Seven forbs and a grass-clover mixture were sown in a field trial with two replicates. Forage was grown in two years and cut four times per year. Analyses of variance were performed. In 3 forbs and grass-clover, a negative relationship was found between total FA and herbage yield. Across eight harvest dates, ALA concentrations were positively related to digestibility and with concentrations of lutein, *β*-carotene, protein and *α*-tocopherol, and negatively with fibre concentrations in grass-clover and in 6, 6, 6, 5, 4 and 5 forbs, respectively, while for total FA, figures were 2, 6, 4, 4, 3 and 4. Implications for management and utilisation of forbs with potential for enhancing the FA profile in forage diets and animal-derived products are discussed.

Keywords: *α*-linolenic acid, digestibility, fibre, forb, forage legume, linoleic acid, protein, vitamin

Introduction

Forbs are important compounds of grasslands in marginal environments. Ruminant products produced on species-rich grasslands and alpine pasture have higher contents of n-3 fatty acids (FA), mainly *α*-linolenic acid (ALA), than from ruminants fed pure grass or oilseed-rich feed (Leiber *et al.*, 2005). An *in vitro* experiment revealed a lower biohydrogenation of ALA from salad burnet (*Sanguisorba minor* Scop.), plantain (*Plantago lanceolata* L.) and lucerne (*Medicago sativa* L.) than from chicory (*Cichorium intybus* L.), grass-clover (*Lolium perenne* L. – *Trifolium repens* L.) and birdsfoot trefoil (*Lotus corniculatus* L.) (Petersen and Jensen, 2014). *In vivo* feeding experiments showed that feeding a mixture of the above-mentioned forbs doubled the amount of n-3 FA in milk compared with traditional total mixed ration feeding (Petersen *et al.,* 2011).

Concentrations of FA vary among and within species; effects of regrowth stage, nitrogen, temperature, day length, and leaf proportion in the harvested herbage have been reported. ALA is the dominant FA in grasses and legumes, and the relative content of ALA increases with increasing total content of FA (Elgersma, 2015). As the FA profile of feed has a direct impact on the FA profile of animal products, FA concentrations and proportions, *α*-tocopherol, *β*-carotene, yield, and quality compounds such as fibre and protein concentrations and digestibility were studied for eight cuts during two years (Elgersma *et al.,* 2013, 2014). In this paper, relationships between these compounds, within and across species, are presented. We hypothesized that relationships between FA and vitamins, yield, digestibility and quality compounds would be similar for species belonging to non-leguminous and leguminous forbs, and the grass-clover mixture. The aims of this study were to quantify seasonal patterns in FA concentrations and proportions across harvests and years, and to examine relationships with vitamin concentrations, yield, and chemical composition.

Materials and methods

Pure stands were sown in Denmark of four non-leguminous forb species, three legumes, and a mixture of perennial ryegrass and white clover. The non-leguminous forbs were: salad burnet, caraway (*Carum carvi* L.), chicory and ribwort plantain. The legumes were: yellow sweet clover (*Melilotus officinalis* (L.) Pall.), lucerne and birdsfoot trefoil. The experimental design was a randomized block with two replications. Net plot size was 1.5×9 m. Cutting dates in 2009 were 29 May, 9 July, 21 August and 23 October, and in 2010, 31 May, 13 July, 19 August and 21 October. After cutting, herbage samples were taken to determine dry matter (DM) content and for chemical analyses of ash, nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and *in vitro* organic matter digestibility (IVOMD) (Elgersma *et al.*, 2014). A sample of approximately 0.5 kg of the total herbage was taken from each cut in both years, immediately frozen, freeze dried and stored in an air-tight plastic bag at -20 °C until analysis. Samples were later lyophilized and milled. Of this material, 2 g was saponified in alcohol. FA were extracted in a mixture of chloroform, methanol and water (Elgersma *et al.*, 2013). Analysis of variance procedures were applied using the MIXED procedures of SAS. There were eight 'species' (the seven forb species plus the mixture) and four harvests per year. Differences detected among main effects and interactions were assessed using the PDIFF option in the least-squares means statement. Regression analyses were conducted between FA and yield, forage quality parameters, vitamin concentrations and weather variables. All tests of significance were made at P=0.05.

Results

There was a negative relationship (P<0.05) between total FA concentration and herbage yield in chicory, ribwort plantain, lucerne and grass-clover. Similar relationships were found for ALA content in these species and also in salad burnet. Patterns were similar for FA and ALA in relation to vitamin contents *in planta*in, chicory, birdsfoot trefoil, lucerne and grass-clover. However, in caraway and salad burnet, ALA was associated with β-carotene (P<0.05) while FA was not, and in salad burnet, ALA was associated with lutein (P<0.05) while FA was not.

In chicory, ribwort plantain, lucerne, birdsfoot trefoil and grass-clover, positive linear relationships (P<0.05) were found between concentrations of total FA and N (Figure 1). ALA was related as well to N in chicory, lucerne, birdsfoot trefoil and also in salad burnet and caraway (P<0.05), but not in ribwort plantain and grass-clover. Relationships of FA and ALA with IVOMD were quite different: for FA, positive associations (P<0.05) were only found in lucerne and birdsfoot trefoil, whereas ALA was positively associated with IVOMD in all species (P<0.05) except salad burnet.

Discussion

In most species, we found that concentrations of ALA were negatively related with NDF concentration and positively with IVOMD. In single-species stands as examined here it would be possible to optimise harvest dates for each species but, as grassland species are grown in mixtures, fixed cutting dates reflect agronomic practice. The new knowledge from this study, i.e. the generally positive relationships between FA, vitamins and protein concentrations across harvest dates can be used in developing forage production systems with increased FA and vitamin concentrations in the herbage. While in grazing systems animals would select leaves over stems and intake would deviate from herbage collected in bulk samples, in cutting systems for silage swards all herbage above cutting height is harvested as in our study and, in total mixed rations with chopped silage, diet selection is limited.

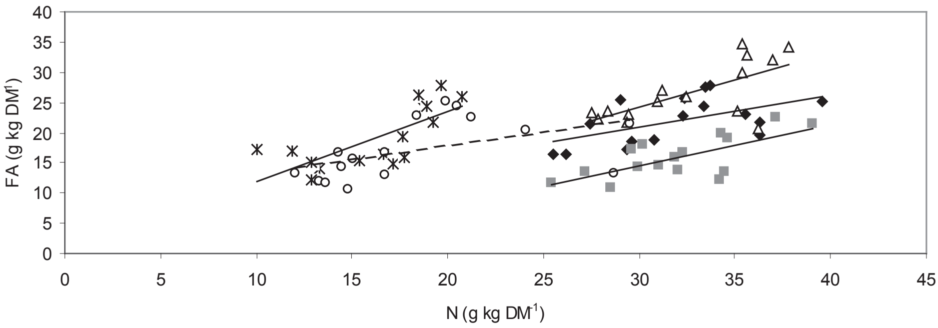

Figure 1. Relationships between concentrations of total fatty acids and nitrogen during 4 cuts in 2009 and 2010 in lucerne (■), chicory (*), birdsfoot trefoil (Δ), grass-clover (◆), and ribwort plantain (o); linear regression lines: R^2=0.48, b=0.68; R^2=0.53, b=1.14; R^2=0.46, b=0.91; R^2=0.30, b=0.53; R^2=0.25, b=0.46 (dotted line).

No differentiation between leguminous and non-leguminous forbs was found regarding the relationship between N and fatty acid concentration. This relation must be indirect, since FA are N-free (Witkowska *et al.*, 2008). N fertilization stimulates chlorophyll synthesis and lipids occur in chloroplast membranes, which might explain the observed increasing FA concentration with higher N content. The lack of such a relation in caraway and yellow sweet clover would need further investigation. The positive trend (P>0.05) found in salad burnet (not shown) was non-significant due to two outliers with a low N content, sampled at one harvest date. In general, relationships between FA and vitamins and quality compounds were rather similar for non-leguminous and leguminous forbs and the grass-clover mixture, confirming our hypothesis. ALA concentration was positively related with forage quality compounds in the 4-cut system applied in this study in most species. Agronomic management practices aiming for herbage with high protein and low fibre concentrations and a high digestibility at the moment of cutting would therefore also increase concentrations of vitamins and ALA that have perceived health benefits.

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Forage quality of shrub-encroached marginal pastures does not impair quality of carcass and meat

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Abstract

In view of the decline in the number of farmers and the ongoing shrub encroachment on mountain pastures the valorisation of products and services provided is gaining importance for the conservation of alpine and subalpine ecosystems. *Alnus viridis* comprises 70% of the shrublands in Switzerland and has been shown to be a serious threat to species diversity. In a grazing experiment, we investigated how robust breeds valorise these abundantly available resources while simultaneously controlling the encroachment or even regaining lost pastures and assessed the forage quality of the *Alnus* understorey. The species and breeds used were Dexter cattle and Engadine sheep. Both breeds feed on leaves and buds of *A. viridis* while the sheep even browse the bark of the shrubs. Although digestible organic matter did not differ among vegetation types, crude protein content was highest in the *Alnus* understorey. Compared to the animals kept on pastures without shrubs there was no decline in meat and carcass quality, and no differences in average daily weight gain. The sheep grazed on the encroached pastures even had a higher dressing percentage. We conclude that appropriate breeds grazing on shrub-encroached marginal pastures can concurrently produce meat, without loss of quality or animal performance, while preventing shrub encroachment and thus, conserving plant species richness.

Keywords: carcass quality, meat quality, shrub encroachment, forage quality, *Alnus viridis*

Introduction

Land use change in mountain ecosystems in recent decades has led to an increase in reforestation and shrub encroachment, primarily on abandoned pastures. From 1999 to 2010 the shrubland area in the Swiss Alps increased from 542 to 659 km^2 (Brändli 2010). 70% of all shrublands in the Alps are dominated by *Alnus viridis*, a nitrogen-fixing pioneer species that releases nitrogen in surrounding soils and streams (Bühlmann *et al.,* 2014). The fixed nitrogen is eventually released into ecosystems that consists largely of plant species adapted to nutrient-poor conditions, which can have dramatic effects and inevitably leads to a decrease in species richness (Zehnder *et al.*, 2016a). In the project EG4BM (Extensive grazing on subalpine pastures: integrating biodiversity and the production of meat with special quality) we therefore aim at developing strategies to stop or even reverse shrub encroachment while maintaining a viable meat production. Here we show results of the forage quality analysis and how grazing of *A. viridis* affected carcass and meat quality of lamb and cattle.

Materials and methods

A grazing experiment was set up using Dexter cattle and Engadine sheep on two neighbouring areas on both sides of the Albula pass (Canton Grisons, Switzerland, 46°34'N, 9°50'E) at an elevation of 1,900-2,200 m a.s.l. The animals were evenly split into four groups, each grazing pastures with different degrees of encroachment ranging from 0 to 80% cover of *A. viridis*. Each group grazed three paddocks in two rotations. Before each change of paddock, the animals were weighed. Forage biomass was sampled from grazing exclusion cages (1.2×1.2 m) placed in three different vegetation types classified according to Dietl *et al.* (1981). Digestible organic matter (DOM) was analysed according to Tilley and Terry (1963). After 8 weeks, the animals were slaughtered and carcass quality assessed. Meat quality was measured on the *Longissimus thoracis* after a 21 d aging period. Significance of differences was tested by linear mixed effect models. Random factors were date of slaughter for meat and carcass quality parameters and date of harvest for forage quality parameters.

Results and discussion

Forage quality: There was no significant difference (P=0.22) in DOM content of the vegetation types between pastures (Figure 1A). Crude protein (CP) content was significantly higher (P<0.001) in the *A. viridis* understorey than in the other vegetation types (Figure 1B). This may be due to the additional nitrogen input provided by *A. viridis* (Bühlmann *et al.*, 2016). High CP and DOM content combined with the high productivity of the *A. viridis* understorey (Wiedmer and Senn-Irlet, 2006; Zehnder *et al.,* 2016a) shows that this vegetation type provides an underestimated forage resource.

Feeding behaviour

Dexter cattle browsed leaves and buds of *A. viridis* and were not hindered in movement by the shrubs. In addition to leaves and buds, Engadin sheep also made use of the bark and seemed to have a preference for some of the plants abundantly available in the *Alnus* understorey (*Adenostyles alliariae, Peucedanum ostruthium*).

Carcass and meat quality

Dressing percentage of the animals did not decrease with higher cover of *A. viridis* (Figure 2A). Engadine sheep even performed significantly better (P<0.001) on pastures with medium and high covers of *A. viridis* and hence a high proportion of *A. viridis* understorey. This agrees with the high biomass production (Zehnder *et al.,* 2016a, Wiedmer and Senn-Irlet, 2006) and the high DOM and CP contents of the *Alnus* understorey (Figure 1), although the average daily weight gain did not differ (Zehnder *et. al.,* 2016b). Pasture type did not have an influence on the shear force of the meat (Figure 2B), where low values coincide with tender meat.

Conclusions

Our data show that forage provided from stands of *A. viridis* has been underestimated. Its comparatively high forage quality makes it a valuable resource for organic meat production in marginal mountain areas. Extensive grazing of either of the tested animal species will offer a way to valorise these resources while maintaining a rich biodiversity.

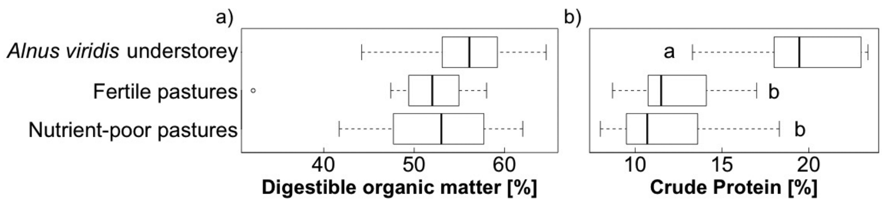

Figure 1. Digestible organic matter (A) and crude protein concentration (B) in the dry matter of different vegetation types. Bold lines show median values, boxes are 50% quantile range and whiskers are 1.5 times the interquartile range. Letters indicate significant differences of pairwise comparison with Tukey contrasts.

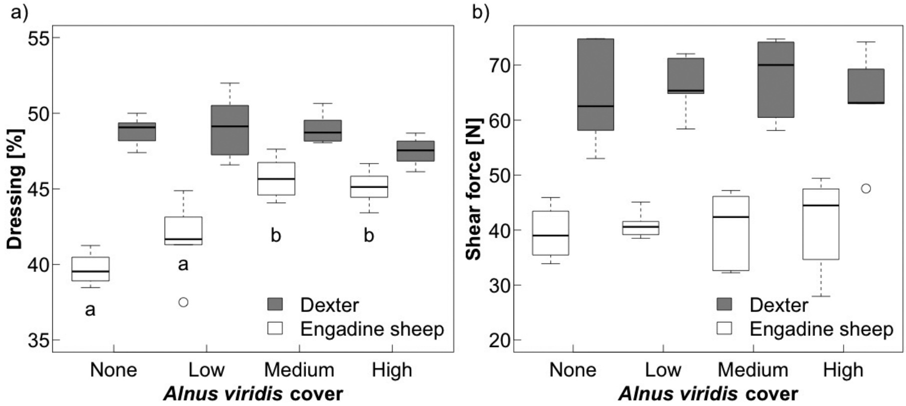

Figure 2. Dressing percentage of the bodies (A) and shear force of the *Longissimus thoracis* (B) of Dexter and Engadine sheep. Bold lines show median values, boxes are 50% quantile range and whiskers are 1.5 times the interquartile range. Letters indicate significant differences of pairwise comparison with Tukey contrasts.

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Developing perennial shrubs to fill feed gaps on marginal soils in Australia

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Abstract

This paper presents data and discusses work being conducted in Australia to improve the feeding value of drought-tolerant shrubs through simple plant selection. The programme targets saline, sandy and infertile soils in low rainfall zones that are marginal for cropping. The shrubs are used to complement the abundant and poor-quality crop and annual pasture residues during the summer and autumn seasons. Plant improvement criteria were determined through whole-farm economic modelling, with increasing energy and protein content the key target. Biomass and nutritional value data of 19 accessions from 7 species growing in a moderately saline environment are presented. The data show there is significant opportunity for selection of improved cultivars. This approach has already been used to commercialise Anameka™ saltbush *(Atriplex nummularia)*, a cultivar with higher energy concentrations, greater biomass production and improved 'palatability'. Work is continuing with the development of elite *A. nummularia* seed lines (to halve the cost of establishment) and to identify improved accessions of *Rhagodia preissii* for sandy and infertile soils in dry areas. Adoption of these shrubs will lead to improved profitability and environmental health.

Keywords: perennial shrubs, extensive grazing systems, sheep, cattle, supplement

Introduction

The south-west of Australia is characterised by a Mediterranean-type climate with hot, dry summers and mild, wet winters. The mixed crop/livestock agro-ecological zone covers approximately 20 million ha and annual rainfall varies from 300 to 650 mm. Cereal crops predominate land use and the majority of farmers manage sheep flocks for lamb and wool production. Dryland salinity is a problem; ~1.1 Mha of agricultural land is severely salt affected and a further 1.7-3.4 Mha is at risk (George *et al.*, 2008). The summer/autumn feed gap, and subsequent reliance on grain supplements, is the critical production cost within livestock systems. Herbaceous perennials struggle to persist and reduce cropping flexibility.

Australian native shrubs from the *Chenopodiaceae* family are broadly adapted to dry, saline, sandy and nutrient deficient conditions. These shrubs offer an opportunity to provide the nutrients that are constraining ruminant production in summer and autumn (Masters *et al.*, 2001; Norman *et al.*, 2004). Economic studies demonstrate that these shrubs, without any genetic improvement, can lift whole-farm profitability, reduce cost of production by reducing supplementary feed and reduce whole-farm risk by buffering feed supply (Monjardino *et al.*, 2010; O'Connell *et al.*, 2006). As they are productive on soils that are marginal for cropping, adoption is associated with a low opportunity cost. Other benefits include provision of vitamin E and improved meat quality (Pearce *et al.*, 2010) and reduced methane emissions intensity (Li *et al.*, under review). The aim of this experiment was to compare 19 accessions from 7 of the most promising Australian shrub species. Over 3 years we measured biomass production, nutritional value and sheep preferences. We tested the hypothesis that there would be significant variation in shrub productivity traits between and within species.

Materials and methods

The research site was established on a moderately saline, clay-loam soil in August 2013 in Tammin, Western Australia (annual rainfall 340 mm). The randomised experimental design consisted of 19 shrub accessions within 12 replicated blocks (8 adjacent shrubs/accession within blocks). The ~2,000 experimental shrubs were hand planted in scalped rip lines that were spaced 5 m apart. Shrubs within rip lines were 1.5m apart (1,333 shrubs ha^{-1}). Fertiliser (100 kg N ha^{-1}) was applied in August 2015. The species (and number of accessions) that were used are as follows: *Atriplex nummularia* (10), *Atriplex amnicola* (1), *Atriplex rhagodioides* (1), *Chenopodium nitrariaceum* (1), *Maireana brevifolia* (2), *Enchylaena tomentosa* (2) and *Rhagodia preissii* (2). The site was completely defoliated by mature ewes in autumn 2015 and autumn 2016 (15 sheep ha^{-1} for 42 days). During grazing, preference was assessed fortnightly using a defoliation ranking method (from 0=untouched to 5 = completely defoliated). 'Edible' biomass (EDM; Andrews *et al.*, 1979) and nutritional value was assessed every 4 months over the 3 years. Samples of EDM were bulked for accessions within blocks, dried for 48 h at 60 °C and ground to 1 mm in a FOSS Cyclotech mill. Nutritional value was estimated using a combination of chemistry and calibrated near infrared spectroscopy. *In vitro* digestible organic matter in the dry matter digestibility (DOMD) and acid detergent fibre (ADF) were measured as described in Norman *et al.* (2010) and shrub standards with measured *in vivo* digestibility values were used for calibration. Total N was determined using a Leco CN628 Analyser and anions by HPLC. ANOVAs were performed in GenStat® 16.

Results and discussion

The primary focus of our research, as determined by whole-farm economic and biophysical modelling, has been identifying species and genotypes with improved energy and protein value. A secondary goal has been to increase biomass production and the relative palatability of these species as this encourages sheep to select shrubs before the understorey is overgrazed. The most productive species during the establishment phase were *A. amnicola* and *A. rhagodioides* (equating to ~2.3 and 1.5 t EDM ha^{-1}, Table 1). It was apparent that *A. nummularia* was able to recover and grow faster than *A. amnicola* after the first grazing. *R. preissii* grew only 1.15 t EDM ha^{-1} by autumn 2016; this species is generally very productive but has a preference for sandy, non-saline soils. There were significant differences in EDM production within the *A. nummularia* accessions (data not presented). As a general rule, nutritional value of the shrubs was relatively constant across seasons and years (data not presented). There were significant differences between species and within accessions of *A. nummularia* for all of the nutritional traits measured. DOMD ranged from 45% (*A. amnicola*) to 66% (*R. preissii*). These differences are biologically and economically very significant. ADF was generally low (13-20%) and crude protein high (14-22%), indicating the shrubs would be a good complement to poor quality, fibrous crop residues. The mean oxalate level of *M. brevifolia* was extreme (102 mg g^{-1} DM). Sheep preferences were relatively consistent between years. Unless there is biodiversity goal, *C. nitrariaceum* and *E. tomentosa* appear to have little to offer in this region.

Australian native chenopods are not the most productive or nutritious forage plants available. They do, however, offer a reliable energy, protein, sulphur, mineral and antioxidant supplement for ruminants grazing poor quality cereal stubbles and pasture residues during summer and autumn. In many systems, lambs have been sold at this time and farmers are seeking to maintain Merino ewes in early pregnancy with minimal grain supplements. With high nitrogen and sulphur, these shrubs improve wool growth. A recent experiment found that replacing 25% of a moderate quality cereal hay diet with either *A. nummularia* (Anameka™). or *R. preissii* led to 24% greater clean wool growth (Li *et al.*, under review). These shrubs utilise out-of-season rainfall and have been linked to a reduction in dryland salinity (Norman *et al.*, 2008). If improved profitability results in greater rates of adoption by farmers, the environmental health of agricultural landscapes in southern Australia will be improved.

Table 1. Mean 'edible' dry matter per shrub (EDM; leaves and stems <3 mm) and nutritional profile in summer/autumn 2016.[1]

Species	g EDM shrub^{-1} 3/03/2015	g EDM shrub^{-1} 1/03/2016	DOMD %	ADF %DM	N %DM	oxalate mg g^{-1} DM	Ash % DM
A. nummularia	698 (15)	1,369 (27)	54 (0.2)	16 (0.1)	3.6 (0.04)	34 (0.9)	21 (0.3)
A. amnicola	1,751 (111)	1,038 (62)	45 (0.6)	20 (0.7)	2.3 (0.09)	18 (1.4)	18 (0.9)
A. rhagodioides	1,060 (101)	2,000 (124)	49 (0.5)	20 (0.5)	2.7 (0.08)	29 (2.2)	18 (0.7)
C. nitrariaceum	13 (1)	53 (4)	57 (0.6)	19 (0.7)	3.0 (0.14)	37 (1.7)	11 (0.6)
E. tomentosa	229 (13)	313 (15)	55 (0.5)	17 (0.4)	3.2 (0.09)	53 (3.6)	18 (0.6)
M. brevifolia	363 (19)	187 (11)	49 (0.4)	17 (0.5)	3.9 (0.10)	102 (2.5)	20 (0.7)
R. preissii	424 (35)	866 (62)	66 (0.5)	13 (0.3)	2.7 (0.06)	24 (1.2)	12 (0.4)
LSD	100	135	1.3	1.0	0.13	6.1	1.7
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1] ADF = acid detergent fibre; DOMD = digestible organic matter in the dry matter digestibility; DM = dry matter; LSD = least significant difference.

Conclusions

In this paper we have provided a snapshot of data from one research site within a shrub domestication programme. The data demonstrate that there is significant variation between and within species and therefore an opportunity to improve nutritional value and agronomic performance of these species. To optimise success, it is critical that shrub improvement targets are defined within ruminant production systems and whole-farm economic contexts.

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Evaluation of precision technologies for measuring cows' grazing behaviour

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Abstract

Grazing behaviour sensors can aid successful grazing management of cows in conventional milking systems, but are likely to be more important in automatic milking systems. Currently, most commercially available sensors for monitoring feeding behaviour are calibrated for indoor systems and are not validated in a grazing environment. Therefore, the objective of this study was to validate two grazing behaviour sensors against visual observation in different time resolutions (1 h or 15 min summaries). The sensors included (1) 'RumiWatch' halter, which measures grazing behaviour by recording jaw movements using a noseband pressure sensor, and is designed for research use; and (2) 'MooMonitor+' collar, which measures grazing behaviour by an accelerometer placed on the neck and is developed for commercial use on farms. The RumiWatch halter versus visual observation showed a correlation of r=0.96 and r=0.98 for grazing and ruminating behaviour, respectively. The MooMonitor+ had corresponding r-values of r=0.94 and r=0.98. In conclusion, both devices demonstrated a high accuracy.

Keywords: sensor technology, grazing behaviour, feeding behaviour

Introduction

Potential for application of sensor technologies on commercial farms is increasing. Different sensor technologies have been developed to monitor behavioural and physiological parameters of cows, with specific focus on cow health and welfare issues within milk production systems. Feeding behaviour, in particular, may be considered as an appropriate indicator for animal health and welfare, as feeding is very much influenced by the health or disease status of the animal, e.g. ketosis, milk fever and hoof lesions (Bareille *et al.*, 2003), calving events (Pahl *et al.*, 2014) and stress (Herskin *et al.*, 2004). A further potential benefit of monitoring cow-feeding behaviour may be achieved by using the data to optimize feed allocation and identify factors that would increase efficient utilization of feed by the cow and increase profitability. The requirement for assistance in herd management in this area increases with growing dairy herd size, while, at the same time, farmers face a shortage in labour availability. Therefore, the application of easy-to-use sensor technology to monitor feeding behaviour of dairy cows continuously would be very valuable. Most of the sensors have been developed and tested for indoor-housed cows. However, in this study, two feeding behaviour sensors were evaluated in an intensive grazing situation. The first sensor was the MooMonitor+ collar (Dairymaster, Tralee, Ireland). This sensor device had an integrated accelerometer, which recorded the activity of the cow. This system was primarily developed for identifying oestrous events of cows, since it is proven that increased activity is associated with onset of heat in cows. Subsequently, the collar device has been further developed to monitor feeding behaviour. The second sensor device investigated in the current study was the RumiWatch noseband pressure sensor (Itin+Hoch GmbH, Liestal, Switzerland). This sensor was previously calibrated for indoor systems (Ruuska *et al.*, 2016), but has not yet been validated on pasture-based systems. Therefore the objective of this study was to validate the automated sensors against human visual observation to assess their reliability for accurately monitoring feeding behaviour of grazing dairy cows. Two behaviour categories, grazing and rumination were validated.

Materials and methods

The study trial was conducted at the Teagasc, Animal and Grassland Research Centre, Moorepark, Fermoy, Co. Cork, Ireland. A group of 12 spring-calved dairy cows were fitted with the RumiWatch noseband sensor, integrated into a halter, and the MooMonitor+ collar placed around the cow's neck. The cows had an average bodyweight of 477±65 kg and consisted of two different breeds, Holstein-Friesian and Jersey-crossbreds. All cows were maintained on a grass-only diet and followed a similar milking routine, that is, twice a-day milking, with approx. 1.5-2.0 h away from the paddock during each milking event. Two observers monitored three cows each over three 2 h periods day^{-1}, for a total period of 6 days. Behavioural classifications of grazing and rumination were recorded by 1 min scan sampling. The values were then totalled for 15 min or 1 h periods. The sensor systems recorded data continuously over the experimental period. MooMonitor+ collars submitted pre-processed 15 min summaries to a base station, while the cows were in the milking parlour. The RumiWatch halter recorded raw data in a 10 Hz resolution. The data were converted by the RumiWatch Converter V.0.7.3.36 into 1 h summaries when the trial was completed. The data analysis involved comparison against visual observation data. The MooMonitor+ data in summary periods of 15 min and 1 h were also compared to the visually observed data. Statistical analysis was performed using R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria), calculating Concordance Correlation Coefficient (CCC), Spearman's Rank Correlation Coefficient (r_s), and Bland-Altman-Analysis. The original values of automatically and visually measured data were not normally distributed thus the non-parametric calculation of Spearman's Rank Correlation Coefficient was used. Alternatively, the differences between the automatically and visually measured values were normally distributed and the Bland-Altman-Analysis was applied.

Results and discussion

The concordance between the MooMonitor+ collar and visual observation for the behaviour classifications of grazing and rumination was high, with r_s-values from 0.90 to 0.97 observed (Table 1). The detection of rumination was slightly more accurate than the detection of grazing with CCC=0.98 for 15 min and 1 h summaries observed for rumination, while CCC-values of 0.95 and 0.97 were observed for 15 min and 1 h resolutions for grazing. The performance of the RumiWatch halter against visual observation was also good with CCC values of 0.96 and 0.99 observed for grazing for rumination, respectively. The results may indicate that rumination behaviour is easier to detect than grazing behaviour. This may be due to the strong rhythmicity of the rumination process resulting in it being easier to detect by the sensors. The Bland-Altman-Analysis in Table 1 showed a mean difference in rumination time of 0.27 min 15 min^{-1} and 0.00 min h^{-1} between MooMonitor+ and visual observation. This indicates that the MooMonitor+ sensor is accurate and not over- or underestimating the rumination time within either time resolution. A similar situation can be applied to the RumiWatch halter regarding rumination time, as a mean difference of 0.03 min h^{-1} was observed between that sensor device and visual observation. The time associated with grazing behaviour was slightly overestimated by both sensor devices. Mean differences of 1.01 min h^{-1} and 4.41 min h^{-1} were observed between the sensors and visual observations. The RumiWatch halter had a slightly higher concordance with visual observation for rumination time, but the MooMonitor+ performed slightly better in grazing time measurement in 1 h summaries. Both sensor devices are sufficiently accurate to be applied in a practical farm setting. However, the MooMonitor+ collar might be more suitable and robust for long-term use on farms as the RumiWatch noseband sensor is integrated into a halter. Additionally, the MooMonitor+ can save 15 min data summaries for 48 h, which is a potential benefit for use on commercial farms. Alternatively, the RumiWatch halter has the ability to detect and record each individual jaw movement of the cow. This is particularly important when using the device in a research scenario and the raw data can be saved for up to 4 months.

Table 1. Comparison of automated measurements of feeding behaviour using the MooMonitor+ and RumiWatch with visual observations. Grazing and rumination times were outputted in 15-min periods for MooMonitor+ and in 1-hour periods for both MooMonitor+ and RumiWatch.

	Behaviour	r_s	CCC	Mean difference	Lower limit of agreement	Upper limit of agreement
MooMonitor+ 15 min period	Grazing time (min 15 min^{-1})	0.90	0.95	0.27	-3.76	4.31
	Rumination time (min 15 min^{-1})	0.93	0.98	0.10	-2.09	2.30
MooMonitor+ 1 h period	Grazing time (min h^{-1})	0.94	0.97	1.01	-10.18	12.21
	Rumination time (min h^{-1})	0.97	0.98	0.00	-4.93	4.93
RumiWatch 1 h period	Grazing time (min h^{-1})	0.96	0.96	4.41	-4.69	13.51
	Rumination time (min h^{-1})	0.98	0.99	0.03	-3.04	3.10

Conclusions

The evaluation of the two sensor technologies indicated their ability to accurately determine feeding behaviour of cows (in terms of grazing and ruminating) in a pasture environment. Both technologies tended to be slightly more accurate in detecting rumination behaviour than grazing behaviour. Nevertheless, the concordance with visual observations was very high for both feeding behaviour categories. From experience gained through working with the technologies, it may be suggested that the MooMonitor+ collar may be more suited to a practical farm setting, whereas, the RumiWatch halter may have a greater potential in a research setting due to its data management characteristics.

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Dynamilk: a model at farm-scale to explore the balance between forage and milk production

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Abstract

Leaning towards better feed and forage self-sufficiency enables farmers to improve dairy farm competitiveness and their ability to cope with climatic changes and production constraints. Finding the proper stocking rate to the potential production of grass, including effect of climate change on yields, is important to maintain milk production and secure the forage system. Building a model at farm-scale enables different dairy systems to be explored for contrasting production strategies, in order to understand which are the possible trade-offs between levels of animal production, feed and forage self-sufficiency, and sustainable grassland uses. Such a dynamic model, called Dynamilk, has been developed. A typical dairy system based on productive permanent grasslands has been simulated, with different levels of stocking rate, in order to investigate its performance (milk yield, forage self-sufficiency and herbage utilization). Two different periods have been simulated to evaluate the impacts of weather conditions. Results have shown that an adapted stocking rate can be found, allowing a high level of milk production through a better valorisation of grass during the grazing without compromising forage self-sufficiency. Current climatic conditions imply a reduction of the stocking rate compared with stocking rates considered during the 1990s.

Keywords: dairy farming system, modelling, grasslands, forage system, forage self-sufficiency

Introduction

Dairy farming systems located in mountain areas often produce milk and cheese that conforms to Protected Geographical Status (PGS) specifications, which enable the promotion of quality production and the special link between farming practices, milk production and 'terroir'. In order to reinforce this link, cheese PGS specifications require forage systems to be based on grassland utilization through production constraints, such as better forage self-sufficiency and limits on feed concentrate supply. Dairy farming systems located in mountain areas are based on grasslands with a predominance of permanent pastures managed with low chemical inputs. Thus, these systems are very sensitive to environmental variations, climatic events and changes in production constraints (Lee *et al.*, 2013). It has been demonstrated that climatic changes have effects on yields of grass-based forages and pastures and their nutritive value due to higher risks of spring and summer droughts (Delaby and Fiorelli, 2014; Lee *et al.*, 2013). In this context, investigating the possible leeway to maintain or increase milk production while maintaining forage and feed self-sufficiency, abiding by the PGS specification demands and facing climatic changes can be important for maintaining dairy systems in mountain areas. Adapting the animal needs through an adequate stocking rate to the potential production of grasslands, taking into account climate change effects, is often seen as a lever (Delaby and Fiorelli, 2014; Lee *et al.*, 2013). Raising stocking rate at herd-level could also allow increasing annual milk production at farm-scale.

In this context, a model at farm-scale called Dynamilk (Jacquot *et al.*, 2012) has been built in order to explore different dairy systems under geographical and production constraints, with contrasting production strategies, in order to understand which are the possible trade-offs between animal production, feed and forage self-sufficiency and sustainable grassland use. Dynamilk has been built to take into account climatic variations and events.

Materials and methods

Dynamilk is a dynamic model which mimics the farming system functioning of a grassland dairy farm. It is a deterministic model with a daily time step. Dynamilk is based on a bio-technical approach focused on dynamic relationships among farmer management and production system components such as dairy cattle, grasslands and feed resources. Dynamilk is designed to consider: (1) dynamic animal needs and production determined by dairy cattle characteristics and farmer management (Jacquot *et al.*, 2015); and (2) dynamic herbage production depending on grassland type, forage management and weather conditions. Dynamilk is made up of three sub-systems, i.e. the farmer and his decisions, the dairy herd, and the resources of grasslands, forage stores and feed. The inputs of Dynamilk are weather data, paddock and herd characteristics and management rules. Farm management strategy and parameters of management practices are defined in a simulation script called 'scenario'. Dynamilk outputs are milk yield in relation to herbage, forage and concentrate offered, annual herbage yields and energy values of different kind of forages, annual biomass utilization rates of grasslands, daily biomass intake, bodyscore and daily weight variations of dairy cows. In addition, Dynamilk calculates the year-to-year evolution of forage self-sufficiency and annual milk production. To investigate the balance between animal needs and herbage offer in order to optimize milk production, a typical system of French mountain areas has been simulated with Dynamilk based on farm survey data (Jacquot *et al.*, 2010). The main characteristics of this system are: (1) the majority of milk is produced during wintertime due to an autumn-calving distribution (AC) from August to February; (2) the farm area is made of 80 ha with grasslands dominated by productive grasses and 48% of the farm area is dedicated to forage harvesting; and (3) dairy cattle comprise 51 dairy cows with a potential milk production of 7,000 kg cow^{-1} y^{-1}, a stocking rate of 0.94 livestock units (LU) ha^{-1} and concentrate amount of 1,200 kg cow^{-1} year^{-1}. The system performances have been analysed on predicted results generated from weather data over the 1995-2011 period. Several simulations were accomplished subsequently to evaluate the effects of stocking rate increase (from 0.94 to 1.54 LU ha^{-1}) on animal production, herbage utilisation and forage self-sufficiency rate on two different periods, 1995-1999 and 2007-2011, in order to investigate climate change effects on dairy system performances.

Results and discussion

Raising stocking rate from 0.94 LU ha^{-1} to 1.54 LU ha^{-1} (in other words, from 51 cows to 82 cows) did not result in deterioration of average milk yields, with similar level of production per cow. At farm-scale, it increased total production from 342,947 to 555,964 kg of milk per year. Raising the stocking rate also implied a better valorisation of grass during the grazing period with an increase of the net energy for lactation (UFL) value of ingested grass (1 UFL = 1,700 kcal kg^{-1}), from 0.84 UFL for 0.94 LU ha^{-1} to 0.88 UFL for 1.54 LU ha^{-1}. This better valorisation was due to a higher speed of rotation on the paddocks with a rotational grazing management and it allowed the milk production level to be sustained. It follows, however, that logically this intensification of the system also considerably reduced forage the self-sufficiency rate for the same farm area with a positive balance until a level of stocking rate of 1.34 LU ha^{-1} for the 1995-1999 period and of 1.14 LU ha^{-1} for 2007-2011. When this average rate was close to 0 Mg DM LU^{-1}y^{-1}, forage self-sufficiency was unsecured because some forage had to be purchased from outside the farm in some years.

Figure 1 indicates that there was a decrease of system performances between the two simulated periods. Indeed, there was a decrease of milk yields between the 1995-1999 and 2007-2011 periods, with an average loss of 110 kg per cow per year for each stocking rate levels. The same decrease was observed for forage self-sufficiency rate with an average difference of 0.18 Mg DM LU^{-1} y^{-1} between the two simulated periods. With the exact same conditions, except weather conditions, the balance for forage self-sufficiency was reached at 1.34 LU ha^{-1} over the 1995-1999 period and only at 1.14 LU ha^{-1} over the 2007-2011 period. Taking into account that there was no effect of the simulated periods on UFL value of ingested grass at grazing, the observed decrease of performances depended on harvested forage.

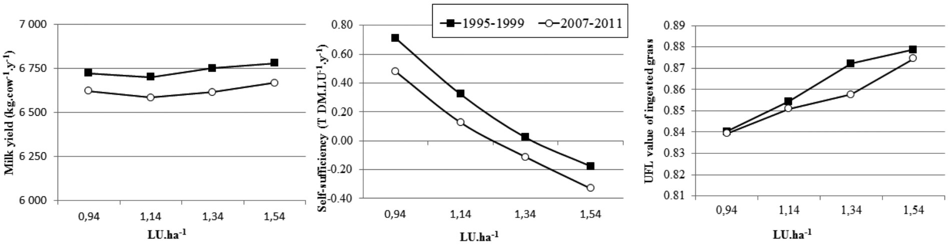

Figure 1. Evolution of average milk yield, self-sufficiency rate and net energy for lactation (UFL) value of ingested grass at grazing according to different levels of stocking rate, from 0.94 to 1.54 livestock units (LU) ha^{-1}, over two simulated periods 1995 to 1999 and 2007 to 2011.

Indeed, in this dairy system, the majority of milk is produced during wintertime from a forage-based diet. The decrease of forage self-sufficiency indicated that there was a decline of forage yields, on average, with 4.63 Mg dry matter (DM) ha^{-1} for the 1995-1999 period and 4 Mg DM ha^{-1} for the 2007-2011 period. Similarly, with same amount of feed concentrates between the two simulations, the difference of milk yields was explained by a decline of the quality of the forage. The digestibility of the forage was 0.666 g g^{-1} corresponding to an energy value of 0.79 UFL for 1995-1999 and 0.649 g g^{-1} (0.76 UFL) for 2007-2011.

Conclusions

In dairy farming based on grasslands with a predominance of permanent grassland, these simulations indicate that there is a little leeway to improve milk production through an increase of stocking rate. Under current climatic conditions, this leeway is reduced due to a degradation of harvested forage, in quantity and quality. Adapting the grassland management and especially using crop plants able to cope with climatic events could be an interesting way to sustain or improve the potential production of grasslands. Another potential adaptation could be optimizing the match between animal needs and grass on offer by producing more milk during grazing time through, for example, early spring-calving period.

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Discriminant analysis to identify ruminant meat produced from pasture or stall-fed animals

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Abstract

The decision to purchase a specific food, especially in developed countries, is increasingly influenced by its putative 'healthiness'. In the case of meat, this is largely related to its fatty acid (FA) composition. Animal feeding strategies play a major role in determination of meat FA and extensive grass-based livestock production systems are recognized for producing 'healthier' products than intensive systems based on conserved forage and concentrates. This work aims to evaluate the reliability of discriminating ruminant meat production systems (pasture-feeding vs stall-feeding) from the FA profile of intramuscular (*Longissimus dorsi*) samples, using a multivariate statistical approach, independently of animal species, sex, and age. FA composition of 53 meat samples from animals raised in different production systems (pasture- and stall-fed animals), of different species (ovine and bovine), sex and age was determined. The database was submitted to a Stepwise procedure to select the FAs that would better contribute to the discrimination. These were then used to classify the source of meat samples, using a Linear Discriminant Analysis. The preliminary results (99% of correct classification) show that meat FA profile can be used to track beef and sheep meat source.

Keywords: sheep, cattle, meat, traceability

Introduction

Consumer preference for a food, especially in developed countries, is increasingly driven by information on its origin and method of production, the former usually detailed in food labels (Prache *et al.*, 2005). The feeding system of ruminants largely determines the intramuscular fat content and its fatty acid composition (FA), which, in turn, influences the perception of meat 'healthiness' by consumers (Alfaia *et al.*, 2009; Dias *et al.*, 2008; Wood *et al.*, 2003). Extensive grass-based systems are recognized as producing 'healthier' meat than intensive systems based on conserved forage and concentrates. Therefore, it becomes important, especially from a consumer's point of view, to discriminate animal products obtained from grazing systems. However, a comparison of retail meat samples on the basis of differences in quality does not allow these samples to be assigned to a particular feeding system, since many factors influencing meat quality (breed, sex, management, nutrition and their interaction) are unknown. The objective of this work was to evaluate the reliability of the multivariate statistical analysis of intramuscular FA profile of meat samples to track the feeding system of the source animals when data such as nutrition, breed, sex, age at slaughter are unknown.

Materials and methods

53 meat samples collected in different experiments were used: 20 were obtained from 7 young bulls and 6 bulls fed natural pasture, and 7 male lambs fed *Lolium multiflorum* Lam. The other 33 came from animals (20 young bulls, 6 bulls and 7 male lambs) fed at stall with hay and commercial concentrate. The grazing period lasted 60 days in spring for young bulls and lambs, and 16 months for bulls. The latter were supplemented with hay and concentrate for short periods, when pasture availability was low. The duration of stall-feeding period mirrored that of grazing for young bulls and lambs while for the bulls lasted 20 months. The animals were slaughtered in a commercial abattoir according to standard

procedures at an age of 416±25 days (lsmeans ± standard deviation) for the young bulls, 94±9 days for the lambs, and 22.6±4.7 months for the bulls. 24-h post-mortem, samples of *Longissimus dorsi* were removed between sixth and tenth thoracic (T6 and T10) vertebrae from each left half-carcass, ground, homogenized, vacuum-packaged and stored frozen at -20 °C until analysis. The fatty acids composition was determined in intra-muscular fat according to Addis *et al.* (2013). The FA profile (expressed as percentage of total Fatty Acid Methyl Esters, % FAME) was submitted to a preliminary analysis of variance (ANOVA), using the lm procedure of R (R version 3.3.2 2016). The model included the fixed effects of animal species, feeding system and their interaction. A stepwise discriminant analysis was then applied to the full set of FA to select the variables that best discriminated between feeding systems. Only variables with significance level lower than 0.02 were retained. The selected variables were then submitted to a canonical discriminant analysis, a dimension reduction technique that performs both univariate and multivariate one-way analysis to derive new variables. If *k* is the number of involved groups, the canonical discriminant analysis derives *k-1* linear equations, called canonical functions (CAN), used to predict the group an object belongs to. The structure of a CAN is:

$$CAN = d_1X_1 + d_2X_2 + ... + d_nX_n,$$

where X_i are the original variables and d_i, called canonical coefficients, indicate the contribution of each original variable in composing the CAN. The effective separation between groups was evaluated with the Mahalanobis' distance and the corresponding Hotelling's T-squared test (De Maesschalck *et al.*, 2000). Moreover, the ability of the canonical functions to assign each meat sample to the correct dietary group of the source animal was reported as the percentage of correct assignment using the discriminant analysis. All multivariate analyses were run using SAS (SAS Institute, Inc., 2014).

Results and discussion

From the 62 initial variables detected in the FA profile of meat, only 5 were retained in the stepwise discriminant analysis: C18:1 12c, eicosapentaenoic acid (EPA), linolenic acid (ALA), C18:1 15t, that coelutes with C18:1 10c and CLA 9t 11t. Some of the selected variables (C18:1 15t/10c, CLA 9t 11t) did not differ between diets or species after running the ANOVA (Table 1).

This is a plausible result as the stepwise discriminant analysis evaluates the contribution of the single variable in relation to the others, to delineate the optimal variable profile to separate groups. Hence, a variable that is not significant for group separation in the univariate approach could be important in the multivariate sense (Vasta *et al.*, 2011). The canonical discriminant analysis separated the two dietary groups (Hotelling's test; P<0.01) and the canonical coefficients are reported in Table 2. In particular, the most discriminant FA were C18:1 12c, EPA, ALA. The high discriminative power of EPA and ALA in

Table 1. Effects of animal species, feeding system and their interaction on fatty acid composition of intra-muscular fat of *Longissimus dorsi* sampled at 6th-10th vertebrae in Sarda lambs, young Sarda bulls, and Sarda bulls fed pasture or stall-fed (Lsmeans ± standard error).

Fatty acids	Diet		Species		Diet	Species	Species × Diet
(% FAME)[1]	Pasture	Stall-feeding	Ovine	Bovine			
C18:1 15t/10c	0.532±0.139	0.357±0.119	0.303±0.163	0.586±0.101	0.32	0.14	ns[2]
C18:1 12c	0.196±0.016	0.391±0.014	0.349±0.018	0.238±0.011	<0.001	<0.001	<0.001
C18:3 (ALA)	2.416±0.213	1.513±0.182	2.355±0.249	1.573±0.155	0.001	0.01	ns
CLA 9t 11t	0.083±0.008	0.071±0.007	0.095±0.009	0.059±0.006	0.29	0.003	ns
C20:5 (EPA)	0.374±0.049	0.167±0.042	0.381±0.058	0.160±0.036	0.001	0.002	ns

[1] EPA = eicosapentaenoic acid; ALA = linolenic acid.

[2] ns = not significant; P>0.05.

Table 2. Canonical coefficients of the original variables.

Fatty acid[1]	Canonical coefficient
C18115t/10c	0.875121146
C18112c	-1.516.953.546
C18:3 (ALA)	1.300.193.171
CLA9t11t	0.550870018
C205 (EPA)	1.012.887.142

[1] EPA = eicosapentaenoic acid; ALA = linolenic acid.

differentiating the feeding systems was expected (Blanco *et al.,* 2010; Scollan and Huws, 2005; Scollan *et al.* 2006) and the ANOVA analysis seems to confirm this (Table 1) showing greater ALA and EPA in pasture-fed animals. The discriminant analysis classified meat samples, in a bootstrap resampling procedure, with only around 1% misclassification rate.

Conclusions

The results showed the reliability of using multivariate analysis of meat FA profile to track the feeding system of source animals with good accuracy, even if important effects, such as animal species, breed, sex, age at slaughter were overlooked. Nevertheless, further studies are needed in order to reinforce this conclusion.

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Coagulation properties and composition of milk from cows fed grass with different amounts of concentrates

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Abstract

Grasslands represent an important resource for milk production in many alpine European regions. Therefore, the aim of this study was to evaluate (1) the impact on milk composition and cheese-making properties of feeding regimes based on high but different dietary proportions of fresh grass during the vegetation period, and (2) to quantify changes due to seasonal variation. Three feeding regimes were tested: (C-0) full-time grazing plus mineral supplementation only, (C-low) partial grazing, fresh grass fed indoors with low concentrate level across the lactation, and (C-high) partial grazing, fresh grass fed indoors with a higher level of concentrate across the lactation. Milk from four, three and four cows (regimes C-0, C-low and C-high, respectively) was sampled during four periods in 2015 and analysed with a Lattodinamografo for various traits describing cheese-making properties. Gross constituent composition and cell counts in the milk were measured via mid-infrared spectroscopy. Milk composition and cheese-making properties were not affected by the feeding regime. However, there were significant interactions of sampling month and regime in the measured traits.

Keywords: grazing, fresh grass fed indoors, concentrate level, milk composition, cheese making properties

Introduction

A large share of the milk produced in many alpine European regions is transformed into cheese. In Switzerland 41% of marketed milk is sold in the form of cheese (BLW, 2016). As cheese is a highly valued product it is important to ensure its quality and milk-use efficiency (solids recovery) by regimes enhancing the contents of gross constituents and the coagulation properties of the milk. Cheese-making properties are influenced by several livestock-related factors including stage of lactation, nutrition, animal health, breed and genotype (Kreuzer *et al.*, 1996). As grassland represents the main feed source in alpine regions, it was the aim of the present study to evaluate (1) the impact of three different fresh-grass based regimes on milk composition and cheese-making properties, and (2) to quantify changes due to seasonal variation.

Materials and methods

The experiment was performed at the experimental farm of the Vocational Education and Training Centre for Nature and Nutrition in Hohenrain (Lucerne, Switzerland). In total 11 selected cows were monitored which originated from three separate herds consisting of 21 to 27 members each. Each herd was subjected to a different feeding regime: full-time grazing plus mineral supplementation only (C-0); partial grazing, fresh grass fed indoors with low concentrate level (C-low); and partial grazing, fresh grass fed indoors with a high concentrate level (C-high). Concentrate intake and milk yield are summarized in Table 1. At the beginning of the experiment the cows were 64±23 d, 64±9 and 37±8 days in milk in groups C-0, C-low and C-high, respectively.

Milk was sampled on three days during four successive periods in April, July, October and November (indoor period) in 2015. Milk samples obtained in the evening were immediately cooled and mixed with the milk from the morning for each cow proportional to the respective milk amounts produced. A

Table 1. Concentrate intake, days in milk and milk yield of the cows subjected to the three feeding regimes.[1]

	Regime[2]	April		July		October		November (indoor period)	
		Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD
Concentrate (kg DM/day/cow)	C-high	3.5	0.5	2.8	0.7	1.0	0.7	0.0	0.0
	C-low	3.0	1.4	0.0	0.0	0.0	0.0	0.0	0.0
	C-0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ECM (kg/day)	C-high	27	1.9	21	2.4	20	3.3	20	6.2
	C-low	27	5.8	19	3.2	19	3.5	16	3.2
	C-0	22	3.3	16	2.9	17	3.4	13	2.7

[1] ECM = energy corrected milk; SD = standard deviation.

[2] C-high (n=4); C-low (n=3); C-0 (n=4).

subsample of the milk was stabilised with Bronopol for mid-infrared analysis (Foss Milkoscan FT 6000) to determine contents of fat, protein, casein and lactose. Non-stabilised samples were transferred to the lab and analysed for milk coagulation properties within 24 h after sampling using a Lattodinamografo (Foss, Italy). Rennet coagulation time (RCT), curd firmness (A_{30}, amplitude measured after 30 min) and coagulation rate (k_{20}, time measured from starting point of coagulation until the amplitude measured reached 20 mm) were analysed. To do so, 10 ml of milk were warmed to 35 °C during 30 min, before adding 200 µl of diluted (1:50) rennet (Clerici Standard liquid, 1:19,000). Data were analysed using the MIXED procedure of SAS (version 9.1 Inst. Inc., Cary, NC, USA). The statistical model included sampling month, feeding regime and their interaction as fixed factors. Sampling month was considered as repeated factor, with cows as a random factor nested within feeding regime. Differences with probability levels of $P<0.01$ were considered significant.

Results and discussion

Milk constituents (fat) were not affected by the different feeding regimes but showed a typical increase with progressing lactation and sampling month over the seasons. Interactions were significant, thus regimes responded differently to sampling month. In July, contents of fat, protein, casein and lactose were lowest. Cell counts showed an increase with progressing sampling month over the year for regime C-0 and C-low, whereas in regime C-high a maximum was reached in July. Cell counts remained stable in April, October and during the indoor period in November (data not shown). Milk coagulation properties were not significantly affected by the different feeding regimes (Table 2), but by sampling month and regime×sampling month interaction. This is in accordance with other findings, where sampling month and stage of lactation had a strong impact on the coagulation properties (Auldist *et al.*, 1998; Jõudu *et al.*, 2008). Feeding regimes were affected differently during the sampling months. Under feeding regime C-high and C-low the RCT was lowest in April and reached the maximum in July. RCT was constantly shorter in C-0 during all sampling months. Coagulation rate was highest in July, where it took 3.5 min (average over all systems) to get the amplitude of 20 mm Curd firmness measured as A_{30} was lowest in July, whereas it recovered in October and November especially under regime C-low and C-0.

Conclusions

The findings demonstrate that, under our conditions, milk coagulation properties were not affected by the regimes investigated. However, sampling month had a major effect on milk composition and milk coagulation properties, affecting the properties differently in the three regimes. Milk coagulation properties and major milk components were unfavourable in July. This was most pronounced in regime C-high. Further evaluation of the data is needed to determine the cause for the decreased content of the major milk constituents during summer period.

Table 2. Milk coagulation properties during the four sampling months as measured in cows subjected to the three feeding regimes.[1,2]

	Regime	Month				SE	P-values		
		April	July	October	November (indoor period)		Regime	Month	Regime×month
RCT (s)	C-high	869 [def]	1,371 [ab]	1,145 [ce]	1,205 [abc]				
	C-low	805 [bef]	1,146 [acd]	1,080 [abcd]	1,004 [abcd]				
	C-0	792 [cde]	956 [abcd]	976 [acd]	954 [abcd]	121	ns	<0.001	0.002
Coagulation rate (k_{20}, s)	C-high	85.0 [c]	188 [ab]	142 [bc]	132[bc]				
	C-low	83.9 [bc]	294 [a]	92.1 [bc]	115[bc]				
	C-0	89.4 [bc]	147 [abc]	85.6 [bc]	96.1 [bc]	29.2	ns	<0.001	<0.001
Curd firmness (A_{30}, mm)	C-high	56.5 [ace]	26.5 [dg]	42.4 [bf]	40.8 [bf]				
	C-low	57.3 [abde]	29.5 [cfg]	44.9 [abcde]	49.9 [abde]				
	C-0	57.7 [abcd]	42.3 [efg]	58.0 [abcd]	50.3 [abcde]	6.96	ns	<0.001	0.011

[1] RCT = rennet coagulation time; SE = standard error.

[2] Within the same measured trait, least square means without a common superscript differ (P<0.01); ns = not significant (P>0.05).

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Feeding value of Portuguese Mediterranean annual-type rainfed pastures

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Abstract

Subterranean clover pastures (SC) have a higher nutritive value than Portuguese natural pastures (NP). However, this assumption does not include anti-quality factors and intake (determined by stocking rates – SR). Therefore, we tested the hypothesis that advantages of SC over NP are different if we consider feeding value (FV = nutritive value × intake) instead of nutritive value (NV) alone. Productivity of SC and NP was compared in the Castelo Branco region, Portugal. NV was determined by dry matter (DM), metabolisable energy, crude protein (CP) and acid detergent fibre (ADF) concentrations. Nutrient requirements of a merino ewe (50 kg live weight, 0.53 kg milk day^{-1}) were considered and a daily intake of 1.48 kg DM with 21% ADF. Apparently, NV limits milk production only at the end of spring, due to CP%. However, considering FV and the limitations of intake, autumn and winter periods presented stronger limitations to sheep production on SC than NP. Decreasing SR or a correct supplementation strategy can alleviate FV negative balances.

Keywords: biodiverse pastures, natural pastures, subterranean clover, grazing, nutrition

Introduction

Mediterranean pastures consist mainly of annual species which undergo strong variations in their nutritional value during the year (Molle *et al.*, 2008). In Portugal, since 1989, the surface area of Mediterranean pastures has increased from 828,691 ha to 1,738,185 ha (2009), mainly due to natural pastures (NP) – 77% of the total (INE, 2012). During the same period, the area of sown mixtures (SC), e.g. annual legume species (*Trifolium*, *Medicago*, *Ornithopus*), with *Lolium rigidum* Gaudin, *Dactylis glomerata* L. or *Phalaris aquatica* L., has remained almost constant (INE, 2012). Research reports (from Almeida, 1988 to Barradas *et al.*, 2006) highlighted the advantages of these mixtures on DM yield and on their nutritive value (NV), which would allow higher stocking rates (SR) compared with NP. However, until now, no research has been presented concerning the feeding value (FV) of these pastures, i.e. the interaction between DM intake (allowed by pasture growth limited by SR) and NV. Therefore, considering that intake is determined by both factors – NV and pasture DM availability per animal (Avondo *et al.*, 2002) – we tested the hypothesis that 'feeding value' of SC relative to NP would be different from the nutritive value comparison *per se*, assuming a constant SR during the year. Lactating merino ewes (50 kg live weight) were used as reference.

Materials and methods

In a randomized complete block design the natural pasture (NP) was compared with a sown subterranean clover mixture (SC), on a lithosol under a Mediterranean climate at Castelo Branco, Portugal. Three replicates of 660 m^2 plots, were grazed by lactating ewes in five cycles for the vegetative phase of pastures (autumn – end spring); herbage samples were collected before and after grazing, for growth and nutritive value determination. Total dry matter (DM) and crude protein (CP) were determined according to AOAC (2000); acid detergent fibre (ADF) according to Van Soest *et al.* (1991); metabolisable energy (ME) by prediction equations (NRC, 2007 and Bath and Marble, 1989, cit. by Coppock, 1997).

During the pasture vegetative period (201 days) we considered the following daily nutritional requirements of a merino ewe (50 kg live weight, 0.53 kg milk day^{-1}): 1.48 kg DM intake, 11.81 MJ ME, 153 g CP and 311 g ADF (NRC, 2007). The SR for each pasture type was derived from 50% annual total DM production (Dikman, 1998) divided by annual ewe intake. These SR remained constant along year; for each growth period, FV was obtained through the difference between pasture allowance (daily kg DM × nutritive value kg^{-1} DM) and ewe requirements (SR × DM intake ewe^{-1} × nutrient). For the pasture dry herbage phase (201-365 days), sheep grazed herbage surplus from later phases (FV of this phase was not studied). Univariate anova (GLM) and Bonferroni tests for LSMeans were used for statistical analysis.

Results and discussion

Except for the winter period (73-116 days), pasture growth was significantly higher in SC, as compared with NP (Table 1). NV (Table 1) showed higher CP concentrations in SC after 117 days of growth; SC had statistically higher ME concentrations and lower ADF concentration but no significance on Pasture type × growth period. From the NV, as suggested by Molle *et al.* (2008), we expected unbalanced CP:ME ratios on both pastures: for the first 150 days above requirements (12.96 g CP MJ ME^{-1}) and below them, after day 166, in NP, thus limiting milk production, reinforced by the highest ADF values.

Differences in total pasture DM production resulted in SR of 3.55 (NP) and 6.74 ewes ha^{-1} (SC). Considering these SR, pasture growth for the first 116 days was not enough to ensure daily DM intake per ewe (Table 2); this effect seems to be enhanced by the very low fibre content, revealed through an apparent unbalance on ADF requirements. For pastures with CP higher than 16% DM, Avondo *et al.* (2002) observed an intake limitation if biomass falls below 1 t ha^{-1}; that was the case in our results (447 and 547 kg DM ha^{-1} in NP and SC, respectively for the initial 116 days). After day 117, CP was higher in SC, and ME was higher in NP for 151-165 but lower for 166-201 days and ADF higher in NP for 166-201 days of growth. However, in these growth periods, NP feeding values were still enough to ensure ewe nutritional requirements, at the studied SR level (3.55), therefore not limiting animal production. Negative FV highlighted from Table 2, can be alleviated by decreasing SR or by roughage and concentrates supplementation.

Table 1. Natural pasture (NP) and subterranean clover pasture (SC) growth (kg DM ha^{-1} day^{-1}) at different periods after germination (0 = average germination date) and nutritive values.[1,2]

Period (days)	Pasture growth		CP (g kg^{-1} DM)		ME (MJ kg^{-1} DM)		ADF (g kg^{-1} DM)	
	NP	SC	NP	SC	NP	SC	NP	SC
0-72	4.1	5.4	223	179	9.6	9.5	248	255
73-116	3.5	3.7	221	223	9.6	10.1	248	200
117-150	11.0	23.0	155	232	9.2	9.8	287	235
151-165	42.0	59.1	100.0	186	9.0	9.5	305	255
166-201	11.8	38.1	68	78	7.9	8.0	399	392
SE	3.0		11		0.2		14	
Past	P<0.001		P<0.01		P<0.01		P<0.01	
Past × per	P<0.01		P<0.001		ns		ns	

[1] CP = crude protein; DM = dry matter; ME = metabolisable energy; ADF = acid detergent fibre; SE = standard error.

[2] Past = statistical significance for pasture type; Past × per = statistical significance for pasture type × growth period effects. ns = not significant.

Table 2. Natural pasture (NP) and Subterranean clover pasture (SC) daily feeding values per ewe (i.e. differences between requirements and pastures allowances at stocking rates of 3.55 and 6.74 ewe ha^{-1}, respectively).[1,2]

Period (days)	Pasture intake (kg ewe^{-1})		CP (g ewe^{-1})		ME (MJ ewe^{-1})		ADF (g ewe^{-1})	
	NP	SC	NP	SC	NP	SC	NP	SC
0-72	-0.33	-0.68	103	-32	-0.8	-4.2	-26	-107
73-116	-0.50	-0.94	64	-5	-2.3	-6.3	-73	-202
117-150	1.62	1.93	314	634	16.5	21.5	588	490
151-165	10.35	7.29	1,006	1,465	94.0	71.7	3,309	1,917
166-201	1.84	4.17	71	301	14.4	33.2	1,011	1,902
SE	0.57		71		5.2		195	
Past	ns		$P<0.01$		ns		ns	
Past × per	$P<0.01$		$P<0.01$		$P<0.05$		$P<0.001$	

[1] CP = crude protein; DM = dry matter; ME metabolisable energy; ADF = acid detergent fibre; SE = standard error.

[2] Past = statistical significance for pasture type; Past × per = statistical significance for pasture type × growth period effects. ns = not significant.

Conclusions

Nutritive value alone seems to be a weak indicator of Mediterranean pasture production potential. In our study, the lower nutritive value of NP as compared with SC contrasts with the feeding values, at the different SR levels studied. Therefore, pasture herbage intake (determined by herbage growth and SR) should be considered together with nutritive values, to evaluate the feeding value of Mediterranean pastures production more accurately.

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Response of pasture-fed lambs to supplementary feeding of forage legumes or concentrated feed

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Abstract

A two-year grazing study compared the effect of supplementary grazing of alfalfa, birdsfoot trefoil (BFT) or feeding concentrated feed on the performance of pasture-fed suckling lambs. Continuously stocked Anatolian merino lambs with their dams on pastures rotationally grazed the adjacent alfalfa or BFT plots with a creep feeding system. The creep feeding of the lambs with concentrated feed was also realized in the adjacent bare plots. The sheep that grazed only pastures without any supplementary feeding served as the control group. In both years, supplementary feeding commenced in June following a 42-day spring grazing period and continued for a period of 42 days until mid-summer. Lambs that had access to creep supplementation grew faster than lambs grazing pasture alone. On average, the extra liveweight that was gained through creep supplementation was 78, 84 and 95 g lamb^{-1} day^{-1} for concentrate, BFT and alfalfa, respectively.

Keywords: pasture, lamb fattening, creep feeding, alfalfa, birdsfoot trefoil

Introduction

Grazing systems that employ creep feeding can be an efficient management tool for maintaining high pre-weaning lamb growth rates (Moss *et al.*, 2009). Concentrate supplementation to grazing animals, even at small quantities, has been reported to increase the feeding efficiency of pastures through improved fibre degradation (Doyle *et al.*, 2006). Alternatively, complementary feeding with forage legumes may fill the void of summer feed gap with more affordable costs of feeding than concentrates. It was hypothesized that the concentrate may provide reliable support for lamb growth but forage legumes may lead to more efficient and cost-effective production than the concentrate.

Materials and methods

The research was conducted at Bahri Dagdas International Agricultural Research Institute in Konya, Turkey. The experiment was a randomized complete block design with three replicates. The treatments were: (1) pasture grazing + creep grazing of alfalfa; (2) pasture grazing + creep grazing of BFT; (3) pasture grazing + creep feeding concentrate and (4) pasture grazing – no creep feeding (Control). A group of 60 Anatolian Merino ewes (44.7±6.0 kg in 2013 and 51.8±4.2 kg in 2014) with their suckling single lambs (7.2±1.2 kg in 2013 and 11.3±1.3 kg in 2014) were stratified into 12 groups (4 groups in each block) of 5 ewe-lamb pairs based on body weight and lamb sex. The groups of ewe-lamb pairs were randomly assigned to one of twelve 0.18-ha pastures where they continuously grazed over a period of 84 days at the stocking rate of 27.7 ewes + lambs ha^{-1}. From day-42 onward, each of the two groups of five lambs in each block had access to either an additional area of 0.045 ha pure alfalfa or BFT stands. Herbage allowance, targeted to be at least 1.0 kg dry matter (DM) d^{-1} per lamb, was calculated by dividing pre-grazing pasture mass by the number of animals per unit area and the number of grazing days. Another group of lambs in each block were creep-fed pelleted concentrate *ad libitum* (170 g/kg crude protein (CP) and 11.3 metabolisable energy (ME) MJ/kg DM) through a grain feeder placed in the adjacent 0.015 ha uncultivated plots while the remaining one group of lambs which served as the control did not receive supplementary feeding. Grazing experiments ran for 84 days in both years from 18 April to 11 July in 2013 and from 23 April to16 July in 2014. Liveweight gain (LWG) was determined by weighing the lambs prior to and following each grazing period. All lambs were weighed after 15-h withdrawal from feed and water. LWG per head was calculated from the change in weight between each liveweight measurement date. Apparent DM intake (DMI) (herbage disappearance) in creep-fed pure legume pastures was measured through exclosure cage cuts to a stubble height of 20 mm. The concentrate feed was provided once each morning and any unconsumed feed was collected from the feeders and weighed prior to the following morning feeding to determine feed consumption. Herbage mass on offer in the grazed areas was measured fortnightly using a calibrated rising plate meter in both years. The liveweight gain per head of lambs, DMI and herbage mass were analysed by one-way ANOVA with repeated measures for each measurement period.

Results

A creep feeding × period interaction (P<0.01) occurred for the lamb growth rates in 2013 (Table 1). Average daily liveweight gains (ADG) of the lambs decreased from 268 g per head d^{-1} at 0-42 d to 131 g per head d^{-1} at 63-84 d. Creep-fed lambs grew faster than control lambs in the second (42-63 d) and the third (63-84 d) periods. In 2014, a creep feeding × period interaction (P<0.01) was detected for the lamb growth rates. ADGs of the lambs were comparable during the non-creep feeding period (0-42 d) but the lambs that creep-grazed BFT had greater ADGs than those had access to alfalfa or the control lambs in the second (42-63 d) period. In contrast, lambs that creep grazed alfalfa grew 28 and 43 g per head d^{-1} faster than the lambs that were creep-grazed BFT and creep-fed concentrate, respectively in the last period of grazing.

In 2013, the average herbage mass on offer of the pastures decreased (P<0.01) from 1.6 to 0.84 t DM ha^{-1}. In 2014, the decline was less sharp than in 2013 with the herbage mass remaining above 1 t DM ha^{-1} in the final week of the grazing. In both years there was no differences (P>0.05) in herbage mass among the pasture treatments. The herbage mass in the creep-fed legume stands ranged from 1.87 to 2.9

Table 1. Liveweight gain per lamb (g head^{-1} d^{-1}) stocked on pasture with and without creep grazing of alfalfa (Alf) or birdsfoot trefoil (Bft) or concentrate (Cnc) feeding.

Year	Treatments	0-42 d	42-63 d	63-84 d	0-84 d
2013	Control	269	157b	51c	159
	Cnc creep	266	209a	149b	208
	Bft creep	260	214a	147b	207
	Alf creep	277	226a	177a	227
	P_{CF}[1]	0.001			
	P_{P}	0.001			
	$P_{CF \times P}$	0.001			
	$SE_{CF \times P}$	10.3			
2014	Control	237	198c	74c	170
	Cnc creep	254	270ab	164b	229
	Bft creep	249	276a	179b	235
	Alf creep	238	248b	207a	231
	P_{CF}[1]	0.001			
	P_{P}	0.001			
	$P_{CF \times P}$	0.001			
	$SE_{CF \times P}$	12.2			

[1] P-values are from ANOVA for effects of creep feeding (CF), period (P) and interaction (CF × P). SE = standard error.

t DM ha^{-1}. On average, alfalfa stands had 0.5 and 0.47 t DM ha^{-1} more herbage mass than BFT stands in 2013 and 2014 respectively ($P<0.05$). In both years, there were interactions ($P<0.05$) between creep feeding and grazing periods for the DMI of the lambs. DMI of lambs was greater at 63-84 d (635; 642 g per head d^{-1}) than at 42-63 d (226; 312 g per head d^{-1}). In 2013, the DMI of lambs was similar for all creep-supplemented lambs at 42-63 d, whereas lambs that creep-fed BFT and alfalfa consumed more than those were creep-fed concentrate at 63-84 d ($P<0.05$). In 2014, lambs that were creep-fed legumes had similar DMI but their intake was greater than those consumed concentrate at 42-63 d. The lambs consumed alfalfa had greater DMI than those consumed BFT and concentrate at 63-84 d ($P<0.01$).

The consistently poor ADG of control lambs in summer compared with the first 42 days of grazing in spring emphasized the need for supplementation. The lambs that had access to a form of creep supplementation had 54-94% greater ADG than control lambs from the onset of supplementation period to weaning (42-84 d) and, consequently, they had 4-6 kg greater final LW than control lambs in both years. On average across the years, the cumulative weight gain of lambs was 13.8 kg $lamb^{-1}$ for control, compared with 18.4, 18.6 and 19.2 kg $lamb^{-1}$ for concentrate, BFT and alfalfa, respectively. In line with this finding, Moss *et al.* (2009) reported that creep-grazed twin lambs were 1.5 and 3.4 kg heavier than non-creep lambs at weaning.

The findings of the current study indicated that alfalfa with superior feeding value can be used successfully in a creep grazing system to finish the pastured lambs. BFT was a viable alternative to alfalfa as a summer forage and it has bloat-free characteristics. In the present study, lambs consumed considerably less concentrate feed than either of the forage legumes. The lower DM intake of these lambs compared with those that creep-grazed forage legumes was probably due to the slow process of lambs becoming accustomed to a novel feed offered in the separate area which their mothers could not access (Thorhallsdottir *et al.*, 1990).

Acknowledgements

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Feed-base strategies that reduce risk of feed-gaps in livestock systems across Australia's mixed farming zone

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Abstract

Australia's highly variable climate means there is large variability in the supply of forage for livestock. Mixed farmers utilise a range of feed sources on their farms to help mitigate the risks associated with this climate variability and to maximise their livestock production. Using simulation models of diverse forage options, monthly whole-farm energy balance was calculated from the supply of energy from forages and the energy demand from a typical livestock enterprise in 6 locations across Australia's mixed farming zone. This analysis showed that diversifying the feed-base to include combinations of forage sources provides the capacity to increase stocking rate significantly at the same time as reducing or maintaining the risk of feedgaps occurring on mixed farms. This demonstrates that there is significant potential to build forage-based feed systems that overcome critical feed gap periods and thereby mitigate the risks of increasing farm stocking rates required to improve the total productivity from livestock systems.

Keywords: simulation, whole-farm, energy, balance, demand, model

Introduction

Year-to-year variability and seasonality in forage supply cause a mismatch between forage supply and animal demand in many livestock production systems in Australia (Moore *et al.,* 2009). This induces inefficiencies in production due to excess feed that is wasted or to unmet animal demand. Producers are often compelled to adopt more conservative stocking rates to ensure the risk of feed deficits or the associated costs (e.g. supplementary feeding) remain low. Hence, strategies and tactics that can be employed by farmers to provide feed at times when forage quantity and quality are low and fill these feed gaps can offer significant opportunities to increase livestock productivity by enabling better utilisation of pastures and reduce risk and costs of production. Approximately 40% of Australia's livestock equivalents occur in the mixed farming zone where a diversity of forage sources can contribute to the farm feed-base (Bell *et al.,* 2014). This analysis explores the potential to design feed systems that utilise a mix of these forages to overcome periods of feed deficits across a range of production environments.

Materials and methods

Using a range of pasture and forage models (APSIM, Holzworth *et al.,* 2015; GRASP, McKeon *et al.,* 2000; and GRAZPLAN, Moore *et al.,* 1997), long-term simulations predicted monthly forage supply from key forage sources on mixed farms across six locations representing the key agro-climatic zones spanning Australia's mixed farming regions. The monthly whole-farm energy balance was calculated from the supply of energy provided from these forages and the energy demand from typical livestock enterprises in each district. This was used to predict 3 complementary statistics of feed-gaps or periods of feed supply shortages; the frequency of months when feed supply was less than feed demand, the frequency of periods when the farm feed balance was negative, and the frequency of months when the stock of available forage on a farm fell below a minimum threshold of 500 kg DM ha^{-1}. Using this approach we examined the risk of feed gaps occurring under a range of combinations of livestock production intensity and mixtures of forages contributing to the farm feed-base.

Results and discussion

This analysis showed that incorporating a diversity of forages into a farm feed system can greatly reduce the frequency of feed gaps on mixed farms in a range of agro-climatic regions differing in timing of feed gaps. At all locations examined there were benefits for potential farm productivity and risk mitigation by increasing the diversity of forage sources available on mixed farms (Figure 1). Systems relying on only one feed source were prone to higher risk or feed gaps and hence would often have to reduce stocking rates to mitigate these risks. Higher stocking rates could be maintained while limiting risk when combinations of other feed sources were introduced into the feed-base. In all cases we found diminishing returns from making the feed-base more complex, with combinations of 2-3 components typically achieving the maximum benefits in terms of reducing the risk of feed gaps.

Even in stubble-retention farming systems in southern Australia, stubbles were found to be a particularly important forage resource for managing periods of low feed supply from other source during summer

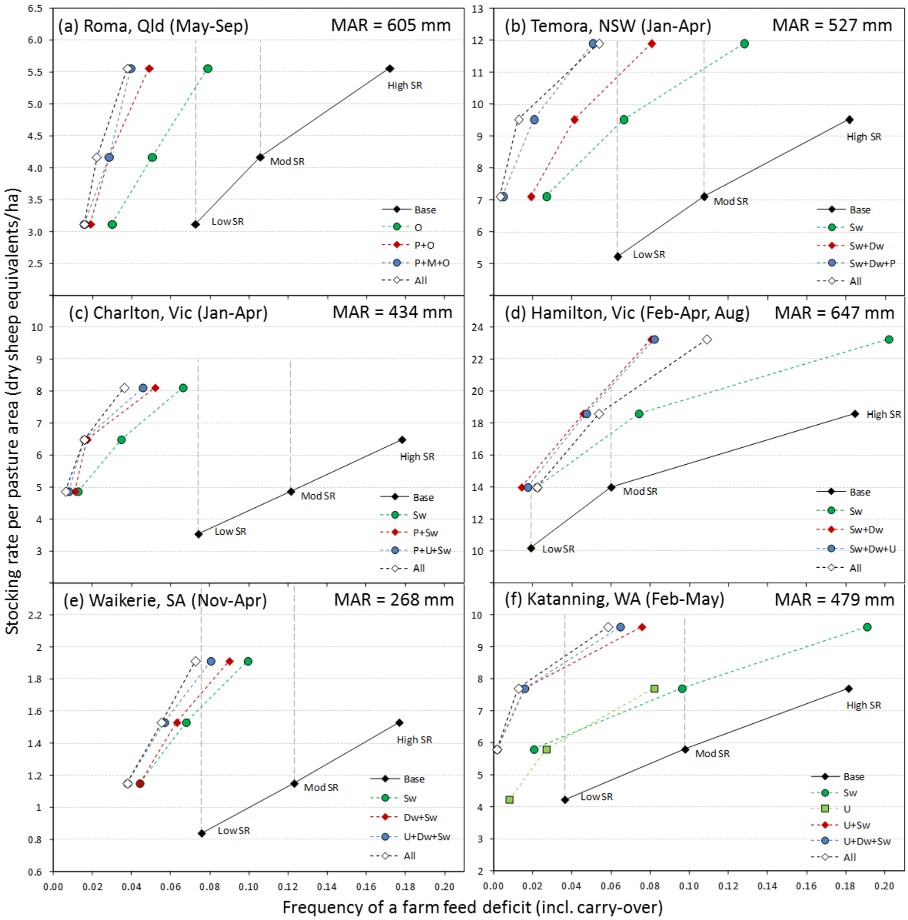

Figure 1. Relationship between frequency of a whole-farm feed deficit and farm stocking rate per pasture hectare (not including area of crop grazed) under the best combination of feed sources for each level of feed-base complexity across 6 locations spanning Australia's mixed farming zone. Mean annual rainfall (MAR) and periods of frequent feed gaps (in brackets) are indicated. Different combinations of the farm feedbase include: Base – an annual grass-legume pasture at all locations, except Roma (a buffel grass pasture) and Hamilton (ryegrass-white clover mix); Sw – wheat stubbles; U – Lucerne; Dw – dual-purpose wheat; O – oats, M – medic pasture; P – improved summer-active perennial grass (panic grass at Roma and phalaris/fescue at other locations).

and autumn (Figure 1B-F). Making stubbles available was highly valuable for avoiding whole-farm feed deficits where supplementary feeding or destocking would be necessary and reducing grazing pressure and the occurrence of low pasture availability on other parts of the farm. Dual-purpose crops and perennial pastures such as lucerne and temperate grasses which provided autumn and winter forage were also effective at providing more continuous supply of fresh forage and complimented stubbles to further reduce risk in mixed farm feed systems. In contrast to the southern locations, in the subtropical location (Roma) feed gaps were more common in winter and spring and hence the addition of summer feed sources had little benefit (Figure 1A). On the other hand, oats providing high quality winter forage was found to be highly valuable.

Conclusions

Diversifying the feedbase to include combinations of improved pastures, crop residues and forage crop grazing demonstrates the capacity to increase stocking rate significantly at the same time as reducing or maintaining the risk of feed gaps occurring on mixed farms. While higher stocking rates increase the risk of a feed deficit, diversified feed-bases were still able to maintain these higher stocking rates at the same time as lowering the frequency of farm feed deficits than would be achieved under low stocking rates (i.e. 45% of the high rate) on a feed-base consisting of a pasture-only. This demonstrates that there is significant potential to build forage-based feed systems that overcome critical feed gap periods and thereby mitigate the risks of increasing farm stocking rates required to improve the total productivity from livestock systems.

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Meta-analysis of historical pasture legume evaluations in northern Australia

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Abstract

There has been a large and long effort in the past to evaluate and develop pasture legumes suited to a range of environments and production systems in northern Australia. Over 180,000 records of evaluation data of pasture legumes from 567 sites in the tropics and subtropics of Australia were collated and analysed to see if this revealed genera, species and/or accessions that might offer further candidates for further evaluation and/or potential commercialisation. Initial interrogation of this data with high power statistical approaches, across a range of past evaluation locations and conditions, has revealed several tropical legume species that have higher productivity potential than commercially successful species. In particular, several *Desmanthus* species showed high levels of persistence and higher year-3 productivity than other species across a range of environments, indicating they many have wider potential for development. Some *Macroptilium* species demonstrated wide potential, with *Macroptilium lathyroides* in particular showing higher productivity levels in both year 1 and year 3 and performed relatively better than other species at locations with lower site yields. Further examination of variation within species or comparisons amongst individual accessions may reveal further information on genotype performance across the full set of evaluation experiments.

Keywords: statistics, forage, G × E, database, tropical

Introduction

The addition of legumes to tropical pastures has the potential to have large benefits for the productivity and profitability of beef production enterprises in northern Australia, with potential to increase farm profitability by >85% (Ash *et al.,* 2015). This potential has long been recognised and a large effort has been made in the past to develop forage legumes suited to a range of environments and production systems. Systematic evaluation and development of improved legumes has stalled over the past 10-20 years. However, there is renewed interest from beef producers and the pasture seed industry in reinvigorating efforts to improve the range of legumes available. A great deal could still be learnt from re-examining these past efforts in order to identify further opportunities and guide current and future directions and priorities for legume development. This study collated tropical legume evaluation data and analysed genotype and environmental effects to identify priority genera and species that may warrant further future evaluation.

Materials and methods

First we compiled a prioritised list of published and unpublished data arising from past and current legume evaluation work in northern Australia. Over 100 data sources were identified with potential information that might be included in the database. The data collated primarily focused on measures of plant productivity and persistence in field evaluation activities, but some data on other significant traits such as seed production, frost and disease tolerance and palatability were also included. Upon completion, the database contained over 180,000 measurements across 950 putative species, 7,061 accessions, at 567 sites (site may include the same location but different soil/projects).

A meta-analysis using high power statistical methods was undertaken to explore genotype by environmental (G × E) interactions to explore relative performance of legume species using the data collated in the database. Not all accessions were grown at all sites so the analysis is moderately unbalanced, and so a subset of the sites with >10 accessions and accessions occurring at >5 sites are included. The analysis was performed using the R packages ASReml and MYF (Butler *et al.,* 2009) and followed those used by Smith (2015) for cases in which there were sufficient genotypes to perform these analyses. The results were presented using the Finlay-Wilkinson (FW) approach to illustrate genetic by environmental effects (Finlay and Wilkinson, 1963).

Results and discussion

Statistical analysis across the 12 locations and years revealed several tropical legume species that have higher productivity potential than commercially successful species (Figure 1). The analysis demonstrates that while several *Desmanthus* species had lower production in year 1 they performed well in year 3 across a range of environments. This genus may offer new options across a range of environments. A notable species here was *Mactoptilium lathyroides* which had a high mean yield in year 1 and 3 but also performed well in less favourable environments. *Chamaecrista (Cassia) rotundifolia* also performed better in less favourable environments, but had a lower mean yield than *M. lathyroides*. Several *Rhynchosia* and *Vigna* species (e.g. *V. oblongifolia, V. decipiens, V. hosei*) showed high production in year 1 but were highly location specific and they were not persistent. Species with the highest year 1 production across multiple sites were *Macroptilium lathyroides, M. martii,* and *Centrosema pascuorum.* Several *Alysicarpus* species, *Desmanthus pubescens*, *D. subulatus* and *Stylosanthes sympodialis* showed higher year 3 production in more favourable environments.

Conclusions

There is great potential to further build on pasture legume evaluation work conducted in northern Australia. The database of historical legume evaluation developed here enabled the first cross-site genotype × environment analysis of pasture legume performance in northern Australia. This revealed some species (e.g. *Macroptilium lathyroides*) and genera (e.g. *Desmanthus*) which performed well across a range of environments and warrant further investigation. However, this analysis only focussed on analysis at the species level of biomass yield related traits, and further in-depth analysis of accession performance in species or genera of interest and for a wider range of traits of interest is likely to be valuable.

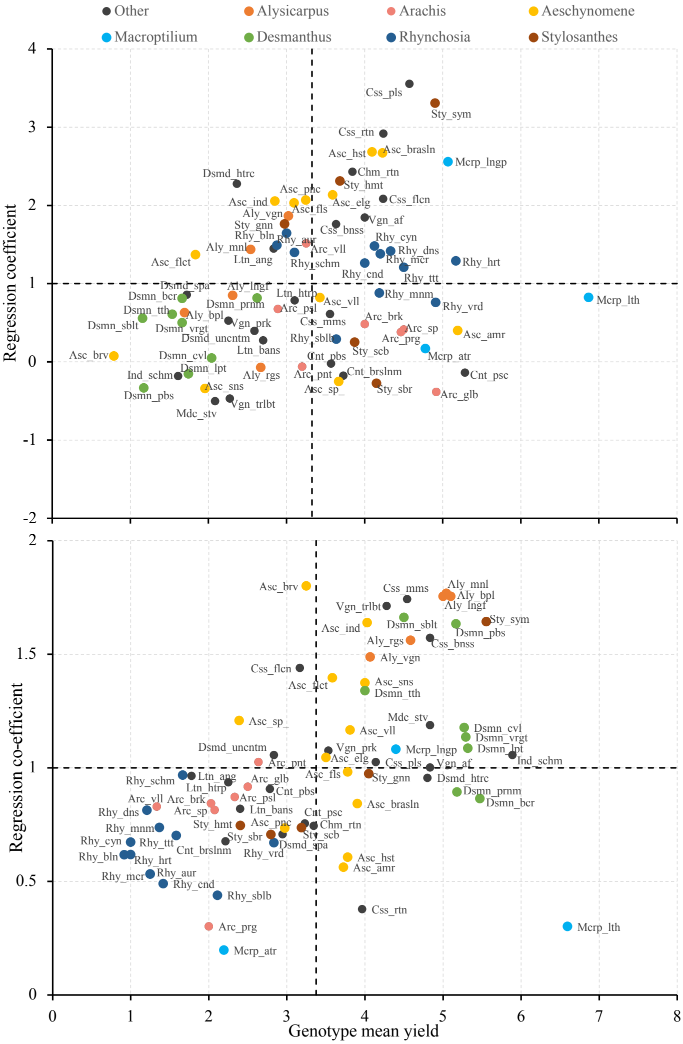

Figure 1. Finlay-Wilkinson plot for first (top) and third (bottom) year biomass production amongst species and environments. Across all sites, species noted to the left lower means performance and to the right higher performance (dotted line indicates the mean of all species); Species indicated to the top (above a regression coefficient of 1) perform better in more favourable conditions and to the bottom (below 1.0) in less favourable conditions. The genus species label has been abbreviated to the first 3 letters.

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Testing a novel custom-built low-cost platform for measuring crop height

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Abstract

Crop height can be used as a proxy for yield to provide fast, non-destructive estimates of farm-based forage supply. In this work we developed a novel low-cost ultrasonic sensor (US) based platform for measuring crop height on-the-go. The tool was tested on a well-developed broad-leaf row crop spaced 0.05 m in the row and with a 0.3 m inter-row. US data were able to reproduce canopy profile, as high correlation (r=0.7) between sensor and ground-truth series was found. The sensor slightly underestimated crop height but well discerned within row height broad variability.

Keywords: ultrasonic sensors, grasslands, cross-correlation, on-the-go measures

Introduction

Plant height is a very useful parameter in agronomy. It is related to crop yield (Yin *et al.*, 2011) and to forage quality and is an indirect method for assessing stand establishment homogeneity (Boomsma *et al.*, 2010) and can be used for estimating farm-based forage supply (Fricke and Wachendorf, 2013). Developing user-friendly, low-cost, ICT-based crop monitoring tools can help increase grassland profitability (Hopkins *et al.*, 2015) filling a gap in a sector traditionally characterized by a low level of technological innovation (Schellemberg *et al.*, 2008). In this work we tested a novel low-cost platform for measuring plant height based on a very cheap ultrasonic sensor and Arduino microcontroller board. The platform was tested in a Chia (*Salvia hispanica* L.) stand. Chia can be considered a novel forage, it is in fact receving increasing attention as a possible source of polyunsaturated fatty acids (PUFA) in ruminant nutrition (Peiretti and Gai, 2009).

Materials and methods

The platform was composed of an Arduino microcontroller (Arduino uno R3: €22.5), connected to an ultrasonic sensor (US) (SRF05 Devantech: €13.5) issuing a sound wave at a frequency of 40 kHz with a cone of 45° from the sensor. A custom-built program triggers the sensor and converts the time intercurring between US pulse and the reflection into a centimetric distance (Fisher and Sui, 2013). Data are transmitted via expansion shield (RS232 Shield: €10.11) to a roughed pc. For the on-the-go setting, the platform was equipped with an external RTK GPS with an accuracy of ±2 cm and mounted on a straddle-wheeled chassis manually towed along the row, pointing the sensor vertically at distance of 0.53 m above the ground (Figure 1A). Sensor data were calibrated on a well developed broad-leaf row-crop (*Salvia hispanica* L.) measuring crop eight at regular distance (5 cm) on a transect of 2.8 m along the row (total number of observations = 57). Data were were analysed as a bivariate time series (Figure 1B) and measures of similarity between the two series were computed. We calculated the cross-correlogram (Figure 1C) and the correlation distance based on Pearson's correlation between a pair of numeric time series of the same length (Warren Liao, 2005). Statistical analysis were performed within the 'R' environment for statistical computing (version 3.1.2; R Development Core Team, 2014).

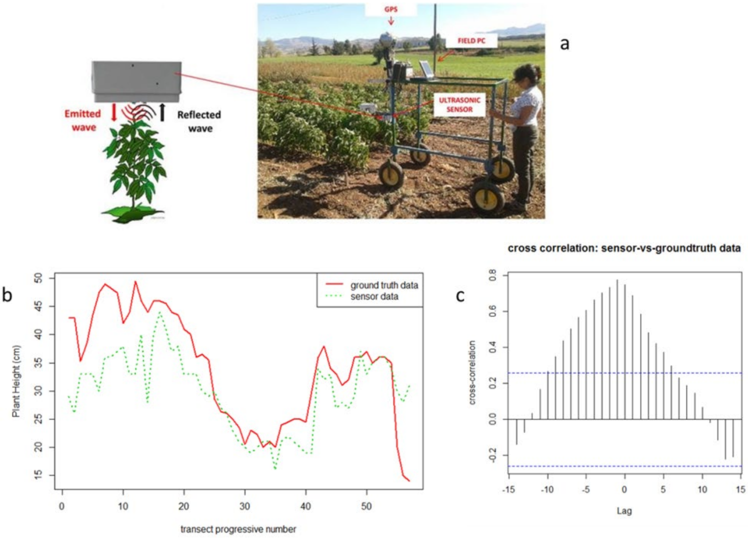

Figure 1. (A) Field setup: wheeled chassis equippd with the GPS and the sensor shield mounted in a protective plastic case. (B) Comparison of sensor-determined and measured height: ground truth data (green dotted line) overlaid by sensor data (red dotted line). (C) cross-correlogram plot.

Results

In our trial sensor data were able to reproduce the canopy profile with reasonable accuracy (Figure 1B). The correlation distance is 0.7 and the cross correlogram reaches 0.8 at lag 0 (Figure 1C).The accuracy seems to increase toward the centre of the transect, possibly due the interference of neighbouring features at the edges of the row. Plant height was underestimated by 4.4 cm on average but broad variation in crop height along the transect can be well discerned. Discrepancies between sensor and ground-truth data depend on microtopography as well as on the position of the sensor relative the the closest leaf that echoes back the signal (Sui and Thomasson, 2006).

Conclusions

In our trial ultrasonic data followed ground-truth data pattern, reconstructing canopy height variation in space. High sample cross correlation coefficients and minor shifts between the series were found. The platform needs to be tested and validated on multiple crops/planting densities; however, the US sensitivity and the speed of on-the-go measures coupled with low investment and labour costs make this equipment a promising tool for mapping crop height with broad variability at field scale.

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The long-term effect on cow's milk quality of methods for improving subalpine grasslands

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Abstract

In Romania, during the summer, the subalpine grasslands located above the upper limit of spruce forests, are an important source of fodder for ruminants feeding. Economic situations, such as decreased wool price in the last 25 years, have led to significantly increased numbers of dairy cows grazing these surfaces. In this paper we present the effects on productivity and quality of cow milk of improved methods, applied since 1995 on degraded grassland (*Nardetum strictae montanum* association). The research was carried out at the Mountain Grasslands Research Centre (MGRC) from the Bucegi Plateau on gently sloping land situated at the base of Blana Peak (1,875 m altitude). The data presented in this paper refer to results achieved in the period 2014-2016, 20 years after the establishment of the experimental field. Chemical and organic fertilizations, liming and reseeding have been applied as variants of improvement. Of the factors tested, liming leads to a significant increase of milk production by 25-29% and of milk protein content by 4-5%.

Keywords: subalpine meadows, improvement methods, quality of cow's milk

Introduction

Grasslands located above the upper limit of spruce forests are of particular importance in Romania. This is due to the large areas that are extended and the possibilities for grazing them for approximately 3 months per year, with large herds of animals, and with minimal costs (Bărbulescu and Motcă, 1983). Mountain grasslands used for many years by grazing are exposed to degradation processes of floristic composition if they are not well maintained, fertilized and used rationally, being dominated by the worthless species *Nardus stricta* (Marusca and Frame, 2003). This degraded grassland is totally inadequate for dairy cow grazing, such that after 1990 cows are present in large herds on subalpine pastures (Marusca *et al.*, 2002).

In 1995, at the Mountain Grasslands Research Centre (MGRC) Blana Bucegi, experiments were initiated in which the efficiency of improvement variants for *N. stricta* degraded pasture is estimated by assessments of cow milk production. In this paper, in addition to determining milk production, as an indicator of technological options for improvement, the dry matter (DM) production, floristic composition of grassland, chemical composition of fodder and milk and agrochemical indices of soil were assessed in order to know in detail the factors of relationship soil – plant – animal product.

Materials and methods

The research was conducted at the MGRC Blana from Bucegi Mountains on a *N. stricta* grassland (40% *Nardus* contribution in the sward), located in a subalpine zone, at 1,800 m altitude. The experimental emplacement contains complex technology variants realized during 1995-2016, with the following experimental plots: T – semi natural pasture, unimproved, grazed in summer with cows, control plot; A – semi natural pasture, fertilized with chemical fertilizers in periods 2000-2002, 2010-2012, 2014-2016 with an average 100 kg ha^{-1} N + 50 kg ha^{-1} P_2O_5 + 50 kg ha^{-1} K_2O; B – semi-natural pasture, chemically fertilized in 1996-1998 with 150 kg ha^{-1} N + 75 kg ha^{-1} P_2O_5 + 75 kg ha^{-1} K_2O and organically fertilized in 2004, 2010 and 2016 by paddocking with dairy cows; C – semi-natural pasture, calcium liming in 1995, chemically fertilized similar to the B variant and paddocked in 2003, 2009 and 2015; D – reseeded pasture in late summer of 1995, after herbicide Roundup at 5 l ha^{-1}, calcium liming and chemically fertilized similar to C variant and paddocked in 2002, 2008 and 2014.

All experimental variants were grazed in a continuous system with cows (Maramures Brown breed) at a stocking rate of 4 livestock units (LU) ha^{-1} in plots A, B, C and D, and 1 LU ha^{-1} in plot T. In order to determine DM production and sampling for quality analysis, three exclosure cages, each of 2 m^2, were placed on each plot. Feed sampling was assessed once per year in the first decade of August, during the flowering of the grass species. In each grazing season weekly measurements were made of milk production, and every two weeks, samples were taken to determine milk quality. Statistical interpretation of results was achieved by applying ANOVA, followed by least significant difference (LSD) mean test using CoStat software.

Results and discussion

As results of the application of technologies to improve pasture with *N. stricta* from MGRC Blana Bucegi, for a period of 20 years, changes in the agrochemical soil indices, productivity and quality of pastures and livestock production have been detected.

Agrochemical analysis of soil shows that acidity (pH) decreased by 0.2 in the variants A and B, unlimed, and by 0.4 in the variants C and D where calcium liming was applied (Table 1). Regarding botanical composition, after 20 years of rational grazing, the proportion of *N. stricta* in the sward decreased from 40 to 15% in control plot T, to 2-3% in variants A and B. In variants C and D, where calcium liming was applied, *N. stricta* was removed. Concerning the pastoral value, all the improved variants increased productivity and forage quality. Calcium liming and paddocking lead to the largest increases in the value of this indicator: +47-55% compared with the control plot variants. By applying improvement measures, DM yield, averaged for 2014-2016, increased from 2.19 t ha^{-1} in control plot T up to 4.16 t ha^{-1} for the D variant, this occurred in parallel to sward quality.

Consistent with increased DM yields and crude protein, the milk production per unit area, in relation to the intensity of measures for improvement of degraded *N. stricta* grasslands, also increased. The average milk production of 4,498-4,365 l ha^{-1} obtained in variants C and D are 29-25% higher than the unlimed variant B (3,500 l ha^{-1}) and 235-226% higher than the control variant T (1,341 l ha^{-1}) (Table 1).

Table 1. The influence on subalpine grasslands of improvement factors on soil pH, floristic composition, dry matter, crude protein and milk yield ha^{-1}.

Specification		Unit	Plot				
			T	A	B	C	D
pH in soil	1995	value	4.6				
	2016	value	4.6	4.8	4.8	5.2	5.2
Floristic composition (Mean of years 2014-2016)	Total grasses:	%	72	80	68	60	77
	of which *Nardus stricta*	%	15	2	3		
	Total legumes	%	12	5	17	14	11
	Other families	%	16	15	15	26	12
	Pastoral value	value	38	45	48	56	59
Average yield 2014-2016	Dry matter yield	t ha^{-1}	2.19	2.94	2.18	2.77	4.16
	Crude protein	kg ha^{-1}	241	360	248	334	413
	Milk yield	l ha^{-1}	1,341	4,286	3,500	4,498	4,365

In addition to production, improving the technological factors for nardetum grasslands has had a significant influence on milk quality. Thus, the main indicators of quality of milk were higher on the improved variants compared with the control treatment. The fat content of milk was higher by 13% in chemically fertilized variant A (37.4 g kg^{-1}), with 11% in chemically and organically fertilized variant B (3.67%), with 8% in variant C (3.56%) and 9% in variant D (3.60%) compared with the control plot T (3.30%) (Table 2). All these differences are statistically significant. Regarding milk protein content, for the calcium liming variants (C and D), we observed differences with statistically significance, respectively with 4% (var. C, 3.40%) and 5 % (var. D, 3.45%) higher compared with the control (T, 3.28%). In our opinion, this is the most important result of this research. Lactose content is highest in variant D (4.9%), the difference compared with the control T (4.80%) were also significant (Table 2).

Conclusions

Improvement solutions by fertilization, calcium liming, paddocking and reseeding applied on subalpine grassland of *N. stricta* species from the Romanian Carpathians, lead to adjustment of chemical deficiencies of the soils on which they are located, preserving the grassland biodiversity and also increasing their productivity. The effect of these measures can be detected after 20 years of application if these areas are included in a rational grazing system and repeating fertilization with moderate application rates of fertilizers.

Calcium liming and reseeding of degraded grasslands influence positively the conversion rate and quality of animal products, such as cow's milk cheese quality, even after 20 years of application.

Table 2. The influence of improvement factors on milk quality parameters (2014-2016).

Specification		Plot				
		T	A	B	C	D
Fat content	g/100 g	3.30	3.74	3.67	3.56	3.60
	Difference, significance	Control plot	0.44***	0.37***	0.26**	0.30**
	LSD 5% = 0.19; LSD 1% = 0.26; LSD 0.1% = 0.33					
Protein content	g/100 g	3.28	3.30	3.30	3.40	3.45
	Difference, significance	Control plot	0.02	0.02	0.12***	0.17***
	LSD 5% = 0.06;LSD 1% = 0.07; LSD 0.1% = 0.09					
Lactose content	g/100 g	4.80	4.79	4.80	4.78	4.90
	Difference, significance	Control plot	-0.01	0.00	-0.02	0.10*
	LSD5% = 0.09; LSD 1% = 0.11; LSD 0.1% = 0.15					

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Performance of lambs fed maize, soybean or maize-soybean intercrop silages

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Abstract

Our objective was to determine the performance of lambs fed diets based on silages from maize, soybean or maize-soybean intercropping. Twenty-four crossbred lambs (average initial BW = 22.7 kg) were housed in individual pens, blocked by sex and body weight (BW), and assigned to three dietary treatments: MS – diet containing 300 g kg^{-1} of maize silage produced in monoculture, SS – diet containing 320 g kg^{-1} DM of soybean silage produced in monoculture, and MSS – diet containing 310 g kg^{-1} DM of maize-soybean silage produced in intercropping. Diets were iso-nitrogenous (14% crude protein (CP)) and iso-forage-neutral detergent fibre (NDF) (16.5%). The DM intake (DMI), average daily gain (ADG) and feed efficiency (ADG/DMI) were 1,098, 968 and 1,057 g day^{-1}; 225, 171 and 213 g day^{-1}; and 0.204, 0.179 and 0.204 for MS, SS and MSS, respectively. Soybean silage produced in monoculture decreased the performance of finishing lambs. The use of silage from maize and soybean intercropping has the potential to be used without affecting intake and performance of the lambs.

Keywords: feedlot, intercropping, protein source

Introduction

In many farming systems, feeding costs are the key to the economic viability of production, especially in relation to the use of concentrates rich in protein, which are a large part of direct expenditure, especially in tropical areas. Therefore, improving farm-grown forage production and the use of forage with high protein content could be a strategy to increase profitability and sustainability of ruminant production systems, especially in tropical areas.

The inclusion of soybean in the form of forage in the diet is an alternative approach for increasing the protein content, and intercropping soybean with other crops such as maize, sorghum or grass for the production of silage may guarantee good silage conservation. Legume silages are considered difficult to ensile due to their chemical characteristics, such as low dry matter concentration and high buffering capacity at harvesting (McDonald *et al.*, 1991).

The use of intercropped crops for silage production is a worldwide reality but studies are still needed to evaluate the proportion of legumes in the diet with the aim of improving the performance of the animals and the success of the production system. This paper presents results on the performance of finishing lambs fed maize, soybean or maize-soybean intercrop silages.

Materials and methods

The experiment was conducted at the Experimental Farm of the Maringá State University, Paraná, Brazil. Maize and soybean were cultivated in the same area in October 2015 and harvested for silage in January 2016, resulting in three different silages: maize silage produced in monoculture, soybean silage produced in monoculture and maize and soybean silage produced in intercropping. After 90 d of storage in 200 litre drums, silages were used to compose three diets: MS – diet containing 300 g kg^{-1} of maize silage produced in monoculture, SS – diet containing 320 g kg^{-1} DM of soybean silage produced in monoculture, and MSS – diet containing 310 g kg^{-1} DM of maize-soybean silage produced in intercropping (Table 1). Diets were iso-nitrogenous (14% crude protein (CP)) and iso-forage-neutral detergent fibre (NDF) (16.5%).

Table 1. Experimental diets containing maize, soybean and maize-soybean intercrop.[1]

Ingredient (g kg^{-1} DM)	MS	SS	MSS
Maize silage	300	-	-
Soybean silage	-	325	-
Maize-soybean silage	-	-	310
Dry ground corn	580	643	575
Soybean meal	88	-	83
Mineral and vitamin mix	10	10	10
Limestone	15	15	15
Urea	6.8	6.8	6.8

[1] DM = dry matter; MS = maize silage in monoculture; SS = soybean silage in monoculture; MSS = maize-soybean silage in intercropping culture.

The proportion of soybean harvested in the mixture harvested from maize-soybean intercropping was approximately 15%, as estimated by agronomical measurements.

Twenty-four crossbred lambs (½ Dorper × ½ Santa Inês) with average initial body weight (BW) of 22.7 kg were blocked by sex and initial body weight, housed in individual pens and assigned to dietary treatments for 60 d (20 d of adaptation and 40 d for data collection). During the adaptation, all animals received the same diet with 650 g kg^{-1} of concentrates and 350 g kg^{-1} of forage, with equal proportions of maize and soybean silages.

Animals were fed twice daily, and orts were recovered every morning to determine the DM intake (DMI). The BW was recorded every other week and the average daily gain (ADG) determined by the slope of the linear regression describing the BW across the comparison period. Feed efficiency was computed as ADG divided by DMI.

Data were analysed using the PROC MIXED of SAS, including the fixed effects of treatment and sex. Initial BW was included as covariate for all variables, except for the initial BW. Means were compared by Tukey test (α=0.05).

Results and discussion

The use of soybean silage produced in monoculture (SS) decreased the DMI, ADG and feed efficiency in comparison with diets containing maize silage produced in monoculture (MS) or intercropped with soybean (MSS) (Table 2).

Table 2. Performance of lambs fed diets based on maize, soybean or maize-soybean intercrop silages.[1,2]

Item	MS	SS	MSS	SEM	*P*-value
DMI (g d^{-1})	1,098 a	968 b	1,057 a	39.3	0.05
Initial BW (kg)	23.1	22.4	22.6	1.33	0.93
Final BW (kg)	34.8 a	32.2 b	34.2 a	0.59	<0.01
ADG (g d^{-1})	225 a	171 b	213 a	9.03	<0.01
Feed efficiency	0.204 a	0.179 b	0.204 a	0.008	0.03

[1] DMI = dry matter intake; ADG = average daily gain; BW = bodyweight; MS = maize silage in monoculture; SS = soybean silage in monoculture; MSS = maize-soybean silage in intercropping culture; SEM = standard error of the mean.

[2] Means within a row with different superscripts differ (α=0.05).

The dietary treatments reduced the dry matter intake (DMI) by 110 g d^{-1}. DM intake is the parameter that inhibits the weight gain and the profitability of the system. Previous studies have also reported lower performance of ruminants fed SS in comparison with MS (Martínez-García *et al.*, 2015; Rigueira *et al.*, 2015). The poorer silage fermentation and lower fibre digestibility are plausible explanations for the modest performance of lambs fed SS diet.

The lower DM intake for the SS directly affected the weight of the animals at the end of the feedlot period, with 32.2 kg. MS and MSS had a similar final BW, 34.8 and 34.3 kg, respectively. At the end of the same feedlot period, heavier animals represent savings of food and improved profit for the production system.

The lowest ADG in diet with SS, only 171 g d^{-1} in relation of the others diets, also contributed to a reduction in the final BW. Feed efficiency for the SS diet of only 0.179 is an indicator of lower energy density in the diet and, because of this, the animals need to ingest more food to increase their weight gain.

Due to the reduction by 10% in DMI and 15% in feed efficiency, lambs fed SS had lower final BW than lambs fed MS and MSS diets. In the context of the productive systems, this lower ADG of SS would represent about 15 additional days of the animals remaining in the feedlot in order to reach the same final BW, with higher labour costs. Additionally, there is a market demand for a shorter feedlot period. Therefore, diets containing maize silage would improve the profit of the production system.

Conclusions

Soybean silage produced in monoculture promoted a lower performance of finishing lambs. Silage of maize intercropped with soybean has the potential to be used without affecting the intake and performance of the lambs.

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Fatty acids, carotenoids and fat-soluble vitamin contents in dairy cow milk from autumn grazing in Galicia (NW Spain)

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Abstract

Pasture growth in Galicia (NW Spain) follows a bi-modal curve, with a maximum in spring followed by a summer drought period, after which the pasture regrowth in autumn makes it possible to extend the cows' grazing season until the winter. The aim of this study was to evaluate the fatty acids (FA) and lipo-soluble antioxidants (AO) content in the milk of dairy cows during the autumn grazing period. A group of eighteen Holstein-Friesian dairy cows rotationally grazed from October to December a 9 ha grass-clover sward where perennial ryegrass was the dominant species. Grazing took place between morning and evening milkings and animals were supplemented indoors during the night with a unifeed mixture made up of maize silage, grass hay and a commercial concentrate. The experiment was divided into two periods, corresponding to the two rotations given to the grazing area. Milk FA and AO concentrations during the grazing period remained fairly constant with no significant difference observed for the main constituents between the two rotations. Average concentrations of polyunsaturated FA, *trans*-vaccenic FA and *c9t*11CLA in milk were, respectively, 4.30, 1.62 and 0.81 g 100 g^{-1} total FA, whilst the milk contents in all-*trans*-β-carotene, retinol and α-tocopherol were, respectively, 181, 363 and 992 μg l^{-1}.

Keywords: pasture, fatty acids profile, β-carotene, retinol, vitamins

Introduction

The fatty acid (FA) composition of cows' milk has become less favourable to human health in the last four decades due to changed feeding and management practices, notably by higher proportions of concentrates and silages in diets with less pasture grazing (Elgersma *et al.*, 2006). Milk contains FA considered essential, as omega-3 and omega-6 and particularly conjugated linoleic acid (CLA), specific to milk and meat from ruminants (Chilliard *et al.*, 2001). Intake of milk and dairy products also contributes to the increase of antioxidant microconstituents such as carotenoids (beta-carotene, lutein, zeaxanthin) in the human body (USDA, 2003) and is an important source of fat-soluble vitamins in the human diet, especially retinol (vitamin A) and tocopherol (vitamin E). Havemose *et al.* (2004) reported that milk from cows that consume fresh or ensiled grass usually has higher levels of these vitamins compared with milk from cows fed maize silage. The aim of this study was to evaluate the FA and lipo-soluble antioxidants (AO) content in dairy cow milk during the autumn grazing period.

Materials and methods

This study was carried out in the autumn of 2014 at the Centro de Investigacións Agrarias de Mabegondo (CIAM) research station farm (Galicia, NW Spain, 43° 15′ N, 8° 18′ W, 100 m above sea level). Eighteen Holstein-Friesian dairy cows with milk yield of 32.3±5.4 l day^{-1}, 81.2±15.1 days in milk, and live weight of 600.5±56.3 kg were selected from a group of 40 cows which had calved in July-August 2014. Cows were turned into pasture in mid-October on a 9 ha grass-clover sward where perennial ryegrass was the dominant species, divided into 14 paddocks with a grazing period of 2-3 days and a rest period of approximately four weeks. After a preliminary period of two weeks, the experiment took place from 27 October to 23 November and from this date until 21 December (periods 1 and 2, respectively), where cows were allowed to graze between milkings, from 9:00 h to 19:00 h and kept indoors after the evening milking, receiving a supplementation made up of 5 kg of maize silage, 0.5 kg of grass hay and 2.5 kg of a commercial concentrate, weights expressed on a dry matter (DM) basis. The concentrate was formulated to provide 14,000 UI kg^{-1} DM of vitamin A (expressed as β-carotene) and 18 UI kg^{-1} DM of vitamin E (expressed as RRR α-tocopherol). Individual milk yield was recorded daily at the milking parlour using the DeLaval Alpro System and individual milk samples were taken per animal in the morning milking of 3 consecutive days in the final week of every period. The collected milk samples were immediately stored at 4 °C and transported to the Laboratorio Interprofesional Galego de Análise do Leite (LIGAL) where they were subjected to routine FTMIR analysis (milk composition) using a MilkoScan™ FT6000 (Foss Electric A/S, Hillerød, Denmark) or immediately frozen (-20 °C) until posterior analysis (FA and AO). The thawed samples were tempered (water bath, 34 °C) prior to analysis. Milk fat was extracted according to ISO 14156:2001/IDF 172 and FA methyl esters (FAME) were prepared following a basic methylation using methanolic KOH (ISO 15884:2002/IDF 182). The FAME were analysed by gas chromatography (GC) equipped with a flame ionization detector (FID) and a BPX70 capillary column. The procedure for the simultaneous extraction of carotenoids and vitamins (E and A) was conducted according to the methodology proposed by Gentili *et al.* (2013). Final extracts were evaporated to dryness and reconstituted in the mobile phase before being injected on the HPLC system. Separation and quantification of compounds was then carried out on an HPLC system coupled with a photodiode array (carotenoids) and a scanning fluorescence (vitamin-E and vitamin-A, excitation-emission at 295-330 nm and 330-480 nm, respectively) detectors, under similar chromatographic conditions to those described by Chaveau-Duriot *et al.* (2010). A one-way repeated measures analysis was performed using Proc GLM of SAS (SAS Institute, 2009) where the period was the within subjects (repeated) variable.

Results and discussion

As shown in Table 1, yield per cow of milk and milk constituents was slightly reduced in the second half of the experiment, but this difference did not reach statistical significance ($P>0.05$), with average daily values per cow of 33.2 kg of milk, 1.21 kg of fat, 1.00 kg of protein and 2.76 kg of total non-fat solids. Amongst the main FA and FA groups reported in this paper, only the polyunsaturated FA content and the omega-6 to omega-3 ratio were different between periods ($P<0.05$) with average values of 4.47 and 4.12% total FA and 2.34 and 1.87, respectively, for the first and second half of the experiment. Average values for saturated FA, mono-unsaturated FA, vaccenic acid (C18:1 *t*11), *c*9-*t*11 CLA and α-linolenic acid (C18:3n3), expressed as % total FA were 70.8, 24.3, 1.62, 0.80 and 0.93, respectively. No significant differences were detected between periods in the milk content of fat-soluble vitamins and main carotenoids, with average values (in μg l^{-1}) of 181.4 for all-*trans*-β-carotene, 19.9 for lutein, 366.0 for retinol, 1001.2 for α-tocopherol and 15.7 for γ-tocopherol (Table 2). Only 13-*cis*-β-carotene showed a significantly ($P<0.05$) higher content in milk in the second half of the experiment compared with the first period (7.5 and 10.5 μg l^{-1} respectively).

Table 1. Effect of grazing period on milk yield and fatty acid (FA) composition in grazing dairy cows' milk.[1,2]

Period	n	Yield (kg cow^{-1} day^{-1})				Fatty acids (% total FA)						
		Milk	Fat	Protein	Solids	SFA	MUFA	PUFA	C18:1 *t*11	*c*9-*t*11 CLA	C18:3n3	ω6:ω3[3]
PER1	54	33.8	1.24	1.01	2.81	70.5	24.3	4.47	1.61	0.82	0.90	2.34
PER2	54	32.5	1.18	0.99	2.71	71.0	24.2	4.12	1.63	0.79	0.96	1.87
P<		0.099	0.104	0.574	0.128	0.431	0.854	0.004	0.731	0.497	0.127	<0.0001

[1] n = number of observations; PER1 = 27 Oct-23 Nov; PER2 = 24 Nov-21 Dec.

[2] Fat and protein corrected milk production (3.5% fat, 3.5% protein); SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

[3] Omega-6 to Omega-3 FA relationship.

Table 2. Effect of grazing period on the concentration of carotenoids and vitamins in grazing dairy cows' milk.[1]

Period	n	β-carotene (µg l^{-1} milk)		Xanthophylls (µg l^{-1} milk)			Vitamins (µg l^{-1} milk)		
		(All-*t*-β+9-*c*-β) Carotene	13-*cis*-β-Carotene	Lutein	Zeaxanthin	β Cryptoxanthin	Vit. A (retinol)	Vit. E (α-tocopherol)	Vit. E (γ-tocopherol)
PER1	54	176.2	7.5	20.0	2.3	2.8	373.0	1,089.4	17.8
PER2	54	186.6	10.5	19.8	2.0	3.0	359.0	913.1	13.7
P-value		0.738	0.044	0.992	0.491	0.545	0.721	0.298	0.110

[1] n = number of observations; PER1 = 27 Oct-23 Nov; PER2 = 24 Nov-21 Dec.

Conclusions

Milk from autumn grazing showed a good quality in terms of FA profile, carotenoids and fat-soluble vitamins content, and its quality remained fairly constant throughout the final grazing period of the year.

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Liposoluble antioxidants content of spring-grazing dairy cows' milk as affected by sward type and maturity stage

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Abstract

An experiment was performed in Galicia (NW Spain) to evaluate the effects of sward type (perennial ryegrass (PR) vs red clover (RC)) and herbage maturity (from vegetative to reproductive stage) on the contents in dairy cows' milk of carotenoids, retinol and tocopherols during the spring grazing. Two paddocks of 4 ha each, sown with a PR or a RC monoculture were strip-grazed during the day by one of two groups of 10 lactating Holstein-Friesian dairy cows from April until July, with no aftermath grazing allowed. Milk sampling took place every three weeks during the experiment. Sward type did not affect significantly the main carotenoids and vitamin milk contents, with average values for the whole grazing season (in $\mu g\ l^{-1}$) of 132.0 for *all-trans*-β-carotene, 12.6 for lutein, 443.7 for retinol and 711.6 for α-tocopherol, respectively. Herbage maturity significantly affected milk contents of lutein and vitamins, but not that of β-carotene, with average values between the 1st and 4th periods (in $\mu g\ l^{-1}$) of 129.7 and 130.1 for *all-trans*-β-carotene, 15.9 to 9.7 for lutein, 401.4 to 486.8 for retinol and 426.0 to 869.8 for α-tocopherol. It is concluded there is a good antioxidant profile of milk from grazing cows during the spring season, irrespective of pasture type.

Keywords: perennial ryegrass, red clover, carotenoids, retinol, tocopherols

Introduction

Milk antioxidants are of increasing interest as active molecules with beneficial effects on human health. Higher levels of antioxidants as α-tocopherol (vitamin E) and β-carotene (pro-vitamin A) were found by Agabriel *et al.* (2007) in milk from grass-fed cows vs diets rich in concentrate or corn silage. The levels of β-carotene and α-tocopherol in grasses and legumes are high in the young stages of growth and tend to decrease as the plant matures (Ballet *et al.*, 2000). In general, grasses have a higher α-tocopherol concentration than legumes, whereas these are richer in β-carotene (Danielsson *et al.*, 2008). Since there was no previous information about milk antioxidant content in dairy cow grazing systems in Galicia (Atlantic NW of Spain), the objective of this work was to gain insight into the variation of carotenoids, retinol (vitamin A) and tocopherols in spring-grazing dairy cows' milk, assessing the effects of sward type and herbage maturity on the content of these antioxidants in milk.

Materials and methods

A field experiment was performed during the spring of 2013 at the Centro de Investigacións Agrarias de Mabegondo research station farm (Galicia, NW Spain: 43° 15′ N, 8° 18′ W, 100 m above sea level) with 20 lactating Holstein-Friesian dairy cows (75±21 days in milk at the beginning of the experiment) distributed in two equal-sized groups, homogeneous with regard to parity, days in milk and milk yield. Groups were randomly assigned to one of two paddocks of 4 ha each, sown the previous autumn with a monoculture of either *Lolium perenne* L. (perennial ryegrass (PR)) or *Trifolium pratense* L. (red clover (RC)). Swards were strip-grazed by cows from 1 April to 7 July during the first herbage growth cycle, with no aftermath grazing allowed, and cows remained in the same pasture treatment during the experiment. Grazing took place during the day between milkings, from 9:00 to 19:00, and during the night the cows were fed in the barn with a mixture composed of 5 kg of maize silage, 0.5 kg of grass hay and 2.5

kg of a commercial concentrate, weights expressed on a dry matter (DM) basis. The concentrate was formulated to provide 14,000 UI kg^{-1} DM of vitamin A (expressed as β-carotene) and 18 UI kg^{-1} DM of vitamin E (expressed as RRR α-tocopherol). The experiment was divided into four periods of three weeks each, preceded by a pre-experimental period of two weeks. The approximate growth stage of the PR and RC pasture swards during the experimental periods were, according to Metcalfe and Nelson (1985), as follows: vegetative-boot and vegetative-bud (1^{st} period), heading-flowering and bloom (2^{nd} period), anthesis-milk stage and full bloom-green seeds (3^{rd} period) and soft dough-ripe seed and green-ripe seeds (4^{th} period), respectively. Milk samples were taken individually per cow during the morning milking of 3 consecutive days in the weeks 3, 6, 9 and 12 of the experiment (n=240 samples in total). The collected milk samples were immediately frozen to -20 °C until posterior analysis. The thawed samples were tempered (water bath, 34 °C) prior to analysis. The procedure for the simultaneous extraction of carotenoids and vitamins (E and A) was conducted according to the methodology proposed by Gentili *et al.* (2013). Final extracts were evaporated to dryness and reconstituted in the mobile phase before being injected on the HPLC system. Then, the separation and quantification of compounds was carried out on an HPLC system coupled with a photodiode array (carotenoids) and scanning fluorescence (vitamins) detectors, as described by Chaveau-Duriot *et al.* (2010). A two factors (sward type and period) repeated measures analysis was performed using Proc GLM of SAS (SAS Institute 2009) where the period was the within subjects (repeated) variable.

Results and discussion

The most abundant carotenes in milk were *all-trans*-β-carotene and lutein, for which the concentrations in milk were not significantly different between pasture types, with average values for PR and RC of 123.6 and 140.4 μg l^{-1} milk and 12.9 and 12.3 μg l^{-1} milk, respectively (Table 1). In the same fashion, the concentrations of vitamin A (retinol) and E (tocopherols) in milk from cows grazing PR or RC pastures were not significantly different, average values obtained were 406.0 and 480.8 μg l^{-1} milk for retinol, 763.9 and 659.3 μg l^{-1} milk for α-tocopherol and 14.4 and 13.9 μg l^{-1} milk for γ-tocopherol, respectively. The maturity of the pasture clearly influenced the content of lutein in milk, which was reduced (P<0.001) from 15.9 to 9.7 μg l^{-1} milk at the first and the last period of the experiment, respectively, but that of β-carotene remained almost constant throughout the grazing period. Contents of A and E vitamins in the

Table 1. Effect of pasture type and maturity on the concentration of xanthophylls, beta-carotene and vitamins A and E in grazing dairy cows' milk.[1]

	Pasture type			Period (PER)					PT × PER
	PR	RC	*P*-value	PER 1	PER2	PER3	PER4	*P*-value	*P*-value
n	120	120		60	60	60	60		
Beta-carotene (μg l^{-1} milk)									
all-trans-β-Carotene	123.6	140.4	0.106	129.7	129.7	138.5	130.1	0.924	0.762
13-*cis*-β-Carotene	6.0	6.8	0.055	6.5	6.6	6.5	5.9	0.762	0.880
Xanthophylls (μg l^{-1} milk)									
Lutein	12.9	12.3	0.693	15.9	14.0	11.0	9.7	<0.0001	0.836
Zeaxanthin	2.0	1.4	0.011	2.2	2.0	1.3	1.3	<0.0001	0.868
β Cryptoxanthin	2.2	2.4	0.027	2.5	2.6	2.1	2.0	0.033	0.946
Vitamins (μg l^{-1} milk)									
Vit. A (retinol)	406.6	480.8	0.054	401.4	372.7	514.0	486.8	<0.001	0.082
Vit. E (alpha-tocopherol)	763.9	659.3	0.449	426.0	428.0	1,122.6	869.8	<0.001	0.384
Vit. E (ganma-tocopherol)	14.4	13.9	0.842	9.6	7.8	19.2	20.0	<0.001	0.095

[1] n = number of observations; PR = perennial ryegrass pasture; RC = red clover monoculture pasture; PER1 = 15 April-5 May; PER2 = 6 May-26 May; PER3 = 27 May-16 June; PER4 = 17 June-7 July.

milk increased significantly (P<0.001) as the grazing season advanced, presenting the maximum values at the end of May-mid July period, where both the ryegrass and the clover were at full reproductive stage; this effect was more marked in the case of vitamin E, independently of the type of pasture consumed (average values of 401.4, 372.7, 514.0 and 486.8 μg l^{-1} milk for retinol and 426.0, 428.0, 1,122.6 and 869.8 μg l^{-1} milk for α-tocopherol, period 1 to 4, respectively). No significant interactions between sward type and period of the experiment was detected, which shows an homogeneous behaviour for the concentration of liposoluble antioxidants in milk from cows grazing either PR or RC pastures during the course of the different herbage maturity stages. The values obtained in the present work are in line with those cited by other authors (for example, Larsen *et al.*, 2012) for milk from Holstein cows grazing diverse pastures of varying degrees of maturity.

Conclusions

Vitamin A and E content in milk from cows grazing pastures in their first growth cycle increases with the maturity of the herbage, whilst the milk β-carotene content remains unaffected. This model of variation is similar for perennial ryegrass and red clover swards.

Acknowledgements

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Organic pasture-based automatic milking systems: comparison of two systems of pasture allocation

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Abstract

French farmers lack technical advice on the integration of a milking robot into a grass-based system, in particular for organic production. An experiment was implemented during 3 years in Trévarez (Brittany) to compare two grazing management systems: a two-way (AB) versus a three way-grazing system (ABC). Allocating three paddocks per 24 h improved the cow traffic but the herd and grazing conditions of the different years did not allow conclusions about potential impact of the management system on the production of grass or milk. The three-paddock system was more difficult and time consuming to manage.

Keywords: dairy cows, milking robot, organic production, grazing, grass management

Introduction

Robotic milking is becoming a common solution to reduce labour in Europe. In France, the number of farms with an AMS (automatic milking system) has doubled since 2010 with some 4,800 farms equipped (Allain, 2016). However, after the purchase of the AMS, farmers usually increase concentrates to maximize milk production, and grazing is reduced or even suppressed (Poulet and Brocard, 2013). This is despite grazing having a very low cost and a positive impact on animal health (Burow *et al.*, 2011). French farms with AMS and grazing have a higher profit per working unit (WU, Caillaud and Brocard, 2015) than those without grazing. Moreover, grazing is the base of a greater feeding self-sufficiency (Rouillé *et al.*, 2014) and is thus the basis of the feeding system in organic dairy farms. But can grazing and AMS be integrated within the same system? Farmers often lack the technical advice for implementing such combinations. For this reason a experiment was implemented at the Trévarez experimental farm (CA de Bretagne) to test two practical solutions of grazing management and develop 'users' guides' for farmers.

Material and methods

To increase cow traffic without fetching cows, a two-way grazing system (called AB, 2 paddocks per 24h) and three-way grazing system (ABC, 3 paddocks per 24 h) were tested during the 2014 and 2016 grazing seasons. According to experiments made in Australia (Lyons, 2013) and Ireland (O'Brien, 2015), offering fresh grass 2 or 3 times per 24 h facilitates cow traffic to the robot, cows being motivated by the possibility to reach a new paddock after the AMS. The aim of the experiments was to test both solutions under Breton real-farming conditions (high genetic merit Holstein cows, heterogeneous swards), to record working time as well as production figures, and to develop 'users' guides' for the farmer.

Trevarez experimental farm is located in western France and it has an oceanic climate. Grazing is possible 10 months per year and it has always been the basis of the forage system because of its low production cost and high availability. Since 2013, the farm with its 60 Holstein cows herd has been testing the combination of 100% grazing and robotic milking with a very low resort to concentrate (Brocard *et al.*, 2015) and has been delivering organic milk since 2015.

The 84 ha of land is split into several blocks including 21 ha on a summer grazing site located 4.5 km from the barn. The AMS has been made 'mobile' on a trailer (Brocard *et al*, 2015, Cloet, in press) and is moved to the summer site from the end of April till October. During this period, the herd gets a 100% grazed-grass diet with no buffer forage and less than 1 kg of barley per cow per day. The pastures are ryegrass and white clover mixtures. The grazing system is rotational with a front fence. The water points are located in the waiting area of the AMS and in the tracks. The furthest paddocks are located 800 m from the AMS.

In 2014 (AB), the 21 ha of the summer grazing site was divided into two blocks: a day block and a night block. Each block consisted of 12 to 15 paddocks grazed rotationally with a front fence. The access times were from 7 am for the day paddock and 5 pm for the night paddocks. In 2016 (ABC), the area was divided into 3 blocks: morning, afternoon and night blocks. The access times were 5:00 am for the morning paddock, 12:00 am for the afternoon paddock and 19:00 pm for the night paddock. The access to the paddocks after the AMS was monitored through a drafting gate located in the waiting area. In 2014 (resp. 2016) the herd comprised, on average, 50 (resp. 57) cows in mid-lactation (Table 1). Finally, only one milking permission system was implemented in 2014 (Table 2), while it changed during the grazing season in 2016. The first permission system tested (ABC1) was much simpler but also more restrictive than the second one (ABC2), more similar to the one implemented in 2014.

Results and discussion

In 2014 (resp. 2016) cows were kept on a 100% grazed-grass system during 161 days (resp. 149 d) showing the possibility to integrate an AMS into a grass system with no buffer feed. No difference appeared in terms of dairy production between the two grass management systems AB and ABC (Table 1) although it remains difficult to compare both situations: different grazing years, grass growth, herd composition, etc. The year and the system effects were confounded in the statistical analyses (Déprés, 2016). In terms of milking frequency, the results show that the choice of milking permission parameters (Table 2) has more influence than the number of paddocks allocated: altogether we observed no statistical difference between the AB and ABC systems (Table 1). But the milking frequency observed during the period with the first permission system in 2016 led to a significantly lower milking frequency (1.5 milkings per day) than the second permission system tested in 2016 and the one implemented in 2014 (Table 3). Milking intervals were much longer with the ABC1 system, as well as the share of cows with intervals over 16 hours (for Lyons *et al.*, 2013, upper limit of risks in terms of udder health).

Table 1. Herd composition and dairy production in 2014 (AB) and 2016 (ABC).

	2014 – AB	2016 – ABC
# days 100% grazing in season	161	149
Concentrate (kg cow^{-1} d^{-1})	0.9	0.7
# Milking cows	50±3	57±4
Cows in first lactation (%)	24	45
Av. lactation stage (d)	196±13	187±12
Milk per AMS (kg d^{-1})	949±123	1,017±113
Milk per cow (kg d^{-1})	19.0±1.9*	17.7±1.4*
Milking frequency (d^{-1})	1.8±0.1*	1.6±0.3*

* No statistical difference at P=0.05.

Table 2. Milking permissions at the automatic milking system in 2014 (AB) and 2016 (ABC-1 and ABC-2).

Milking permission	Early lactation	Mid lactation	Late lactation
AB	Calving – 90 d: 6 h 30 or 10 kg milk	91-71 d before next calving: 8 h 30 or 11 kg	70 d before calving – drying off: 10 h or 12 kg
ABC 1	10 h or 12 kg		
ABC 2	Calving – 90 d: 8 h or 10 kg milk	91-100 d before next calving: 9 h or 9 kg	70 d before calving – drying off: 10 h or 12 kg

Table 3. Milking frequencies and intervals according to milking permissions.[1]

	AB-2014	ABC1-2016	ABC2-2016
Milking frequency (d^{-1})	1.8[a]	1.5[b]	1.7[a]
Av. milking interval per cow	13 h 16 min	17 h 40 min	14 h 35 min
% intervals over 16 h	21	56	29

[1] Statistical adjusted mean (SAS mixed procedure); same letter = non stat. different at P=0.05.

No effect of the grazing system was found on margin over feeding cost. In both situations the feeding cost was 75% lower than during the winter season in the barn. In terms of working time, having a third fence to move with the ABC system led to 20 extra minutes of work per day for the herdsman compared with the AB system. The cow traffic appeared good with no cows to fetch for most of the time. The ABC system required more time than an AB system to prepare the grazing planning and adjust it according to the grass growth and offer every week.

Conclusions

Both grazing management systems show that it is possible to integrate an AMS in a 100% grazed-grass system with limited resort to concentrates. Even if this experiment could not allow a perfect comparison, as the two management systems (AB or ABC) were not implemented in the same year, it offered practical observations currently used to help farmers who want to integrate an AMS within an organic self-sufficient, grass-based system and who want to 'keep things as simple as possible' in terms of labour. Both milking frequency and milking intervals observed appeared to be more linked to the milking permissions at the robot than to the system of pasture allocation, as summarized by Déprés in 2016. In both situations the margin over feeding cost was high, thanks to the 100% grazed-grass-based system. Finally, the ABC system remains more complicated to manage for the farmer and requires 20 extra minutes of work per day than the AB system. Having three different grazing blocks to monitor and manage appears to be a serious limiting factor for extension to French farmers.

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Growth, carcass and meat quality of Bergamasca light lambs under 'Agnello del Centro Italia' PGI

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Abstract

The study aims to provide additional information to characterise light lambs of the Bergamasca breed produced under 'Agnello del Centro Italia' PGI in terms of growth performance, carcass traits and meat physico-chemical and nutritional properties. For this purpose, 22 lambs, raised with their mothers on lowland pastures in two seasons (winter and spring), were slaughtered at an average age of 60 days. Mean slaughter weight and average daily gain (total rearing period) of lambs were 25.13 kg and 330 g, respectively. Mean carcass weight was 11.22 kg, dressing percentage 44.24 and mean carcass pH 5.49. Results in terms of colour parameters, chemical and fatty acid composition, determined in *longissimus dorsi* muscle are also reported and they are common for meat of unweaned lambs.

Keywords: Bergamasca breed, meat quality, Agnello del Centro Italia PGI

Introduction

In Central Italy lamb production is mainly based on extensive grazing systems and transhumance is still of major importance (Budimir *et al.*, 2016). In summer, most of the flocks graze upland pastures of the central Apennines. From autumn, the sheep are progressively transferred to lowlands where until spring the main forage resources are lucerne meadows, but winter cereals at vegetative stage, crop residues, marginal lands and riverbanks are sometimes also used. Lambs are reared with ewes and supplemented with concentrate and/or hay when needed. Lamb production is mainly carried out in the lowlands for the Easter and Christmas markets.

To promote lamb production from these systems and to protect the local lamb meat market from foreign competition 'Agnello del Centro Italia' Protected Geographical Indication (PGI) label (EU Reg. 475/2013) was recently created and includes different breeds.

The aim of this study was to provide additional information to characterise the Bergamasca light lambs produced under 'Agnello del Centro Italia' PGI in terms of growth performances, carcass and meat quality including fatty acid (FA) composition.

Materials and methods

Twenty-two male, single-born Bergamasca lambs were reared in the Marche region in winter (11 lambs) and autumn (11 lambs) of 2015 according to the traditional grazing system (Caballero *et al.*, 2009). Lambs were fed with maternal milk at pasture that, in both rearing periods, was dominated by lucerne (*Medicago sativa* L.). Mean forage dry matter on offer in the pasture was estimated on three 1.0 m^2-sampling areas (1.0×1.0 m), randomly chosen on each studied paddock (4 in winter and 4 in autumn) at the beginning of grazing (Frame, 1981). The herbage samples (23 in winter and 27 in autumn) were dried at 60°C for 48 h, and used to determine the chemical composition (Martillotti *et al.*, 1987) summarised in Table 1.

Table 1. Dry matter (DM) and chemical composition (% of DM) of pasture plots.[1]

	mean ± SD	min – max
DM yield (t ha^{-1})	2.71±1.14	1.19-4.16
DM %	74.91±8.68	58.81-83.86
Crude protein	17.60±6.15	10.92-27.71
NDF	41.81±4.27	35.20-47.13
ADF	28.58±4.74	22.48-37.71
Ether extract	1.60±0.44	1.05-2.15
Ash	13.13±1.89	11.07-15.84

[1] NDF = neutral detergent fibre; ADF = acid detergent fibre; SD = standard deviation.

In both rearing seasons, dams had free access to hay and were supplemented with corn grain (0.5 kg head^{-1} day^{-1}). From 20 days of age, lambs were given concentrate *ad libitum*. Lambs were slaughtered at an average age of 60 days. After chilling at 4 °C for 24 h *post mortem*, carcasses were weighed and the dressing percentages were calculated. Final carcass pH was recorded 24 h *post mortem* in the *longissimus thoracis* muscle using a portable pH-meter (Eutech Instruments, mod. XS pH 110, Singapore). CIE L × a × b × coordinates ('L*' lightness, 'a*' redness, 'b*' yellowness) were measured on the fresh *longissimus dorsi* samples using a Minolta CR 200 spectro-colorimeter. Chemical determination of crude protein (Kjeldahl, nitrogen × 6.25), fat (extraction with petroleum ether) and ash (incineration in muffle furnace at 550 °C) content was performed in freeze-dried *longissimus dorsi* samples. Fatty acids methyl esters (FAME) of *longissimus dorsi* samples were prepared according to the ISO 5509 method, analysed on a gas chromatograph (HRGC MEGA 2 series, Fisons Instruments, Milano, Italy) and identified using a 37-component FAME standard mixture.

Results and discussion

Growth and carcass characteristics of Bergamasca lambs are reported in Table 2. The lambs had a mean slaughter weight of 25.13 kg with a mean average daily gain (total rearing period) of 330 g, higher than those reported by Santos-Silva *et al.* (2002a). Lambs had 11.22 kg mean carcass weight and were classified as class C according to the European carcass classification system for light lambs (10.1-13.0 kg; EU, 1994). Mean dressing percentage (44.24) was lower than the reported for Apulian light lambs reared in similar conditions (Cifuni *et al.*, 2010). Mean carcass pH (5.49) was acceptable and within the normal ranges, taking in consideration that a final pH greater than 5.8 is regarded as undesirable for a meat quality (Tejeda *et al.*, 2008). Physico-chemical and nutritional parameters of *longissimus dorsi* muscle are presented in Table 3. The chemical composition of meat was comparable to that reported for light lambs of different breeds by other authors (D'Alessandro *et al.*, 2013; Mazzone *et al.*, 2010; Russo *et al.*, 2003). On average, meat of Bergamasca lambs showed lower L* (42.00 vs 43.93), higher a* (19.34 vs 17.34) and lower b* (3.28 vs 7.35) values compared with the values reported by Russo *et al.* (2003) for light lambs of similar slaughter weight. The saturated fatty acids (SFA) were the most abundant (53.48%) in *longissimus dorsi* muscle, followed by the mono-unsaturated (MUFA) (33.81%) and the polyunsaturated (PUFA) (9.77%) FA. The mean values of C14:0 (10.07%), C16:0 (24.88%) and C18:0 (13.52%) FA were comparable to those observed by Alessandro *et al.* (2015) in light lambs of similar slaughter age. The mean value of C16:1 (1.68%) was comparable to the value reported by Mazzone *et al.* (2010) for Apenninica light lambs (1.78%). ∑C18:1c (27.87%) was lower than values of oleic acid of light lambs of other breeds (Santos-Silva *et al.*, 2002b). The mean value of PUFA C18:2 n-6 was lower (3.36 vs 6.19%) and C18:3 n-3 slightly higher (1.47 vs 1.30%) than the values reported for light lambs reared in similar conditions (Santos-Silva *et al.*, 2002b).

Table 2. Growth and carcass characteristics of the Bergamasca lambs.[1]

	mean ± SD	min – max
Slaughter weight (kg)	25.13±4.18	15.00-30.80
Average daily gain (g)	330±61.93	192-411
Cold carcass weight (kg)	11.22±2.58	5.47-15.13
Dressing (%)	44.24±4.41	36.45-52.71
pH	5.49±0.25	5.14-5.99

[1] SD = standard deviation.

Table 3. Meat quality traits in *longissimus dorsi* muscle of the Bergamasca lambs.[1]

	mean ± SD	min – max
Chemical composition (%)		
Moisture	74.81±1.43	72.34-77.84
Protein	19.50±0.71	17.62-20.58
Fat	1.85±0.84	0.52-3.76
Ash	1.31±0.19	1.15-1.96
Colour parameters		
L*	42.00±3.37	35.66-51.16
a*	19.34±1.45	16.59-21.75
b*	3.28±0.83	1.95-5.90
Fatty acid composition[2]		
C 14:0	10.07±1.76	5.50-13.90
C 16:0	24.88±1.97	21.45-28.67
C 16:1	1.68±0.41	1.04-2.43
C 18:0	13.52±2.93	9.50-19.03
∑C18:1c	27.87±3.09	20.66-33.73
C18:2 n-6	3.36±0.75	2.40-5.21
C18:3 n-3	1.47±0.22	1.07-1.91
SFA	53.48±2.21	49.78-58.69
MUFA	33.81±2.66	27.51-38.77
PUFA	9.77±1.22	7.66-12.41

[1] SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = polyunsaturated fatty acids; SD = standard deviation.
[2] % of total analysed FAME;

Conclusions

The present study provides further information on the quality traits of Bergamasca light lambs produced under the 'Agnello del Centro Italia' PGI in addition to those already included in the product specification. Further research is needed to confirm the variability range of the reported traits for the studied breed included in the list of the PGI.

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Tocopherols and tocotrienols in plant species from Atlantic mountain and valley grasslands

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Abstract

The diversity of plant species in mountain and valley grasslands is affected by geographical, agronomical and climatic conditions, and also by animal grazing. Tocols are well-known as lipid-soluble micronutrients because of their role in human and animal health. Pasture plants are an important source of these components in the animal diet and are directly transferred from plants to milk, cheese and meat. Thus, tocols can be proposed as potential biomarkers of grasslands products. The aim of this study was to investigate the tocopherol and tocotrienol composition of plant species from valley and mountain grasslands, located in the Atlantic region of northern Spain, which are usually used as grazing areas by commercial sheep flocks. Most abundant plants from valley and mountain grasslands were collected during the grazing period. Tocols were analysed by normal phase HPLC coupled to fluorescence detection. The main tocopherol isomer in all plant species from both valley and mountain grasslands was α-tocopherol. Small amounts of β-, γ-, and δ-tocopherols, α-tocopherol acetate and tocotrienol isomers were detected in most plant species. Despite differences in the content of tocopherols and tocotrienols among individual plant species during the sampling period, the estimated average content of tocols was significantly higher in mountain than in valley grasslands.

Keywords: tocopherols, tocotrienols, plants, mountain, valley, grassland products

Introduction

Vitamin E consists of four tocopherols and four tocotrienols (α-, β-, γ-, and δ-isomers) which are collectively referred as tocols. Plants with photosynthetic tissues such as most botanical species of grasslands show very different content and composition of tocols (Saini and Keum, 2016). In this regard, botanical species, phenological stage, plant biodiversity, geographical, agronomic and climatic factors can strongly influence the tocol composition of pasture plants (Larsen *et al.,* 2012). The α-tocopherol isomer has an important role to prevent the oxidation of long-chain polyunsaturated fatty acids in cell membranes in order to reinforce the plant stress tolerance (Munné-Bosch, 2005). Pasture plants are an important source of tocols in animal diets managed under extensive grazing, and these compounds are directly transferred from plants to milk, cheese and meat. Thus, tocols can be proposed as indicators of animal's diet and therefore as potential biomarkers of grassland products (Osorio *et al.*, 2013). The aim of this study was to investigate the tocopherol and tocotrienol composition of plant species from valley and mountain grasslands, located in the Atlantic region of northern Spain, which, are usually used as grazing areas by commercial sheep flocks. The potential impact of plant tocols on the traceability of grasslands products from valley and mountain pastures was also discussed.

Materials and methods

The study was conducted during the third week of May and June 2015 in grazing areas of six commercial sheep flocks (150-250 lactating ewes). These areas were located in the mountains and surrounding valleys of the Aralar Natural Park (42°59'48'N and 2°06'51'W; northern Spain). Three flocks remained in valley farms during the whole lactation period and the ewes grazed in the valley grassland located close to each farm at 100-400 m altitude. In the first week of May, the other three flocks were moved to mountain farms located at 700-1,200 m altitude. Grazing areas of the commercial flocks were defined according to fences for valley grasslands, and GPS collars were used to define grazing areas used for mountain flocks (Arzak *et al.,* 2016). The size of valley grassland plots ranged from 0.64 to 6.38 ha, while grazing areas in mountain ranged from 82.04 to 173.2 ha. For measuring the botanical composition, the relative abundances (% cover) of plant species were estimated by direct observation using 0.25 m^2 quadrat. In the valley, two grassland plots were sampled per flock, and 60 quadrats were randomly located on each plot, whereas 100 quadrats were randomly located on each mountain grazing area. The flowering stage was dominant in plant species during the sampling period. Individual plant species were sampled according to their relative abundance in the grazing areas. Seventeen and twenty-one individual plant species (around 70% of the total abundance) were collected in valley and mountain grazing areas. Each botanical species was collected separately and once in the laboratory, the same botanical species samples collected in valley grasslands, or in mountain grasslands, respectively, were pooled and, subsequently, lyophilized and stored at -80 °C. Two aliquots of each plant sample were analysed. Tocopherols and tocotrienols were extracted from 100 mg plant sample and analysed by normal-phase HPLC coupled to fluorescence detection as previously described (Valdivielso *et al.*, 2015). The average amount of tocopherols and tocotrienols in the pastures (valley or mountain) was estimated taking into account the relative abundance of each plant species in the grazing areas as described (Valdivielso *et al.*, 2016). Tocopherol and tocotrienol content of plants (mg 100 g^{-1} DM) was analysed by ANOVA ($P \leq 0.05$) with altitude grassland (valley/mountain) and sampling period (May and June) as fixed effects.

Results and discussion

Major plant species collected in valley grazing areas were *Agrostis* L. (22.4%), *Trifolium repens* L. subsp. *repens* (18.4%), *Holcus lanatus* L. (6.7%), *Lolium perenne* L. (5.6%) and *Poa pratensis* L. (3.9%). In mountain grazing areas, major plant species were *Festuca nigrescens* subsp. *microphylla (St-Yves)* Markgr.-Dannenb. (31.8%), *Agrostis capillaris* L. (10.2%), *Trifolium repens* L. subsp. *repens* (7.5%) and *Thymus praecox* Opiz (4.6%). Other minor plant species collected in the valley and grazing areas are indicated in Table 1. Regarding plant selection by ewes during grazing, a previous study conducted in mountain grasslands showed that, despite the sheep selecting certain plant species over others, the tocol composition of the dominating botanical species in the pasture prevailed in the sheep diet (Valdivielso *et al.*, 2016). As Table 1 shows, the tocol profile of both valley and mountain pastures was dominated by α-tocopherol during the two sampling periods (around 95% of the total tocol content). Effects of grassland altitude and sampling period interaction were significant. Except for α-tocotrienol, increasing grassland altitude resulted in higher mean contents of tocopherols and tocotrienols in mountain than in valley grasslands. Considering those plant species common in both valley and mountain grasslands (*Trifolium repens* L. subsp. *repens* and *Bellis perennis* L.), the content of tocols was higher (around 58%) in mountain than in valley. Taking into account that plant tocols can be directly transferred to animal food products, differences observed in the tocopherol and tocotrienol composition between mountain and valley pastures could contribute to the traceability of grassland products, in particular for mountain foods.

Conclusions

Higher average amounts of tocopherols and tocotrienols were found in mountain than in valley grasslands. Differences observed in the tocol composition were mostly due to type and abundance of botanical species and grassland altitude. These differences could contribute to the traceability of grasslands products, in particular for mountain foods, due to plant tocols being directly transferred to food products from grazing animals.

Table 1. Estimated average tocol composition (mg 100 g^{-1} DM; three significant figures) of valley and mountain grasslands in two sampling periods (May and June).[1]

	Valley[2]		Mountain[3]		SEM	Significance *(P)*		
	May	June	May	June		A	S	A×S
α-tocopherol acetate	0.227	0.00216	0.0801	0.292	0.0437	*	*	*
α-tocopherol	5.32	8.21	13.9	11.5	1.23	*	ns	*
α-tocotrienol	0.0129	nd	0.00664	nd	0.00215	ns	*	ns
β-tocopherol	0.0438	0.0972	0.222	0.0999	0.0250	*	*	*
γ-tocopherol	0.166	0.263	0.363	0.351	0.0307	*	ns	*
γ-tocotrienol	0.00156	0.00644	0.0248	0.0313	0.00469	*	*	ns
δ-tocopherol	0.0176	0.0434	0.110	0.0650	0.0130	*	ns	*
∑ tocols	5.59	8.62	14.7	12.3	1.31	*	ns	*

[1] SEM = standard error of the mean; A = grassland altitude; S = sampling period; A×S, interaction term; nd = not detected; * $P \leq 0.05$; ns = not significant.

[2] Minor plant species collected (abundance lower than 3%) were *Mentha suaveolens* Ehrh., *Dactylis glomerata* L., *Ranunculus* L., *Plantago lanceolata* L., *Festuca arundinaceae* Schreb subsp. *arundinaceae, Cerastium fontanum* Baumg., *Bellis perennis* L., *Festuca rubra* L. subsp. *rubra*, *Veronica chamaedrys* L. subsp. *chamaedrys*, *Carex divulsa* Stokes, *Bromus hordeaceus* L. and *Cynosurus cristatus* L.

[3] Minor plant species collected (abundance lower than 2.5%) were *Carex caryophyllea* Latourr., *Bellis perennis* L., *Lotus corniculatus* L., *Poa annua* L., *Ulex gallii* Planch. subsp. *gallii, Potentilla montana* Brot., *Galium saxatile* L., *Medicago lupulina* L., *Luzula campestris* (L.) DC., *Hieracium pilosella* L., *Potentilla erecta* (L.) Raeuschel, *Danthonia decumbens* (L.) DC., *Jasione laevis* Lam. subsp. *laevis*, *Cerastium arvense* L., *Cerastium fontanum* Baumg., *Erica vagans* L., and *Genista hispanica* subsp. *occidentalis* Rouy.

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Extensive goat production systems in northern Morocco: production and use of pastoral resources

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Abstract

Grazing is a Mediterranean tradition with an unvaried importance in northern Morocco. Rangeland has known profound changes, inducing a major gap between pastoral supply and demand. This study aimed to assess pastoral production of pastures at two sites in northern Morocco (Derdara and Loubar) and investigate their use by goats. Concerning biomass production, we used the method of the reference module. The surveys were realized during the biomass evaluation period. Pastoral species, mainly *Arbutus unedo, Cistus monspeliensis, Erica arborea, Lavandula stoechas* and *Pistacia lentiscus*, represent the most palatable species selected by goats. Grazing is practised throughout the year except during rainy days when breeders practice 'cut and carry' of the branches. The biomass produced was estimated at 1,455 and 333 kg dry matter ha^{-1}, respectively, in Derdara and Loubar. Significant differences were observed depending on the season and according to sampling sites. Furthermore, the research showed the appearance of degraded areas dominated by annual species together with species of low pastoral interest – thorny plant species that characterize degraded areas. Due to climate changes and overgrazing, pastoral resources will have availability and quality reduced. The reasonable use of pastures, including a reduction in grazing pressure, should be developed to insure their sustainability.

Keywords: grazing, northern Morocco, goat, pastoral resources

Introduction

Mediterranean rangelands are rich in plant species and life forms (Le Houérou, 1981). They constitute an important year-round source of feed for livestock. In northern Morocco, pastures are traditionally an integral part of the feeding calendar of goats and consequently of the pastoral systems of the region. Pastoral systems are characterized by integrating pasture with forage and livestock production, and pastures, which can be natural, improved or cultivated (grassland). Rangeland has known profound changes inducing major malfunctions between pastoral supply and demand (FAO, 2011). These pastures are under pressure mainly due to climate change, overgrazing, population and especially from ineffective operating practices of pastoral resources such as cutting the branches (Chebli *et al.,* 2012). For a sustainable and integrated development of pastoral resources, it is essential to establish a resource assessment system.

We conducted this study in two pastoral areas of the Moroccan Rif Mountains in northern Morocco (Derdara and Loubar). This paper attempts to assess botanical composition and biomass production investigating the use of these pastures.

Materials and methods

The areas of study are two rangelands, exploited by goat breeders: Derdara and Loubar. The study was realized over a period of eight months to assess the species composition and the productivity of pastoral plants by evaluating the vegetation qualitatively and quantitatively. The qualitative evaluation of vegetation concerned the floristic diversity. In each sampling period, a herbarium was collected to determine the floristic composition.

For quantitative evaluation, biomass production has mainly concerned the biomass of palatable species. Measurements were performed during three seasons (winter, spring and summer 2016). Plant biomass was measured using the non-destructive method known as the reference module. In order to control spatial heterogeneity we used the stratification method as proposed by Qarro (1996), Kouraimi (1997) and Chebli *et al.* (2014). Twenty quadrats were identified in each pasture. The size of the quadrats adopted for measurement of biomass is 2×5 m for shrub strata and 1×1 m for grass. Several surveys were conducted with breeders during the study period to gather information on modalities of using pastures. For the statistical analysis we used R-Project, quantitative data were analysed using the model:

$$Y_{ijk} = Season_i + Site_j + (Season \times Site_{ij}) + Residual\ error_{ijk}.$$

Results and discussion

The pastures were characterized by an average level of diversity of pastoral flora (81 and 55 species, respectively in Deradra and Loubar) dominated by shrubs and grass. During recent years breeders have noticed an abundance of unpalatable species in degraded areas dominated by annual plant species such as *Arisarum vulgare* (Targioni-Tozzetti) and *Coriaria myrtifolia* L. This situation can be explained mainly by misuse of some part of rangeland, causing over exploitation of pastoral resources and contributing to appearance of low palatability species.

Rangelands in northern Morocco are characterized by dense vegetation mainly dominated by shrub layers. The pastoral production was considerably higher in Derdara than in Loubar. The annual production of biomass was estimated at 1,455 and 333 kg dry matter ha^{-1}, respectively in Derdara and Loubar (Figure 1). The difference in pastoral production, depending on the season and according to sampling sites, is statistically highly significant (P<0.001). Loubar is considered as permanent pasture. This difference can be explained in part by a continuous use of pastoral resources and a lack of appropriate pasture management. Regarding the distribution for each season, higher production is observed in the spring, which coincides with the growth phase of most species groups.

In most cases farmers managed the goat herds themselves. Grazing is practised directly throughout the year from mid-winter to mid-autumn. Indeed, during this time of the year, pastures are in restoration phase of their ground cover with maximum forage production during the months of April and May. Goats spend most of the day on pasture areas (7-8 h day^{-1}). During the rainy period, the intensity of pasture use decreases (2-3 h day^{-1}) (Chebli *et al.,* 2016). Moreover, access to pasture becomes difficult, which explains the use of grassland near farms to reduce the costs of supplementation.

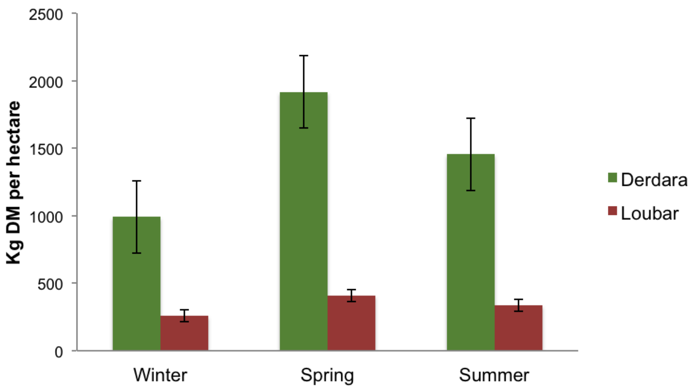

Figure 1. Biomass production of pastoral species in Derdara and Loubar pastures.

Persistent goat concentration on the same pasturing area causes overgrazing and consequently this induces the appearance of unpalatable species and disappearance of palatable species. The movement of goats is based on the availability and accessibility of forage. The average distance travelled by goats was estimated between 8 and 10 km day^{-1} (Chebli *et al.*, 2016).

Conclusions

The pastures in northern Morocco remain as a large area for rangeland. Biomass production varies greatly from one area to another. Farmers are becoming sedentary where the pastures are overgrazed and dominated by unpalatable plant species, and biomass production does not exceed 1455 kg dry matter ha^{-1}.

Pastoral resources on these areas, despite all forms of degradation, continues to play an important role for all agricultural activities of the local population, representing a fundamental source of feed for livestock. The pressure on pastoral resources in the mountain Rif could lead to their irreversible degradation. Consequently, it will be necessary to adopt an effective use of rangelands, on a seasonal schedule and/or depending on the state of resources, convincing farmers to work together for pasture use.

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Partial replacement of concentrate by field pea: effects on milk and cheese fatty acid profile

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Abstract

The aim of our work was to test the effects of partial replacement (17%) of commercial concentrate with pea flour on cows' milk and cheese quality. Milk protein content decreased in a pea-based diet. Proportion of unsaturated fatty acids (FA) increased, medium chain FA (MCFA) decreased by 4% and long chain FA (LCFA) increased by 9%, while short chain FA (SCFA) and conjugated linoleic acid (CLA) did not differ between treatments. Cheese fatty acid composition changed in agreement with milk fatty acids composition but the differences were significant only in a few cases.

Keywords: *Pisum sativum*, milk quality, fatty acid, dairy cows

Introduction

Decreasing the dependence on the import of protein-rich feedstuffs is among EU's priorities (De Visser *et al.*, 2014). The use of farm-grown legumes is a low-cost natural way for modulating milk chemical composition, reducing the use of commercial concentrates (Dewhurst *et al.*, 2006). Pea (*Pisum sativum* L.) is one of the most important food and feed crops in Europe; it is rich in lysine and is also an important source of beneficial fatty acids (Grela *et al.*, 1995). Due to the key role that fatty acid (FA) profile plays in human health, manipulating their profile in milk and cheese is a relevant target for dairy industries all over the world. In this work we tested the effects of partial replacement of concentrates with pea flour in the diet of lactating cows, on gross composition of milk and on milk and cheese fatty acid profiles.

Materials and methods

Six multiparous Holstein cows at the beginning of lactation (45±10 days in milk) were allocated to two balanced groups according to their stage of lactation and milk yield. The groups were randomly assigned to control or experimental diets (P-mix). Control diet (C) (dry matter basis consisted of: 37% mixed grass hay, 17% alfalfa hay, 17% maize flour and 29% of commercial concentrate 'Lattifera 25' (A.I.A. Agricola Italiana Alimentare S.p.A.), where sunflower meal and soybean are the main protein sources. In the experimental diet (P-mix) 17% of concentrate was replaced by pea (*Pisum sativum* L. cv Cheyenne) flour. After a diet adaptation-period of two weeks milk samples were collected at herd level four times across two weeks (from 25 June 2013 to 5 July 2013). Samples were taken from stirred bulk tanks and represented 24 h of production of the two groups. For each group at two dates sampled milk was processed in Caciotta cheese (25 days ripening). Milk samples were analysed for fat, true protein, lactose (MilkoScan FT 6000, Foss Electric, Hillerød, Denmark). FA of milk, cheese and feedstuff were determined following the protocol of Di Trana *et al.* (2005). For each treatment, collection dates were used as replicates. Differences between FA means were compared using a two-sample t-test.

Results and discussion

The 17% replacement of concentrate with pea significantly affected milk chemical composition. Milk protein content decreased (from 3.27% in C to 2.73% in P-mix diet; P=0.030), lactose content increased (P=0.012). Other studies showed that even a 15 to 20% inclusion of pea in dairy cows diets did not affect milk composition (Van de Pol *et al.*, 2008). In our case, however, pea was finely ground and this possibly enhanced nitrogen degradability (Bayourthe *et al.*, 2000). Pea supply positively affected milk

FA composition (Figure 1 and Table 1). Total saturated fatty acid percentage (TSFA) decreased (-11%) in P-mix diet, mono-unsaturated FA (MUFA) increased but polyunsaturated FA (PUFA) did not differ. MCFA proportion decreased by 4% and that of LCFA increased by 9%. The most abundant milk saturated acid (C:16, 30% of total FA on average) decreased in P-mix diet. Milk oleic acid increased possibly due to the desaturation of C:18 (Chilliard *et al.*, 2000), which was more abundant in P-mix compared with the control diet (data not shown). There were no significant differences in CLA and ALA. Cheese FA composition changed in agreement with milk data (Figure 1), though the differences were significant for only a few parameters.

Conclusions

Our work shows that the chemical composition of bovine milk and cheese can be altered even by replacing only 17% of concentrate with pea flour. Milk protein content was reduced, and this requires further investigation on pea flour by-pass protein content and ingestion rates. Pea inclusion increased the content of beneficial MUFA and LCFA, which is a relevant target for dairy industries.

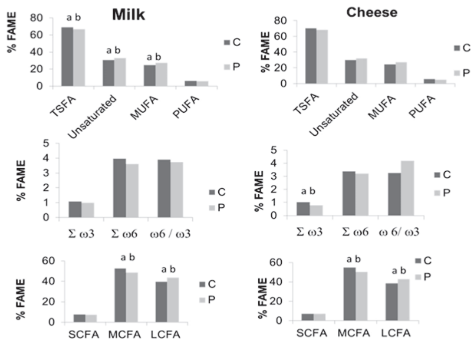

Figure 1. Barplot of groups of fatty acids in milk (left) and cheese (right) under the two diets (Control diet (C) dark grey columns and experimental diet (P) light grey columns). Different letters indicate significant differences at the t-test $P<0.05$).

Table 1. Significant differences ($P<0.05$) between mean proportion of individual fatty acids (g 100 g^{-1} total fatty acids (FA)) of milk produced by cows respectively under control (C) and experimental diet containing pea meal (P-mix).

% FA	C	P-mix	*P*-value
C16:0	31.606	28.621	0.001
C16:0 iso	0.24	0.301	0.043
C17:0	0.639	0.711	0.011
C17:0 ante iso	0.447	0.52	0.005
C17:1	0.232	0.262	0.045
C18:0	10.711	12.259	0.055
C18:0 iso	0.062	0.077	0.006
C18:1 ω7	0.439	0.522	0.052
C18:1 ω9	20.967	23.82	0.002

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Introducing fatty acid profile for milk payment in a dairy plant to valorise milk from permanent grasslands

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Abstract

This work aimed to predict milk fatty acid (FA) concentrations by medium infrared reflectance spectroscopy (MIRS) to calculate a milk FA index usable for milk payment, and to test its application in a commercial dairy plant. Ten FA were selected for their role on human health, cheese sensory properties, or as indicators of animal welfare. A method based on class approach was used. The median of the regional population of 651 bulk-milk samples was set as reference value of Class 0. To each class for each FA a progressive number, and positive or negative signs to the above or below classes, were assigned, depending on their correlation to human health, etc. The sum of the number of each class associated to its sign for each FA generated the milk FA index. This index was multiplied for a supplementary premium, to be added to a base price after reaching a minimum threshold of milk FA index. Milk samples provided by the farms of a commercial dairy plant located in the western Alps were analysed. Farms basing cow feeding on permanent grassland reached a milk FA index greater than zero throughout the year and could potentially increase their standard milk price.

Keywords: fatty acid, milk payment, infrared spectroscopy

Introduction

There is increasing demand from consumers for a greater health-promoting FA composition of dairy products, and an increasing interest by dairy companies to use this as a marketing opportunity. This requires a fast and economic tool for milk FA analysis and the development of strategies to pay supplementary premiums to farmers to move their milk production towards an improved FA profile (Coppa *et al.*, 2014). MIRS can be a useful tool for routine analysis of milk FA, but highly reliable prediction equations have been obtained for only few FA, whereas most FA have been predicetd only approximately (Coppa *et al.*, 2014). The aims of this work were to (1) develop MIRS prediction equation for milk FA composition; (2) to estimate a milk FA index (MFAI) utilizing MIRS developed equations, taking into account their precision, and (3) to test the application of MFAI for milk payment in a pilot commercial dairy plant.

Materials and methods

A total of 651 bulk samples of cows' milk were collected from 245 commercial farms located in north-western Italy to improve calibration equations of milk FA by MIRS presented by Coppa *et al.* (2014). A method based on a class approach to overcome the performance limit of the equations with high standard error of prediction (SEP) was proposed. Each class has a nominal FA concentration value and an upper and lower limit. These limits were calculated as the nominal concentration ± the confidence interval (CI) at P=0.95, calculated as SEP×1.96. The higher the SEP, the larger were the classes. The median of the distribution of a FA in the dataset was set as reference value of the central class (Class 0). The lower limit of the subsequent above (A) class (Class A1) was the upper limit of the previous class (Class 0); the nominal value of Class A1 was calculated as the lower limit of the class plus the CI, the upper limit of Class A1 as the nominal value of the Class A1 plus the CI; and so on for the Class An. The same method was applied for the determination of the classes below (B) the Class 0 (i.e. Class B1).

The wider the range of a FA, the higher the class number. Ten FA were selected and positive or negative signs to the A or B classes were assigned, depending on their role on human nutrition and health (Givens, 2010), on sensory properties of dairy products (Martin *et al.*, 2005), or as indicators of animal welfare (Comino *et al.*, 2015), or of environmental sustainability (Peeters, 2012). The sum of the number of each A or B class, associated with its sign for each of the selected FA, generates the MFAI. For the calculation of milk supplementary premium, the MFAI was multiplied by a unitary monetary value that allowed determination of the amount of the supplementary premium for milk FA composition to be added to a base price. The MFAI was applied to the milk produced in the farms conferring milk to a foothill commercial dairy plant in north-western Italy. Farms were representative of both intensive dairy farming systems of the Po plain, with cow-feeding system based on maize silage and concentrates, and the extensive farming systems of upland areas of north-western Italy, feeding cows with forages from permanent grasslands.

Results and discussion

The validation statistics of the MIRS prediction equations of the FA used for the MFAI calculation are given in Table 1. The classes for the FA used for the MFAI calculation are given in Table 2. The range of variation of MFAI was -4 to 29 (Table 3). Within the intensive dairy farming systems, basing feeding

Table 1. Statistics of the MIRS prediction equations of the fatty acids (FA) used for the MFAI calculation.[1]

Fatty acid (g 100 g^{-1} FA)	Bias	SEP	Slope	R^2V	CI
C18:3n-3	0.06	0.13	1.07	0.77	±0.25
CLAc9t11	0.01	0.13	1.19	0.78	±0.26
ECSFA	-0.04	0.66	0.99	0.95	±0.29
BCFA	0.05	0.21	1.03	0.80	±0.40
MUFA	0.00	0.58	1.03	0.92	±1.13
PUFA	-0.26	0.37	0.90	0.77	±0.73
Total n-3	-0.08	0.15	1.37	0.77	±0.30
n-3/n-6	-0.06	0.09	1.01	0.70	±0.17
C18:1c9/C16:0	0.00	0.05	1.19	0.88	±0.09
C18:1t10/C18:1t11	-0.05	0.12	1.01	0.62	±0.23

[1] SEP = standard error of prediction; CI = confidence interval; ECSFA = even-chain saturated FA; BCFA = branched-chain FA; MUFA = mono-unsaturated FA; PUFA = polyunsaturated FA.

Table 2. Classes for the fatty acids (FA) used for the MFAI calculation.

Fatty acid		Class B1		Class 0		Class A1		Class A2		Class A3		Class A4		Class A5	
(g 100 g^{-1} FA)	limit	value	limit	value	limit	value	limit	value	limit	value	limit	value	limit	value	limit
C18:3n-3			min	0.43	0.68	0.93	1.19	1.44	1.69	1.95	2.20	2.45	max		
CLAc9t11			min	0.49	0.75	1.02	1.28	1.54	1.80	2.07	max				
BCFA			min	1.81	2.21	2.61	3.02	3.42	max						
MUFA	min	26.04	27.17	28.31	29.44	30.58	31.71	32.85	33.98	35.12	36.25	37.39	38.52	39.66	max
PUFA			min	4.50	5.22	5.95	6.68	7.40	8.13	8.86	max				
Total n-3			min	0.62	0.92	1.21	1.51	1.81	2.11	2.41	max				
n-3/n-6			min	0.24	0.42	0.59	0.77	0.94	1.12	1.29	max				
OA/PA	min	0.52	0.61	0.71	0.77	0.86	0.96	1.05	max						
t10/t11	min	0.30	0.53	0.77	1.00	1.23	1.47	1.70	max						
ECSFA	min	49.45	50.74	52.03	53.32	54.61	55.90	57.19	58.48	59.77	61.06	62.35	63.64	64.93	max

[1] ECSFA = even-chain saturated FA; BCFA = branched-chain FA; MUFA = mono-unsaturated FA; PUFA = polyunsaturated FA; OA/PA = C18:1c9/C16:0; t10/t11 = C18:1t10/C18:1t11.

Table 3. Range of variation of the MFAI in the milk from intensive and extensive farms.

Fatty acids[1]	Intensive farms	Extensive farms	
		Winter	Summer
C18:3n-3	0 to 0	0 to 1	1 to 4
CLAc9t11	0 to 0	0 to 0	1 to 3
ECSFA	-1 to 0	0 to 3	2 to 5
BCFA	0 to 0	1 to 2	1 to 2
MUFA	-1 to 0	0 to 2	2 to 3
PUFA	0 to 0	0 to 0	0 to 3
Total n-3	0 to 0	0 to 1	1 to 3
n-3/n-6	0 to 0	1 to 1	1 to 3
C18:1c9/C16:0	-1 to 0	0 to 1	1 to 2
C18:1t10/C18:1t11	-1 to 0	1 to 1	1 to 1
Total MFAI	-4 to 0	3 to 12	11 to 29

[1] ECSFA = even-chain saturated FA; BCFA = branched-chain FA; MUFA = mono-unsaturated FA; PUFA = polyunsaturated FA.

system on maize silage and concentrates, the milk did not reach MFAI values >0, while in the extensive dairy farms applying winter feeding system based on hay from permanent grasslands and a low proportion of concentrates, the MFAI ranged from 3 to 12. The MFAI of milk from pasture-based diets applied in extensive farms during summer ranged from 11 to 29. The dairy plant that tested the MFAI proposed a fixed price for the milk, similar to the average of the current market price (0.35 € l^{-1}), giving an additional 0.01 € l^{-1} at each point of MFAI over 7, with a maximum of 0.15 € l^{-1}. A MFAI of <7 did not give any additional premium and the milk is collected separately. This system could lead to an increased income for dairy farmers using permanent grassland both in winter and in summer.

Conclusions

A tool for milk payment based on a careful use of milk FA prediction equations developed by MIRS was provided, and was shown to help dairy plant managers and farmers to valorise their milk price in relation to FA composition.

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Factors affecting herbage and milk fatty acid composition from permanent grasslands in the Italian Alps

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Abstract

This research aimed to highlight which factors, related to climatic conditions and herbage characteristics, can affect the fatty acid (FA) composition of permanent grasslands and remain determinant in influencing the FA composition of derived milk. The FA profiles of 159 herbage samples from permanent grasslands and their chemical and botanical compositions, and the characteristics of growing site were collected in the Italian western Alps. The milk from grazing herds and the related diet composition were collected. A principal component analysis was performed on herbage FA composition and growing conditions. A correlation matrix was performed on milk FA concentrations, herbage characteristics and diet composition. Concerning herbage FA composition, C16:0, C18:0 and C18:1c9 concentrations were positively related to herbage DM content, legumes proportions and phenological stage, whereas relations were negative for C18:3n-3. The C18:1c9, C18:2n-6 concentrations were also positively related to growing degree days, rainfall, and herbage ADF content. Concerning milk, C18:3n-3 concentration was positively correlated to herbage DM content, whereas negatively to herbage crude protein (CP). The CLAc9t11 concentration was negatively correlated to herbage phenology. Branched-chain FA concentration was positively correlated to herbage DM, NDF, and ADF content and phenology, and negatively correlated to herbage CP and PUFA content.

Keywords: fatty acid, herbage, milk, permanent grasslands

Introduction

Permanent grassland are strategic in the production of typical or labelled dairy products, as they are able to confer specific traits to dairy products (Martin *et al.*, 2005). These traits can vary according to the herbage characteristics, such as botanical composition, nutritive value, phenological stage, etc. Among these aspects, the herbage FA composition can have an important impact on ruminal lipid metabolism (Chilliard *et al.*, 2007), thus changing the milk and cheese fatty acid (FA) profile and sensory properties (Martin *et al.*, 2005), with important implication also for human health (Givens, 2010). This research aimed to highlight which factors, related to climatic conditions and herbage characteristic, can affect the fatty acid (FA) composition of permanent grasslands in the Alps and remain relevant in influencing the FA composition of derived milk.

Materials and methods

The FA profiles of 159 herbage samples from permanent grasslands and their chemical and botanical compositions, and the characteristics of growing site and its climatic conditions were collected in the Italian western Alps. At each sampling a 1 l milk sample from grazing cow herds and the related diet composition were collected. The studied permanent grassland was located in 42 different sites at various altitudes, from valley floors to upland pastures. The botanical composition of permanent grasslands was determined through botanical surveys (Braun-Blanquet, 1932), and the phenological stage of the dominant grasses was determined by using the BBCH scale (FBRCAF, 2001) at each sampling. The herbage chemical and FA composition and the milk gross and FA composition were determined according to Coppa *et al.* (2015a). To highlight the relationship between herbage FA composition and growing conditions, a principal component (PC) analysis was performed on such data. A correlation matrix was performed on milk FA concentrations, herbage characteristics and diet composition to understand the relationships among these factors.

Results and discussion

Herbage fatty acid composition

The PC1 was positively related to herbage dry matter (DM) content, legumes proportion, phenology, and herbage C18:1c9, C16:0 and C18:0 concentrations, and negatively to rainfall and herbage C18:3n-3 concentration (Figure 1). On the other hand, PC2 was positively related to growing degree days (GDD), rainfall, herbage ADF content and herbage C18:2n-6 concentration, and negatively related to herbage ether extract (EE) and crude protein (CP) contents. High concentration of C16:0, C18:0 and C18:1c9 in herbage were associated with herbage DM content, legumes proportions and advanced phenological stage, whereas relations were negative for C18:3n-3. The herbage C18:1c9, C18:2n-6 concentrations were also associated with higher GDD, rainfall, and herbage ADF content and low CP and EE content in herbage. These results on mountain pasture of diverse composition are consistent with observations on lowland grassland (Elgersma *et al.*, 2103).

Milk fatty acid composition

The fresh herbage proportion in the cow diet was one of the main drivers determining milk FA composition (Table 1) (Coppa *et al.*, 2015b). The concentration of C18:3n-3 in milk was positively correlated to herbage DM content and forbs proportion, whereas it was negatively correlated with herbage CP content. The CLAc9t11 concentration was negatively correlated with herbage phenology (Coppa *et al.*, 2015b). The C16:0 and the even-chain saturated FA (ECSFA) concentration in milk was positively correlated with herbage DM content. Branched-chain FA (BCFA) concentration was correlated with high herbage DM, NDF and ADF contents, and late phenology, and with low herbage CP and PUFA contents.

Conclusions

This work has confirmed that climatic conditions and herbage chemical and botanical composition and phenology occur together to affect herbage FA concentrations on botanically diverse upland pastures. Herbage ADF and DM contents (and herbage PUFA content), herbage phenological stage and botanical composition appear relevant also in influencing the FA composition of pasture-derived milk.

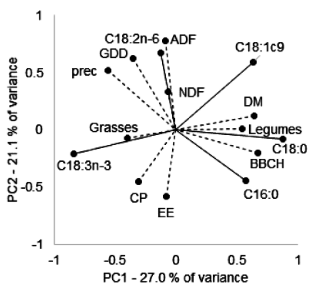

Figure 1. Principal component (PC) analysis on herbage fatty acid composition (full lines) and herbage chemical composition and growing conditions (dotted lines). GDD: =growing degree days; prec = precipitation (mm); BBCH = herbage phenological stage; DM = dry matter content, NDF = neutral detergent fibre; ADF = acid detergent fibre; CP = crude protein; EE = ether extract.

Table 1. Correlation matrix between herbage characteristics and composition (first column) and milk FA composition (first row)[1]; only significant correlation coefficients are shown.

	C16:0	C18:1c9	C18:2n-6	C18:3n-3	CLAc9t11	ECSFA	BCFA	MUFA	PUFA	n-6/n-3
C18:1c9 herb.	-0.229		0.29	0.23		-0.18			0.23	
PUFA herb.	0.21	-0.14				0.27	-0.47	-0.16		0.23
Herbage %	-0.70	0.12	0.34	0.58	0.44	-0.65	0.37	0.37	0.66	-0.51
DM	0.41	0.16		0.36	0.15	0.45	0.42	0.23	0.39	0.36
NDF	-0.15	0.23	-0.29		0.14	-0.23	0.32	0.20		
ADF	-0.14			0.10	0.14	-0.20	0.33		0.17	
CP	0.27	-0.11	-0.13	-0.38		0.30	-0.37	-0.10	-0.33	0.23
EE	0.25	0.15	0.28	-0.21	-0.23		-0.25	0.11	-0.22	0.32
Legumes %			0.14							
Forbs %	-0.11		0.28	0.29			-0.12		0.21	
BBCH				0.12	-0.38		0.38	-0.11		-0.15

[1] herb. = herbage; BBCH = herbage phenological stage; DM = dry matter content, NDF = neutral detergent fibre; ADF = acid detergent fibre; CP = crude protein; EE = ether extract.

Acknowledgements

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Monitoring grazing behaviour of Sarda cattle using an accelerometer device

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Abstract

Monitoring grazing behaviour is increasingly important for understanding the relationships between production and welfare of ruminants and their nutrition and management, particularly in extensive livestock systems. Methods of assessing behavioural activity have changed in recent years, favouring automatic recording techniques and information and communication technologies. A recent device (BEHARUM), equipped with a three-axial accelerometer sensor has been developed coupling the use of MEMS and ICT. The sensor, inserted in a halter, measures feeding behaviour by sampling accelerations due to jaw and head movements. Three acceleration values at 1-s intervals are then sent to a nearby server by a LoRa wireless module that can communicate to a remote computer via GSM. Calibration and validation approach of the system is ongoing in Sarda cattle reared in natural pasture comparing acceleration data automatically classified in three activities, grazing, ruminating and resting (including other activities) with video-recorded data. A data mining approach followed by multivariate analyses procedures allowed correct assignment of 88.2% of minutes to the three activities (88.8%, 89.7% and 86.1% for grazing, ruminating and resting respectively).

Keywords: ICT, accelerometer, beef cattle, feeding behaviour, multivariate analysis

Introduction

Monitoring ruminant behaviour is important to understand how animals meet their requirements in pastoral systems by grazing a dynamic vegetation to achieve optimal plant production, animal forage intake and performances (Carvalho, 2013). Recent managerial tools, based on Precision Livestock Farming approach, focus on the individual instead of the whole herd to control animal grazing, rumination and resting behaviours on pasture (Andriamandroso *et al.*, 2016). The use of animal-attached sensors for this scope is now a common practice; in particular, accelerometers attached to animals allow the measurement of animal behaviour in environments that preclude direct observation, thus saving time and field effort. Moreover, the application of ICT to the world of agriculture has allowed the driving of management decisions in real time. The BEHARUM device, through the use of wireless modules, makes it possible by gathering acceleration data from the cattle's jaw and sending them to a remote computer for post processing. The aim of this work is to investigate if this device, already tested with grazing sheep (Giovanetti *et al.*, 2017), can discriminate the feeding behaviour of grazing cattle raised on natural pasture.

Materials and methods

The BEHARUM device includes a muzzle equipped with a three-axial accelerometer sensor positioned under the lower jaw of the animal. Animal head and jaw movements are detected through accelerations measured in the X (longitudinal), Y (horizontal) and Z (vertical) axis. The acceleration sensor, inserted in a micro-electromechanical compact system (MEMS), samples raw accelerations, converts them in digital values and selects three converted values per second per axis (Giovanetti *et al.*, 2017). Acceleration-converted data are sent (LoRa wireless system) to a nearby receiver computer equipped with an antenna or to a remote computer through a local server using GSM services. A 5-day calibration trial has been conducted at Bonassai experimental farm of AGRIS, located in the NW of Sardinia (40° 40' 16.215" N, 8° 22' 0.392" E, 32 m a.s.l). In each experimental day a Sarda beef cattle was equipped with the BEHARUM device and enabled to free range for 4 hrs in a 2-ha paddock of natural pasture. Acceleration data were recorded and acquired in a receiving computer in a csv file. At the same time cattle behaviour was video-recorded by a fixed camera. The 5-day dataset collected from the animal was used to calculate from the acceleration data the following variables: mean, variance and inverse coefficient of variation (ICV; mean ± standard deviation) per minute for each axis as well as the resultant mean, variance and ICV of the three axes (Watanabe *et al.*, 2008). Moreover, the total number of acceleration per minute and per axis was also calculated. On the basis of the video recording, the behaviour of the animal was classified, at 1 min intervals, into three activities: grazing, ruminating and resting. Resting activity includes cattle lying down, standing or walking in the absence of jaw movement. The dataset generated from the 15 acceleration variables combined with the three behavioural activities was analysed by using three multivariate statistical techniques: stepwise discriminant analysis (SDA), canonical discriminant analysis (CDA) and discriminant analysis (DA). To evaluate the performance of DA for discriminating the three behaviour activities, sensitivity, specificity, precision and accuracy were calculated, as well as k coefficient.

Results and discussion

SDA applied to the entire dataset selected 14 among the 15 original variables. The subsequent CDA significantly discriminated the three behaviours (Hotelling's test $P<0.0001$) by extracting two canonical functions. The variance explained by CAN1 and CAN2 was 0.73 and 0.27, respectively.

Figure 1 shows that canonical functions can separate the three behavioural activities. In particular, CAN1 discriminates the resting activity from the grazing and ruminating, whereas CAN2 differentiates the ruminating from the grazing and resting behaviour. Errors in assignment, specificity, sensitivity, precision and accuracy of DA for discriminating the cattle behaviour activities and k coefficient of agreement between observed and predicted values are displayed in Table 1.

The BEHARUM device correctly classified the three behaviours with a high overall accuracy and statistical agreement (Table 1), as shown by k coefficient, which takes into account the chance agreement between observed and predicted data for each behaviour. In particular, the resting behaviour reported

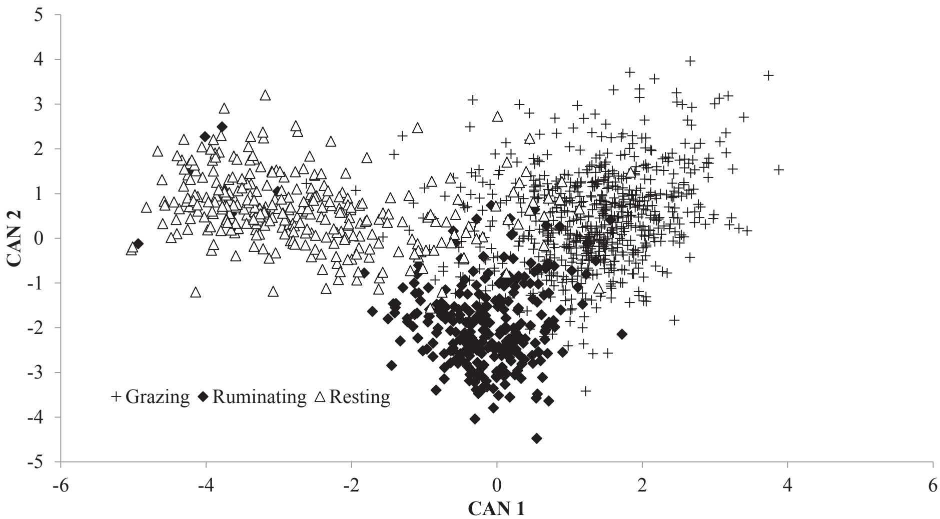

Figure 1. Graph of the two canonical functions (CAN1 and CAN2) obtained in the canonical discriminant analysis applied to acceleration data.

Table 1. Distribution of the total error and performance in the assignment of behaviour activities, predicted on the basis of accelerometer data.

Observed behaviour	Predicted behaviour			
	Grazing	Ruminating	Resting	Total
Grazing (min)	603	65	11	679
Ruminating (min)	22	253	7	282
Resting (min)	26	18	273	317
Total (min)	651	336	291	1,278
Sensitivity (%)	92.6	75.3	93.8	
Specificity (%)	87.9	96.9	95.5	
Precision (%)	88.8	89.7	86.1	
Accuracy (%)	90.3	91.2	95.1	88[a]
k	0.77	0.86	0.82	0.81[b]

[a] Overall accuracy of the discriminant analysis.

[b] Kappa: overall coefficient of agreement ($P<0.001$).

the highest sensitivity and accuracy values, whereas the specificity, precision and k coefficient were the highest for ruminating. These performances are promising if compared to those of similar validation trials based on grazing cattle and the same time epoch (1 min) (Watanabe *et al.,* 2009; Yoshitoshi *et al.*, 2013).

Conclusions

The BEHARUM device has proved to be a useful tool for automatically determining the behaviour of cows at pasture. Further work is needed to strengthen the calibration with more animals tested in different environments. As the next step, validation trials, that could help to understand its applicability even in marginal areas, have to be planned.

Acknowledgements

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Nutritive value and legume content of multi-species swards managed under four cuts per year on organic farms

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Abstract

Increasing forage production is a key strategy to reach feed self-sufficiency on cattle farms. On organic farms, multi-species swards (MSS) are commonly sown and often contain at least 5 plant species. Our objective was to monitor the forage yield, nutritive value and botanical composition of different MSS along the growing season on commercial farms. In 2015, five MSS (MSS1 to 5) were studied on three farms located in Wallonia (Farm1: 3 MSS; Farm2: 2 MSS; Farm3: 2 MSS). MSS3 was present in the three farms. Recorded parameters included the proportion of plant species per cut, and the dry matter (DM) yield, chemical composition, digestibility and nutritive value after pre-wilting. Annual DM yield was mainly linked to sward management and agricultural area (few differences between MSS within farms). Across all cuts, on average (weighted mean), crude protein content (CP) was less than 15% DM, and organic matter digestibility reached 75%. CP increased from cut 1 to cut 3. Plant species proportion varied according to the cut, farm and MSS.

Keywords: grass-legume proportion, yield, nutritive value evolution, organic farming

Introduction

Increasing forage production is crucial to reach feed self-sufficiency on cattle farms. On organic farms, grasslands are often the basis of ruminant diets. In such grassland systems, multi-species swards (MSS) are commonly sown to secure forage stocks. The species in MSS can present various functional and structural advantages and, due to their complementarity, contribute to stabilize grassland yield, both in quantity and quality under low input management systems. This is particularly important under erratic weather conditions. In such a context, this study aimed to follow the performances of several MSS on commercial farms and, in particular, the evolution of forage yield, nutritive value and proportions of grasses and legumes in relation to the number of cuts.

Materials and methods

In 2015, commercial multi-species swards (MSS) from temporary grasslands were monitored on three organic dairy farms in Wallonia. MSS were studied at each cut, and four cuts were taken. All MSS seed mixtures contained both grass and legumes species (Tables 1 and 2). The dry matter (DM) yield (t DM ha^{-1}) was measured after pre-wilting at each cut. Sampling was performed at the cut to determine the MSS botanical composition and after pre-wilting to evaluate the forage quality. Forage samples were oven dried (60 °C, 48 h) to determine the DM content. Botanical composition of MSS was obtained by hand-sorting. Plant species were oven dried (60 °C, 48 h) to determine the proportion of each species in MSS, expressed as % of dry weight. All dried samples were ground in a hammer mill and in a Cyclotec mill (1 mm screen, FOSS), and submitted to Near Infrared Reflectance analysis (FOSS – XDS NIR system). Crude protein (CP), cellulose (CEL) and *in vitro* organic matter digestibility (OMD according to De Boever *et al.,* 1988) were estimated according to NIR model developed at CRAW. Nutritive value of forage was then estimated according to the VEM-DVE system.

Table 1. Proportion of seeds in the multi species swards (MSS) at sowing.[1]

	Trifolium pratense	*Trifolium repens*	*Trifolium* sp.	*Medicago lupulina*	*Lotus corniculatus*	*Lolium* sp.	*Dactylis*	*Festuca*	*Phleum*
MSS1	0.06	0.12				0.20	0.17	0.26	0.19
MSS2	0.06	0.06	0.09			0.31	0.15	0.27	0.06
MSS3	0.20	0.12	0.10	0.05	0.05	0.26	0.08	0.06	0.08
MSS4	0.10	0.10				0.54		0.27	
MSS5	0.09	0.05	0.05	0.02	0.02	0.28	0.31	0.14	0.04

[1] *Trifolium* sp. = *Trifolium resupinatum* and/or *Trifolium hybridum*.

Table 2. MSS per farm, type of soil and date of cuts (C1, C2, C3 and C4).[1]

	Farm1			Farm2		Farm3	
	MSS1	MSS2	MSS3	MSS4	MSS3	MSS5	MSS3
Soil type	Sandy-loam (85%)			Loam (89%)	Loam (82%)	Loam (87%)	Loam (89%)
Surface (ha)	2.5			3.2	3.9	6.2	3.2
Sowing (year)	2012 (autumn)			2014 (autumn); under cover of barley and oat for MSS3		2013 (autumn)	
Date of cuts	20/05; 08/07; 31/08; 29/09			15/05; 29/06; 06/08; 26/09		08/05; 13/06; 30/07; 23/09	
Fertilisation	compost			slurry: 22 m^3 ha^{-1} before sowing (MSS3) and 18 m^3 ha^{-1} after C1		digesta: 20 m^3 ha^{-1} in spring	

[1] MSS = multi species sward; C = cut.

Results and discussion

DM yield, chemical composition and nutritive value at harvest are presented in Table 3. Total annual DM yield was 9.9±1.2 t ha^{-1}. This result was in agreement with the national Belgian statistics (INS, 2013). Annual DM yields were quite similar to each other at the farm scale. On average, they appeared lower on Farm2 probably linked to sward management (sowing year). On average, C1 contributed to 43% of the DM yield. C1 was late and accounted for more than 50% of DM yield on Farm1, which resulted in a lower energy value of the silage. MSS from Farm 2 and Farm3 were cut earlier (C1) and therefore had a lower CEL content and were more digestible than MSS from Farm1. Total DM content was higher than the 45% ideal value for pre-wilted silage.

Table 3. Annual dry matter (DM) yield, chemical composition and nutritive values of multi species sward (MSS).

		DM yield (t ha^{-1} $year^{-1}$)	C1 (Proportion in annual DM yield (%))	C2	C3	C4	DM %	CP % DM	CEL % DM	OMD % DM	VEM kg DM^{-1}	DVE g kg DM^{-1}
Farm1	MSS1	11.6	54.4	20.0	18.8	6.7	52.6	10.2	28.0	72.6	881	69.9
	MSS2	9.3	50.2	18.4	21.1	10.3	50.4	12.4	27.6	74.0	887	76.1
	MSS3	10.7	53.7	19.8	18.0	8.6	52.0	10.9	28.0	71.4	878	71.3
Farm2	MSS4	8.2	38.3	23.2	14.0	24.5	55.5	12.2	23.4	78.5	951	82.4
	MSS3	8.9	34.0	21.5	17.7	26.8	63.0	12.9	24.1	77.0	935	82.6
Farm3	MSS5	10.5	36.3	24.3	17.3	22.1	48.2	10.6	23.5	81.2	972	81.3
	MSS3	10.4	39.1	17.4	23.3	20.2	46.7	12.2	24.4	77.0	928	80.6
Mean		9.9	43.7	20.7	23.3	20.2	52.6	11.6	25.6	75.9	919	77.7
SD		1.2	8.7	2.5	3.0	8.3	5.4	1.0	2.2	3.5	37	5.4

The botanical composition per cut is given in Table 4. On Farm1 and Farm2, the proportion of legumes increased from C1 to C3, regardless the MSS. On Farm3, the proportion of legumes was lower for all cuts and MSS. CP content increased from C1 to C4 for all MSS on Farm1 and Farm2, as a consequence of the higher legume proportion in C2 and C3 and of nitrogen mineralisation (C4). Ryegrass was the most abundant grass in C1. Ryegrass and especially *Lolium multiflorum*, present in all MSS, is a competitive species characterized by a rapid spring growth. In autumn (C4), *Dactylis* showed a good regrowth and even exceeded that of ryegrass. Fescue and timothy were scarce. Their highest proportions were found in MSS1 and MSS2 which contained the highest proportion of these species in seeds mixture. Red clover was the main legume species in C3.

Conclusions

This descriptive study provides an illustration for a group of farmers of changes in grass-legume proportions of MSS. In temporary grasslands, species equilibrium is variable and depends on grassland management, which was not studied here. On this basis, MSS present satisfactory annual DM yields and produce good quality forage. As expected, legume proportion increased from C1 to C3, which influenced both forage quantity and quality. The variability in species proportions among cuts and MSS remained high. The control of these proportions, using adapted management schemes, remains a key challenge for MSS.

Table 4. Grass-legume proportions and crude protein content per multi species sward (MSS) cut.[1]

Parameters	Cut	Farm1			Farm2		Farm3		Mean (SD)
		MSS1	MSS2	MSS3	MSS4	MSS3	MSS5	MSS3	
White Clover (% DM)	C1	3.6	15.4	8.6	5.1	6.0	5.7	4.2	6.9 (4.0)
	C2	13.0	37.7	13.1	12.5	17.2	13.8	8.9	16.6 (9.6)
	C3	5.9	23.0	8.2	12.0	15.0	22.0	18.4	14.9 (6.6)
	C4	20.8	33.5	18.8	20.2	20.9	21.1	3.6	19.8 (8.7)
Red Clover (% DM)	C1	16.9	2.2	12.0	6.6	5.0	8.2	17.0	9.7 (5.8)
	C2	42.9	7.7	40.7	24.4	22.8	1.5	12.0	21.7 (15.9)
	C3	76.3	37.4	65.3	75.8	70.2	10.1	24.6	51.4 (27.0)
	C4	15.7	3.8	16.6	49.3	58.3	10.7	32.6	26.7 (20.6)
Ryegrass sp. (% DM)	C1	70.0	22.7	46.9	45.8	67.8	79.8	67.5	57.2 (19.7)
	C2	36.7	9.9	37.9	32.7	53.1	79.2	70.0	45.6 (23.7)
	C3	4.1	7.7	5.4	10.0	10.4	61.4	32.1	18.7 (21.1)
	C4	2.8	50.6	7.9	5.4	4.4	7.0	2.8	11.6 (17.3)
Dactylis (% DM)	C1	1.1	40.0	30.9	0.3	20.8	6.3	11.2	/
	C2	2.0	34.7	6.4	4.1	2.7	4.8	6.9	/
	C3	12.0	30.9	12.8	0.9	4.4	5.7	19.0	/
	C4	15.4	7.3	41.3	6.9	16.4	38.2	57.0	/
Crude protein (% DM)	C1	7.2	9.6	7.7	8.1	9.9	9.3	10.5	/
	C2	10.3	11.4	10.9	11.2	11.8	8.5	10.6	/
	C3	15.6	16.1	16.0	16.5	13.1	11.2	14.1	/
	C4	20.1	20.2	19.1	14.7	17.5	14.7	14.9	/

[1] C = cut; DM = dry matter; SD = standard deviation

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Parasitism and farmers' practices in heifers under organic farming management

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Abstract

Organic farmers are requested to allow cattle grazing as much as possible. However, this can lead to parasitism issues and thereby affect livestock performance. This study aimed to determine 'good practices' with respect to parasitism control in grazing heifers under organic farming conditions using a participatory approach. Six organic farms, including three dairy and three beef production systems, were monitored in 2015 and 2016. Recorded variables included the heifers' growth and parasitic pressure in terms of eggs abundance in faeces for three distinct parasites, grass availability, the nutritional value of grass and feed supplement, if any, and the farmer's practices regarding the grass and herd management. The relationship between recorded variables was investigated by principal component analysis (PCA). The PCA revealed a positive correlation between heifers' growth and the number of plots. However, heifers' growth and the number of plots were both negatively correlated with stocking rate, occurrence of anti-parasite treatment, and parasitic pressure. In conclusion, this study highlighted the importance of rotational grazing and low stocking rate for control of parasitic pressure and ensuring heifers' development.

Keywords: cattle, grazing, organic, parasitism, participatory research

Introduction

Parasitism is a major challenge to the health and development of grazing cattle. The challenge is even more important in organic agriculture because of its larger reliance on grazing and the restrictions on the use of anti-parasite treatments. However, controlled exposure to parasites helps to build immunity with a limited impact on livestock performance. A livestock production system that manages to control parasite populations is therefore sought. In this context, it has been shown that 'good practices' with respect to herd, grass and feed management can be effective in controlling parasitism (Remience *et al.*, 2013; Singh, 2014). Here, we investigated the relationship between parasitic pressure and farmers' practices in organic dairy and beef farms in order to identify practices to recommend to organic farmers.

Materials and methods

A survey was run on six organic farms, three dairy farms and three beef farms, in 2015 and 2016 (Table 1). All Farms were located in the south-eastern part of Belgium, at elevations ranging from 236 (Farm 1) to 591 (Farm 6) m a.s.l.. Temperature and rainfall were, on average, higher in 2015 than in 2016 during the grazing season (Pameseb, 2016). Lowest temperatures were found in Farm 3, and highest temperatures in Farms 4 and 5. Lowest rainfall was found in Farm 4, and highest rainfall in Farm 1. Loamy-stony soils with shale and/or sandstone load were found in Farms 2 to 6. In Farm 1, the soil was mainly loamy, slightly stony and included clay patches.

Heifers were weighed at the beginning, end, and when possible, the middle of the grazing season. Faeces were collected from five heifers per farm at each weighing time, and the parasitic pressure was noted on scale of 0 to 4 according to eggs abundance for three distinct parasites: *Fasciola hepatica* (FH), *Paramphistomum* sp. (PA), and *Ostertagia* sp. (OS). Also, the level of blood pepsinogen (mUTyr l^{-1}) was measured at the end of the grazing season to assess the acquired immunity against *Ostertagia* sp. The grass

Table 1. Characteristics of the six monitored farms: type of production, breed, altitude, and temperature and rainfall in 2015 and 2016

Farm ID	Type	Breed	Altitude (m a.s.l.)	Temperature (°C)[1]		Rainfall (mm)[2]	
				2015	2016	2015	2016
1	Milk	Holstein (H), Montbeliarde (M), H×M	236	8.2-17	6.2-16.4	533	537
2	Milk	Holstein (H), Montbeliarde (M), H×M	454	7-17.3	6.7-16.9	472	436
3	Milk	Holstein (H), Jersey (J), H×J	569	7.2-16.9	5.2-16.4	423	480
4	Beef	Blonde d'Aquitaine	239	9.6-19.2	8.7-18.9	337	378
5	Beef	Salers	247	9-19.5	8-18.3	415	478
6	Beef	Mixed Belgian Blue	591	8-18.1	6.4-17.1	400	408

[1] Minimum and maximum average monthly temperature between April and October in 2015 and in 2016.

[2] Cumulative rainfall between April and October in 2015 and in 2016.

height and nutritional value were characterized. Recorded farmers' practices included the occurrence of anti-parasite treatment (noted as 0 or 1), the number of plots dedicated to heifers, the stocking rate (number of heifers per acreage), and the nutritional value of possible feed supplements. Nutritional values were estimated according to the VEM-DVE Dutch system (Tamminga *et al.*, 1994; Van Es, 1975).

The relationship between heifers' variables, parasitic pressure, grass variables, and farmers' practices was investigated by principal component analysis. Because the VEM and DVE contents were highly correlated with each other (r>0.92), VEM contents only were considered in the PCA.

Results and discussion

A total of 132 heifers was followed (35, 22, 20, 12, 22, and 21 in Farms 1 to 6). Among these, 86 (55-31), 82 (28-54), and 18 (2-16) were in their first, second and third grazing year in 2015-2016, respectively. Youngest grazing heifers were 4 to 6 weeks old (Farms 1 and 3 in 2015). Heifer's average daily gain (kg day^{-1}) ranged from 0.46 (Farm 1) to 0.66 (Farm 3) in dairy farms, and from 0.43 (Farm 4) to 0.52 (Farm 6) in beef farms.

Lowest parasitic pressure was found in Farms 2 and 3 (20% of heifers on which faeces were collected were positive to FH, PA, or OS in 2015; 0% in 2016), and highest pressure in Farms 1 (100% in both years) and 5 (60% in 2015; 100% in 2016). Heifers were treated in both years in Farms 1 and 6, in 2015 only in Farms 2 and 5, and in 2016 only in Farm 4. No treatment was provided in Farm 3. Rotational grazing was applied in Farms 2, 3 and 5, with 2 to 3 plots dedicated to heifers. The average stocking rate over both years ranged between 1.9 (Farm 3) and 4.3 (Farm 5) heifers ha^{-1} in all farms with the exception of Farm 4, where it was highest with 10.6 heifers ha^{-1}. Feed supplements were provided in Farm 1 [concentrates (cc), grass silage (gs) and hay] and Farm 3 (hay) in 2015 only, and in Farm 4 (cc and hay) and Farm 5 (cc and gs) in both years. A first PCA including all animals with parasitism data was achieved (90 data points, Figure 1). It showed a positive correlation between heifers' growth and the number of plots dedicated to heifers, as well as, to a lesser extent, the grass nutritional value (grassVEM). The heifers' growth and number of plots were both negatively correlated with the stocking rate and occurrence of anti-parasite treatment, as well as, to a lesser extent, with the parasitic pressure (FH and PA, in particular) and supplements' nutritional value (suppVEM). A second PCA including all animals with both parasitism and pepsinogen data was then conducted (57 data points, not shown). The blood pepsinogen level was positively correlated with heifers' growth, suggesting that heifers with relatively high pepsinogen level had acquired immunity, which would have resulted in a higher growth.

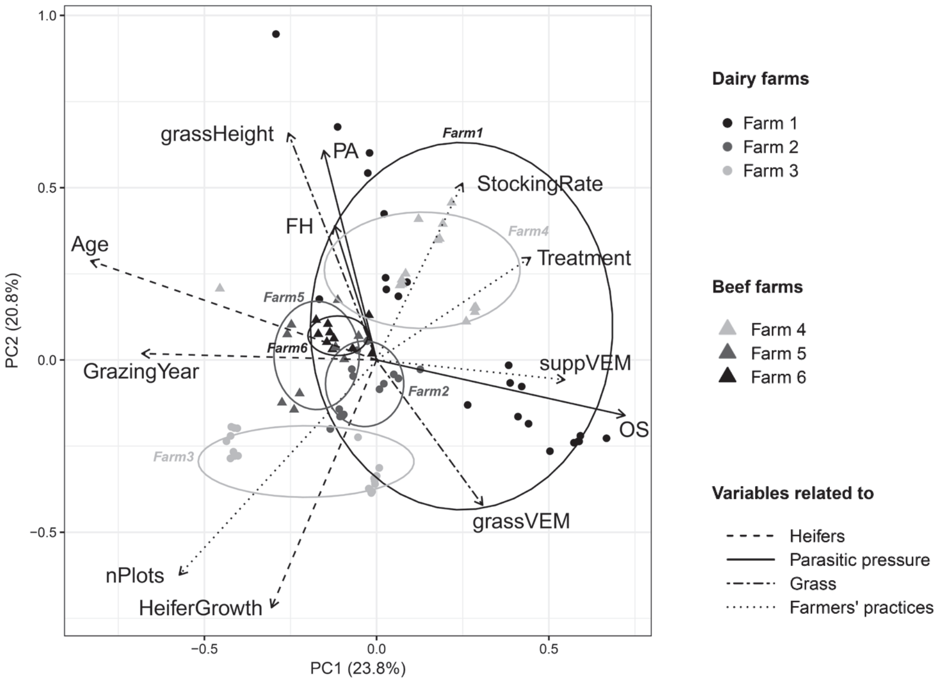

Figure 1. Dispersion of data points according to the first two components resulting from principal component analysis. Each data point corresponds to a given animal observed at a given time in a given farm. FH = *Fasciola hepatica*; PA = *Paramphistomum* sp.; OS = *Ostertagia* sp.

Among the monitored farms, Farm 3 offered an interesting example of recommendable practices for heifers' health management. In contrast, Farm 1 has experienced parasitism issues for a while. Discussion with Farmer 1 led to the decision to apply rotational grazing from 2017, which could provide interesting information on how to reverse high parasitic pressure to a controlled level.

Conclusions

The present study highlighted the importance of rotational grazing and low stocking rate to prevent parasitism issues. Both of these practices were associated with little or no need for feed supplement and anti-parasite treatment as well as with controlled parasitic pressure.

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Productivity of silvopastoral systems under *Pinus radiata* D. Don estimated with the Yield-SAFE model

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Abstract

The Yield-SAFE is a biophysical model to predict the long-term production according to light and water availability in exclusively agricultural and forest systems and also in agroforestry systems. The productivity estimated with the Yield-SAFE model can be used to determine the Land Equivalent Ratio (LER). The LER allows comparison of the productivity of an agroforestry system with the productivity of a monoculture system. The objective of this study was to determine the productivity of exclusively forest and agricultural systems and a silvopastoral system established with *Pinus radiata* D. Don in Galicia (NW Spain) through the Yield-SAFE model to calculate the LER. The results showed that the LER simulated by the Yield-SAFE model was 1.41. This value indicates that the hypothetical silvopastoral system of this experiment produced 41% more than the exclusively forest and agricultural systems, probably due to the diversified production of the silvopastoral systems. Therefore the establishment of silvopastoral systems could be promoted in Europe due to its high productivity compared with the exclusively forest and agricultural systems, which is increased by the non-market products that agroforestry systems support.

Keywords: agroforestry, modelling, LER, sowing, afforestation

Introduction

The silvopastoral systems (SS), in which the woody vegetation is integrated with forage and livestock production on the same land, are characterised by their environmental and economic benefits compared with the exclusively agricultural and forest systems. These benefits are highly difficult to predict due to the interaction of many biophysical and management factors. One option to determine the benefits of SS could be the use of models like Yield-SAFE (Van der Werf *et al.*, 2007). The Yield-SAFE is a biophysical model to predict the long-term production according to light and water availability in exclusively agricultural and forest systems and also in agroforestry systems. The productivity estimated with the Yield-SAFE model can be used to determine the Land Equivalent Ratio (LER). The LER allows comparison of the productivity of an agroforestry system with the productivity of a monoculture systems (Graves *et al.*, 2010). The objective of this study was to determine the productivity of exclusively forest and agricultural systems and a SS established with *Pinus radiata* D. Don in Galicia (NW Spain) through the Yield-SAFE model to calculate the LER.

Materials and methods

The Yield-SAFE model, a process-based modelling concept for agroforestry systems (van der Werf *et al.*, 2007), was previously calibrated for *P. radiata* D. Don and a *Dactylis glomerata* L. pasture in Galicia (NW Spain) (Ferreiro-Domínguez *et al.*, 2014). The Yield-SAFE model was used to determine the production of hypothetical (1) forest systems established with *P. radiata* in which it simulated the management carried out in the yield tables for this tree species in Galicia (Sánchez *et al.,* 2003); (2) agricultural systems with a *D. glomerata* pasture which was harvested three times in the spring and once in autumn to replicate traditional practices in Galicia; and (3) SS in which the pasture was combined with *P. radiata* established at 100 trees ha^{-1} to keep the land eligible for CAP support. In the case of the SS, it was simulated so that the pasture was harvested three times in the spring and once in autumn during the first ten years and then only once in the spring and once in the autumn because the pasture production decreases over time due to the shade provided by the trees. For the simulations we used artificial climate retrieved from the CliPick tool (Palma, 2015) for Lugo (Galicia, NW Spain). The simulations were carried out for 30 years, starting in 2016. The tree and pasture production estimated with the Yield-SAFE model was used to calculate the Land Equivalent Ratio (LER) as:

$$\text{LER} = \frac{\text{pasture production in agroforestry}}{\text{pasture production in agricultural}} + \frac{\text{stand volume in agroforestry}}{\text{stand volume in forestry}}$$

Results and discussion

In agroforestry studies it has been common practice to consider yield benefits in terms of the LER. The predicted LER at the end of this experiment through the Yield-SAFE model was 1.41 (accumulated pasture production in agroforestry = 65.31 Mg ha^{-1}, accumulated pasture production in agricultural = 75.35 Mg ha^{-1}, accumulated stand volume in agroforestry = 4,034.51 m^3 ha^{-1} and accumulated stand volume in forestry = 7,373.37 m^3 ha^{-1}) (Figure 1). This value indicates that the hypothetical SS of this study produced 41% more than the exclusively forest and agricultural systems. A LER greater than 1 has also been observed by other authors, e.g. Dupraz and Newman (1997) in the early years after establishment of a cherry/fescue system (LER=1.6) and by Sharrow (1999) in a young *P. radiata* silvopasture in New Zealand (LER=1.71). In general, the higher productivity of the agroforestry systems compared with the exclusively forest and agricultural systems could be explained by the diversified production of the agroforestry systems. In the agroforestry systems, different species occupy different spaces within the system and therefore their production can be complementary and greater than the sum of production of the individual components if they were grown separately. Moreover, diversified and integrated production of the agroforestry systems can help farmers remain competitive in the markets, as well as provide environmental amelioration and thus social benefits (Rigueiro-Rodríguez *et al.*, 2009). On the other hand, the LER does not take into account the non-market products that agroforestry systems support, such as the regulation of air quality, climate change, water quality or management of pests and diseases, and therefore the productivity of the agroforestry systems is likely to be higher still. Finally, it is important to be aware that the Yield-SAFE model is able to provide useful predictions of yields in agroforestry systems, relative to agricultural and forestry systems, throughout Europe. Therefore, the model could potentially be used to decide how land should be used to meet demands.

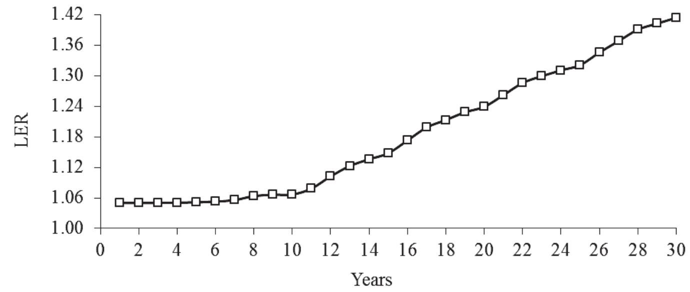

Figure 1. Land Equivalent Ratio (LER) of agroforestry results estimated through the Yield-SAFE model.

Conclusions

The LER simulated by the Yield-SAFE model was 1.41, which indicates that the hypothetical silvopastoral system of this experiment was more productive than the exclusively forest and agricultural systems. This was probably due to the diversified production of the SS. Therefore the establishment of SS could be promoted around the Europe due to its high productivity compared with the exclusively forest and agricultural systems which is increased by the non-market products that the agroforestry systems support.

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Behavioural parameters of suckler cows on outdoor winter grounds in north-east Germany

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Abstract

In many farms in north-east Germany suckler cows are kept outdoors on the pasture area on partly hay or straw-bedded ground during the entire winter season. The herds are continuously supplied with silage, hay, mineral nutrients and water. There are very few behavioural data available for cattle on outdoor winter grounds due to the labour and daylight requirements for such work. During three periods each lasting four weeks, four cows of a suckler cow herd were equipped with collars measuring several behavioural data on winter grounds in 2015/16. The activity, measured by a two-axis activity sensor, was continuously recorded in 5 min intervals over the three periods. The objective of the study was to learn more about the cows′ behaviour on winter grounds during daytime and darkness as well. In comparison with the grazing season, the activity of cows is lower on winter grounds, after the additional grazing has finished and conserved forages are offered alone and *ad libitum*. The day and darkness activity periods proceed similarly. In order to meet the feed requirements, cows must have access to sufficient amounts of conserved feed also during the night time.

Keywords: day light activity, night activity, telemetric measurement, GPS collar

Introduction

Mainly due to economic reasons, in many farms of north-east Germany suckler cow herds are kept outdoors on the pasture area on partly hay or straw-bedded winter ground during the entire winter season, and are continuously supplied with silage, hay, mineral nutrients and water. As long as there is grass growth and no risk of sward damage, cows are allowed to graze on the neighboured pastures until only conserved forage is fed. Many farmers assume that cows are active and covering their feed requirements only during the daylight period and they arrange their feeding system accordingly.

Herd observations are very labour consuming and mostly require daylight conditions. Therefore only a very few behavioural data on cattle on outdoor winter grounds over the whole day are available (Popp, 2010). By means of specific collars behavioural data were recorded in three experimental periods with a suckler cow herd on a winter ground during late autumn and winter of 2015/2016. The objective of this study was to record and analyse the activity patterns of cows and to learn more about the cows' behaviour on winter grounds during daytime and also during darkness.

Material and methods

The study was carried out on an open, unfenced winter ground located on a sandy part within a fen pasture site at Paulinenaue, north-east Germany (52°68'N, 12°72'E, mean annual temperature 9.2 °C, mean annual precipitation 534 mm, Eutric Histosol). At the winter ground the cows had unlimited access to water, mineral feed and grass silage (round bales with a dry matter content of 51 to 82%) offered *ad libitum*. At the feeding place, the cows formed a bed with parts of the grass silage bales and spent most of the time there.

During three 4-week periods, in October-November 2105, December 2015 and January 2016, four cows within our suckler cow herd were equipped with a GPS Collar (Vectronic, 2014). The collar recorded the GPS position of the animals. Its two-axis activity sensor describes the animal's activity, expressed as a scale between 0 for rest situations and 250 for the quickest movements. Data were processed in 5 min intervals. The mean values were checked by t-test and correlated with weather data (Pearson).

Results and discussion

The length of day light decreased during experimental period 1, was shortest in period 2 and increased again in the course of period 3. There was only little rainfall during the entire experiment. The air temperature decreased from 8.7 °C in period 1 to 2.7 °C in period 3 (Table 1). Due to the mild weather conditions, grass was still growing on the surrounding pasture, thus, additional grazing was observed during the period 1 from October to November.

Figure 1 exemplarily shows the activity for cow no. 01 over 24 h in period 2 in December. During sunrise and sunset the activities were higher than in other day times, as well known for normal behaviour of grazing animals. There were also remarkable activities during the darkness. Obviously, the day light time at the end of December was too short for the uptake of the required forage amounts.

The length of the activity periods during day time depended on the day length, with a coefficient of correlation of 0.91 (Table 2). At the beginning of period 1, high activity during day light and low activity

Table 1. Length of day light and mean air temperature during the experimental periods.

period	from – to	day light (h)	air temperature (°C)
1	18 October – 13 November	10.35-8.56	8.7
2	3 December – 30 December	7.59-7.40	7.8
3	13 January – 8 February	8.02-9.23	2.7

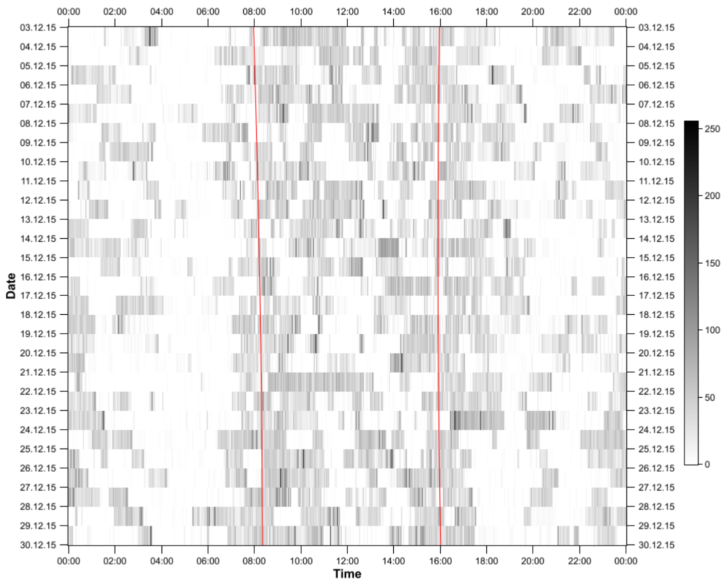

Figure 1. Activity patterns of cow no. 01 for the experimental period 2.

Table 2. Length of activity periods during the three experimental periods.[1]

Period	Activity, day (min)	Activity, night (min)	Activity, day (% of the day light time)	Activity, night (% of the night time)
1	405.9 ^{aA}	284.1 ^{bA}	58.8	37.9
2	254.3 ^{aB}	307.9 ^{bB}	45.2	35.1
3	290.8 ^{aC}	287.5 ^{aB}	50.3	33.4

[1] Small letters indicate significant differences within and capitals between the periods 1-3; t-test (P<0.05).

during darkness was observed. With decreasing day light hours in the experimental periods 2 and 3, the activity periods during day and night became similar and did not differ significantly. During these periods, the activity level was lower than in period 1. Obviously the cows were able to cover their feed requirements more rapidly by consuming grass silage only, than by additional grazing. The cows had to be active and to eat about one-third of the forage during darkness at night time.

There were no significant correlations of activity patterns with air temperature and rainfall. As the results were obtained during a mild winter period the experiment should be repeated under more severe weather conditions, such as snow and severe frost, which are typical for the region.

Conclusions

In comparison with the grazing season, the activity of cows exclusively fed with conserved forage *ad libitum* on winter grounds is lower. The day and darkness activity periods become similar in winter. In order to meet their feed requirements, the cows must have access to sufficient amounts of conserved feed also during the night time. The feeding system has to be adapted accordingly and almost half of the conserved feed amounts must be available during the night time.

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Carcass composition and meat quality of pasture-raised *Mallorquina* sheep in Balearic Islands

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Abstract

Mallorquina, a sheep breed indigenous to Majorca Island (Spain), is adapted to rangeland grazing systems and has made a historical contribution to development of rural areas and maintenance of ecosystems. However, there is dearth of information on the effect of feeding management system on the carcass composition and meat quality of lambs. Management data from 10 farms were collected. Five groups of lambs were differentiated according to feed management and weight: suckling lambs-milk, light lambs-milk, light lambs-pasture, light lambs-concentrate and heavy lambs-concentrate. From each group, 10 lambs were selected randomly and were sacrificed in a slaughterhouse where several carcass measurements were taken: carcass yield, carcass linear measurements and tissue composition. Meat quality attributes such as water holding capacity, cooking loss, Warner-Bratzler shear force and haem pigments were determined. With increased slaughter weight, significant positive differences were observed in yield but other attributes related to quality worsen with the highest carcass weight. Regarding lambs with similar weight and different feed management, significant differences were obtained in weight parameters in lambs fed with concentrate; however, they were worse for carcass yield and some marketing parameters. Lambs raised under traditional grazing systems, which produce commercial medium weight lambs, had the highest quality meat.

Keywords: lamb meat, meat quality, carcass characteristics, pastoral systems

Introduction

Sheep farms, operating under controlled grazing management systems help maintain the balance of ecosystems by reducing the risk of wildfires, soil erosion and loss of biodiversity. Pastoral references of local know-how often prove to be more efficient and better adapted to local conditions than intensive management systems (Dubeuf, 2011). However, these pastoral references are at risk of disappearance mainly due to lack of farm profitability. Differentiation of the production that offers higher quality animal products for human health presents an important strategy to conserve these pastoral systems. A number of studies reported that both milk (Delgado-Pertiñez *et al.*, 2013) and meat (Nardone and Valfrè, 1999) obtained from grazing-based systems offer better attributes of quality than stall-fed production systems.

In Majorca (Spain's largest island in the Mediterranean) there is an autochthonous sheep breed (Mallorquina) raised on pastoral systems. Most Mallorquina sheep are raised on rangelands that consist of grasslands, shrublands and forest ranges. Traditionally, lambs fed on pastures are marketed as light lambs (7-10 kg hot carcass weight (HCW)) fed without external concentrate supply. This type of system also holds special significance as the breed is in danger of extinction, despite having made a remarkable historical contribution to the economic and social development of the Island. Nevertheless, there is little information on the breed management and there is no information on the lamb carcass and meat quality. The objective of this study was to characterize the attributes of carcass and meat quality of Mallorquina in relation to feeding management and in order to improve its conservation.

Materials and methods

Management records from 10 Mallorquina sheep farms were collected monthly during 2015. However, only the data related to feeding management of lambs were taken into consideration in this study. Based on the records, three feeding groups were identified: lambs fed only mother milk (milk); pasture grazing with occasional forage supplementation (pastoral); and pasture grazing with concentrate supplementation (concentrate). In addition, three groups of lambs were classified according to their commercial weights: suckling lambs (less than 7 kg hot carcass weight (HCW)), light lambs (7-10 kg) and heavy lambs (more than 10 kg). Five groups of lambs were classified: suckling lambs-milk, light lambs-milk, light lambs-pasture, light lambs-concentrate and heavy lambs-concentrate. Of each group, 10 male lambs were selected and were taken to the slaughterhouse where different measurements were conducted: carcass yield measurements, carcass linear measurements and tissue composition. The carcasses were then transported to laboratory where other measurements of quality meat were conducted: water holding capacity, cooking loss, Warner-Brazler shear force, haem pigments.

Results and discussion

Regarding lambs within the same feeding group and different slaughter weights (Table 1), we compared two types of lambs: those fed milk only and those fed concentrate. In the group of lambs fed milk we compared suckling lambs and light lambs. Significant differences were found in indicators associated with weight (live weight (LW), HCW, carcass internal length, carcass compactness index) that were higher in heavier carcasses. Higher subcutaneous fat was recorded with the increase of carcass weight because, according to Peña *et al.* (2005), with increase of weight differences are observed in the deposit of fat in the tissues. The dressing percentage was also increased with the increased slaughter weight, leading to significant differences in the water holding capacity, results that agree with Sañudo *et al.* (1997). For concentrate type, we also observed differences in some indicators due to weight (LW, HCW, carcass internal length, carcass compactness index) and, although carcass yield is higher in heavy lambs, they have less muscle in total percentage, which will give carcasses that are less appreciated by consumers who prefer lean meat (Sen *et al.*, 2004).

Table 1. Carcass and meat quality indicators of Mallorquina lambs according to slaughter weight.

Quality indicators	Lambs with the same feed and different carcass weight							
	Milk				Concentrate			
	Suckling lambs	Light Lambs	SEM	*P*-value[1]	Light Lambs	Heavy Lambs	SEM	*P*-value[1]
n	10	10			10	10		
Liveweight at slaughter	11.58	14.34	0.468	**	18.89	22.12	0.464	***
Hot carcass weight	6.14	7.94	0.260	***	8.89	11.02	0.331	***
Carcass yield	53.09	55.56	0.719	ns	47.03	49.73	0.601	*
Carcass internal length	42.46	46.68	0.699	**	49.54	52.69	0.500	***
Carcass compactness Index	144.55	169.77	3.940	***	179.30	209.00	4.969	**
Percentage of muscle	55.25	53.50	0.708	ns	56.85	52.63	0.780	**
Percentage of bone	26.62	26.10	0.430	ns	25.24	25.29	0.504	ns
Percentage of subcutaneous fat	7.17	10.47	0.800	*	6.86	11.84	0.992	**
Percentage of intramuscular fat	6.41	6.08	0.396	ns	6.91	6.13	0.560	ns
Water holding capacity	12.56	14.60	0.439	*	16.08	16.60	0.530	ns
Cooking loss	24.56	22.06	1.035	ns	25.89	23.00	0.844	ns
Warner-Bratzler shear force	5,138	4,474	269.06	ns	5,194	5,215	329.39	ns
Haem pigments	3.20	3.32	0.243	ns	3.93	4.03	0.320	ns

[1] ns = not significant, $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$. SEM = standard error of the mean.

On the other hand, regarding lambs with the same weight and different feeding system (Table 2), we only could compare the light lambs under three feeding management systems. Shepherds fatten lambs with external feed in order to get heavier lambs, and in this sense, lambs that were fed concentrate had higher LW and HCW; however, they produced the lowest carcass yield. In this sense, different studies had demonstrated poor yield in lambs that fed high-energy diets such as concentrates (Russo *et al.*, 1999).

Conclusions

Results indicated that Mallorquina lambs have high meat quality. The meat production was greater under grazing with a semi-commercialization system. This grazing management represents the traditional production systems that are commonly adopted by the island's shepherds. To preserve this system it is necessary to conduct further research to differentiate products by their quality.

Table 2. Carcass and meat quality indicators of Mallorquina lambs according to feed management.[1,2]

Quality indicators	Light lambs with similar carcass weight and different feed				
	Milk	Pasture	Concentrate	SEM	*P*-value
n	10	10	10		
Liveweight at slaughter	14.34	15.42	18.89	0.452	***
Hot carcass weight	7.94	8.03	8.89	0.163	*
Carcass yield	55.56a	52.10b	47.03c	0.507	***
Carcass internal length	46.68	46.07	49.54	0.830	ns
Carcass compactness Index	169.77	174.56	179.30	2.700	ns
Percentage of muscle	53.50	55.50	56.85	0.548	ns
Percentage of bone	26.10	26.00	25.24	0.387	ns
Percentage of subcutaneous fat	10.47a	9.90ab	6.86b	0.548	*
Percentage of intramuscular fat	6.08	5.21	6.91	0.413	ns
Water holding capacity	14.60	13.66	16.08	0.465	ns
Cooking loss	22.06	23.14	25.89	0.725	ns
Warner-Bratzler shear force	4474	4951	5194	253.20	ns
Haem pigments	3.32	4.04	3.93	0.271	ns

[1] Means with different letters (a, b, c) between columns differ significantly. ns = not significant, $P>0.05$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$. SEM = standard error of the mean.

[2] The statistical analyses were realized including hot carcass weight as covariate.

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What is the place of the feed self-sufficiency in the French PDO specifications?

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Abstract

This paper focuses on how feed self-sufficiency is taken into account in French PDO (Protected Designation of Origin) cheese specifications. Farmed areas in France are various: lowland with a major part of grasslands or cereals, pastoral area or mountain area. The local environment determines the way feed self-sufficiency (FSS) is recorded in the various specifications. From the study of all these PDO specifications, we can list the different types of local practices and associated criteria. The study allowed us to build a typology of PDO French cheese specifications based on FSS criteria. With increased constraints, PDO organisations have to reconcile feed self-sufficiency and capacities, building compromises.

Keywords: cheese, PDO, herd feeding, feed self-sufficiency

Introduction

In France, 45 PDO cheeses are produced with cow (28 cheeses), goat (14) or sheep milk (3). The biggest cow-milk cheese productions are *Comté*, *Reblochon*, *Cantal* and *Saint-Nectaire*; *Roquefort* and *Ossau-Iraty* for those made from ewe milk; *Sainte-Maure de Touraine* and *Rocamadour* are made from goat milk. In total, they represent 16% of the French cheese production or 194,322 tons of cheeses in 2015 and 25% of the annual turnover of the French cheese market. In France, the milk for PDO cheese making is provided by 17,950 milk producers (nearly 28% of the French dairy farms). In France, 21% of cow-milk production goes to PDO cheese production, also 93% of ewe milk production is used for PDO cheese production and finally 36% of goat milk production is used for PDO cheese production. PDO cheese productions are deeply ingrained in their environment and are defined as typical products linked to the notions of locally grown/produce and geographic origin (called 'terroir'). The feed self-sufficiency (FSS) is one of the major aspects of the expression of this 'terroir' in the French PDO farming system and also defined by EU legislation (1151/2012, article 5). FSS is defined as the balance between the herd requirements and the forage and feed production on farm and it is currently a major issue in French livestock farming (Brocard *et al.*, 2016). FSS can be also approached on different scales: farms, regions, countries. It is within this framework that the specifications of the 45 French PDO cheeses have been examined in order to identify herd-feeding practices connected to FSS.

Methods

The specifications of the 45 French PDO cheeses were collected from the INAO website (www.inao.gouv.fr) in March 2016. They have been studied by the authors and analysed according a graphic methodology (Bertin, 1977) to highlight the main criteria and their levels related to FSS. Results are presented and discussed hereafter.

Results

A large diversity of criteria to define herd-feeding practices in French PDO cheese specifications

Agricultural areas of French PDO cheese productions are various: lowland with a major part of grasslands or cereals, pastoral area or mountain area, with different local capabilities and practices. For this reason, FSS is taken into account by different ways in the specifications of these PDO cheeses. Criteria recorded in these specifications could be based on a direct measure on the home-grown production of any feed (for example, proportion of the hay produced on the PDO area). Other criteria could also be defined in order to promote practices combined to the valorisation of local resources (for example, a defined pasture period) and promote indirectly FSS. The main FSS criteria are, first, a limitation of the supplementary feed registered in 95% of PDO cheese specifications, and then a fixed level of PDO area FSS registered in 88% of PDO cheese specifications. These criteria, associated with defined thresholds, were identified in the PDO cheese specifications, corresponding to various feeding-herd strategies. For each PDO cheese specification, these criteria should allow the matching of the feed needs of herds and the resources that can be produced at farm or at PDO area level.

Nine main FSS strategies in the French PDO cheese productions

The graphic semiology of Bertin is shown in Table 1 and allows us to highlight 10 types of French PDO cheese specifications, based on FSS criteria analysis. Each type represents a group of PDO cheeses with similar FSS criteria recorded in their specifications. The main group (type number 2 in Table 1) gathers 16 PDO cheeses with a similar FSS strategy based on pasture and PDO area-grown forages. A large diversity of other FSS strategies is observed across all other groups. In 2016, only two PDO cheeses have no recognized specifications of FSS in their standards (they both are working on for a new specification including FSS criteria).

Table 1. Typology of PDO cheeses based on their feed self-sufficiency criteria recorded in their specifications.

	T: Type										
	T 1	T 2	T 2bis	T 3	T 4	T 5	T 6	T 7	T 8	T 9	T 10
Limited supplementary feeding	■	■	■	■	■	■	■	■	■		
Amount of roughage grown on the PDO area	■	■	■	■	■						
Duration of pasture		■	■ pastoralism	■		■			■		
Amount of total feed supply growing on the PDO area				■	■	■	■	■		■	
Rate of concentrates growing on the PDO area					■						
Amount of total feed supply growing on farm								■			
Amount of total roughage growing on farm					■				■		
Number of PDO Cheeses/type	3	16	3	3	3	4	3	3	4	1	2

Three main trends could be identified in these nine different FSS strategies

- Recorded criteria mostly promote the FSS at PDO area level (more than at the farm level): 40 cheeses have to produce milk from home-grown feed (forages and concentrates) produced on their PDO area. Thresholds mentioned are various and also calculation methods (annual, per period, daily).
- Forages are mainly produced in the PDO area: partial or total self-sufficiency for the roughage at the PDO area level is requested in the specifications of 31 PDO cheeses (from 70% to 100%).
- Farming-intensive practices are limited through stringent thresholds: limitation of the supplementary feed for 42 PDO cheeses. The individual animal milk production is consequently limited.

In addition, the specifications of 23 PDO cheeses limit the livestock density (number of animals/ ha), in order to match size of the herd and available resources from the PDO area. Thirty PDO cheese specifications require grazing during a defined period of time, up to pastoralism for some of them.

Conclusions

The study of the 45 French PDO cheese specifications allows the diversity of FSS strategies to be examined. It should be noted, however, that PDO cheese organisations adapt and change these specifications according to new developments and agricultural innovations, and also according to consumer behaviour and demand. Milk and PDO cheese producers should always renew their specifications in agreement with these new trends. Farm FSS is currently a main issue in the French agricultural and food environment. The French PDO cheeses have replied to this challenge at an adequate scale: the PDO area. Innovative initiatives have been identified: forage exchanges between milk producers, supplemented feeds PDO marked, group dealing with information exchange. These could be communicated and developed. PDO organisations have to optimise FSS in a restricted framework. Livestock systems are under constraints, with some limits in fodder productivity and the necessity to maintain natural resources and biodiversity. PDO specifications have to create a coherent framework to find a balance between a set of services and a goal of production. That is why the 'French Terroirs Cheeses Network' (www.rmtfromagesdeterroirs.com) proposes European-wide exchanges on this subject, which can be set up through the EIP (European Innovative Partnership, www.eip-agri.eu).

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Design and assessment of multispecies pastures for sustainable dairy goat production systems in western France

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Abstract

Greater use of multispecies pastures can be a viable option for improving the sustainability and protein self-sufficiency in goat farming. A 3-year study was conducted to measure the forage yield, botanical composition and nutritive value of a multispecies pasture that was established both on-station at INRA (FERLus-Patuchev) and on eight farms (REDCap network) under a large diversity of soil and climatic conditions and of pasture utilization (grazing, cutting or both). In autumn 2012, multispecies seed mixtures containing 9 species were sown on 11 fields (19 hectares). The mixture was composed of 60% grass and 40% legumes. On the experimental farm, total annual dry matter (DM) yield from the ungrazed (exclusive cutting) plots was 10.0±2.3 t ha^{-1}. Average crude protein (CP) content of forages was 17.8±2.8% DM. On farms, the specific diversity increased over the years, thanks to sown species and due to germination of adventitious plants. On average, the DM yield and CP content of the second cut forage were 3.1±1.8 t DM ha^{-1} and 14.1±2.9% DM, respectively. This study presents an initial step for designing multispecies pastures for goat grazing.

Keywords: multispecies pastures, dairy goat, sustainability, western France

Introduction

Achieving self-sufficiency is a major goal for dairy farming to alleviate the negative effects of high input costs and environmental pollution. Increasing home-grown feedstuff and protein production to improve protein self-sufficiency which is currently quite low (38%) are the main challenges of dairy goat farming in western France (Broccard *et al.*, 2016). It is also vital to improve the traceability of feed and reduce the dependency on imported proteins. Multispecies pastures present a potential for improving dry matter production and protein self-sufficiency in goat farming (Lüscher *et al.*, 2014; Protin *et al.*, 2014). The network REDCap and the experimental platform Patuchev of INRA work on these research areas, through applying agroecological principles (Altieri, 2002), such as (1) designing farming systems based on biological regulations and interactions between the components of the farm, (2) increasing local feed resources and inputs of self-sufficiency, (3) working with local actors (Caillat and Jost, 2015). This approach was used to design and assess multispecies pastures for sustainable dairy goat production systems in western France.

A 'passkey' multispecies pasture co-built by breeders, advisors and researchers

Lucerne and red-clover are the most important legumes used in goat diets in western France (Caillat *et al.,* 2016). However, under unfavourable cropping conditions or in the context of more persistent and sustainable grasslands, multispecies pastures are an important alternative for promoting the feed and protein self-sufficiency of goat farms. It is also a way to produce a well-balanced feed suitable for cutting with more flexibility (Huyghe *et al.,* 2008). As there are few references available for western France, our challenge is to design a multispecies pasture that is: (a) suitable for goat farming; (b) productive, sustainable and rich in protein; (c) adapted to different uses (grazing or hay) and soil types. To answer to these objectives, a multispecies pasture was designed by the REDCap, e.g. goat breeders, advisors and researchers.

A same multisward in different environments

A trial was conducted on both the experimental farm (FERLus-Inra) and on eight farms (REDCap) in Poitou-Charentes during three years. In Autumn 2012, a similar sward of 9 species composed of 60% of grass (*Lolium perenne* & *Lolium multiflorum*, *Festuca arundinacea* & *Festuca pratensis*, *Phleum pratense*) and 40% of legumes (*Medicago*, *Trifolium pratense* & *Trifolium repens*, *Lotus corniculatus*). The seed mixture was sown in 11 fields (19 ha), of which three were located on the experimental farm of INRA (1 ha is only cut for hay and 2 ha are used for both hay making and pasture). The seed density was 26.7 kg ha^{-1} (about 1,500 seeds m^{-2}). Selected cultivars were diploid and non-alternative and according to earliest heading date. This study is carried out under a large variety of soil types (dry, hydromorphic, healthy), soil textures (silt, clay-limestone) and forage utilization (cutting and/or grazing) conditions to assess the suitability of the multispecies pasture mixtures for different environments.

Sward measurements

This study was conducted on farms and on the experimental farm, with the same evaluation of botanical diversity and yields before the second cut/grazing of every year. During three years, yield is estimated once a year, through biomass density of 6×0.25 m^2 plots per field. From each plot, two subsamples were collected to measure botanical composition and nutritive value of the forages.

Additional continuous measures were made after each use on the experimental farm, to produce annual references. Yield is measured by harvesting of total biomass. For grazing fields, grass yield has been calculated by multiplication at each cycle the number of days of grazing (effective × time) by the average feeding intake of the herd, estimated by a simplified model of intake capacity of INRA (Delagarde *et al.*, 2017). Botanical composition of each cutting is estimated by proportion of each species from sampling of 6 plots ha^{-1} of 0.2×0.3 m.

For biochemical analyses, all samples are dried at 60 °C for 48 h and ground to pass a 1 mm screen and analysed by the reference methods at LABCO of Surgères. Feeding values are calculated from tables (INRA, 2007).

Results: a short-term productive grassland

On average, the second cut produces 3.1±1.8 t dry matter (DM) and crude protein (CP) content was 14.1± 2.9%. This represents 500 kg of CP ha^{-1}. On the experimental farm, herbage yield was by 9.4±2.2 t DM per hectare per year, with differences between exclusive cutting (10.0±2.3 t DM) and grazing/cutting (8.8±2.5 t DM) plots. Harvesting type had few consequences on CP content, at 17.8%±2.8 DM or on energy levels, at 0.82±0.09 UFL (feed unit for lactation).

During the first and second year, *L. multiflorum* and *T. pratense* represented more than 75% of the botanical composition of the pastures. The proportion of *L. perenne* and *T. repens* were low but stable during the 3-year study (respectively 3% and 6%). *Medicago* did not appear in the botanical composition of pastures, except in three fields during years 2 and 3. In the third year, the proportions of *L. multiflorum* and *T. pratense* decreased, which was favourable for the development of *F. arundinacea* and *F. pratensis* (+28% between year 2 and 3). *Lotus corniculatus* and *P. pratense* rates also improved. With years, we can notice a better balance between species, even if legumes stay low (between 40 to 30%). Finally, we can notice a development of non-sown species which doubled (from 10 to 20%). These non-sown species may sometimes be interesting (in terms of yield or feeding value), as *Rumex obtusifolius*, *Dactylis glomerata*. Finally, during the third year, grass represented 70% of DM. This evolution was also observed on the experimental farm during the fourth year, where 86% of the field consisted of grass. These facts provide answers about the durability of this mixture.

Prospects

Forage and protein yields are high with a low-input management. Yields are mainly made thanks to two short-term-productive species (*L. multiflorum* and *T. pratense*). But these species are also well-known to be aggressive compared with *Medicago* or *Festuca*. This lack of diversity limits the persistence of the mixture.

Moreover, the amounts of legume remain quite low during the experiment (43 to 30%) and *Medicago* was absent, except in 3 fields (<12% of DM diversity). Two facts may explain this evolution: (1) *Medicago* was not inoculated; and (2) the fields were sown late (at the beginning of October 2012). Indeed, autumn 2012 was not favourable conditions for sowing legumes: dry weather before seeding (12.5 mm of rain in August, 60 mm in September) and rainy weather after seeding (183 mm in October) (MeteoFrance data – Lusignan).

This trial allowed us to highlight that multispecies pastures can provide an opportunity to develop protein self-sufficiency for dairy goat farms. But the multisward still needs to be designed, and other mixtures have to be assessed. A second trial is in progress, focusing on two main challenges: persistence and site-specific adaptation. As *Medicago* suffers from the aggressiveness of *L. multiflorum* and *T. pratense*, the main adaptations are to remove *L. multiflorum*, reduce *T. pratense* and increase *Medicago*. Other species will stay in similar amounts in the mixture. Seeding conditions will also be checked, so as to be favourable for legume development.

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Diet selection of three small ruminants: implications for multispecies concepts

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Abstract

It is known that low-intensity grazing of fen grasslands, especially in combination with reduced stocking rates, may cause patch selection and favour the spread of weeds. Multi-species grazing may counteract these detrimental effects since the concept is based on the experience that ruminant species differ in their food spectrum. We studied the browsing selection of a sheep breed (Skudde), mouflon and fallow deer on drained, shallow fen grasslands, located northwest of Berlin (Germany) over two growing seasons. The results of diet selection on paddocks with a single animal species in each case indicated that sheep and fallow deer could be fitting partners for multi-species grazing. The parallel-performed multi-species grazing with these two species resulted in homogenously grazed plant stands. The spread of weeds, such as *Cirsium arvense* (L.) Scop. and *Urtica dioica* L., could be effectively suppressed, particulary by the browsing behaviour of fallow deer. Live weights achieved and reproductive performance were indications that the site conditions accommodated the needs of sheep and fallow deer, whereas mouflon failed to adapt to the local conditions and, consequently, they cannot be recommended for grazing on fen sites.

Keywords: extensive use, forage preference, grazing regime, weed control

Introduction

It is well known that extensive grazing conditions promote food selection, which is associated with the development of patchy vegetation and a mosaic of different sward heights (Ludvíková *et al.*, 2015). This can favour the development of pernicious weeds (Kaiser and Fischer, 1997; Tallowin *et al.*, 2005) especially on nutrient-rich soils. Ruminants are differently specialised in their diet consumption (Hofmann, 1989). The use of mixed herds may facilitate pasture maintenance if the included grazing species complement each other in their food preference. Thus far, there has been relatively little study of the role of wild ruminants in such grazing concepts.

Materials and methods

The study area was located in a drained fen area near Paulinenaue (federal state of Brandenburg), approximately 50 km northwest of Berlin. The site may be described as a shallow-undulated type of fen grassland over sand. The study paddocks were extensively managed for 20 years with the result that a site-adapted grassland vegetation could become established. The grazing experiment was carried out from the beginning of May 2013 to beginning of December 2014. We used three single-species groups (Skudde sheep breed, European mouflon and fallow deer) with each group grazing on 1 ha grassland. Each group included 10 dams with their offspring. Additionally, we studied a mixed group (sheep/fallow deer) on 2 ha of grassland containing 10 females with offspring per species. At the beginning of May, each paddock was separated into two equal sub-paddocks with electric fences. One of these two sub-paddocks was used for early pasturing, and the other was cut in June for haymaking. After a second growth had developed in the mowed sub-paddocks, animals were brought to the second sub-paddock in August. Haymaking and early grazing were alternated in the two units of a paddock annually. From December to April, the sheep were kept in a pen whereas mouflon and fallow deer stayed outdoors on separate paddocks. Outside the growing seasons, the animals were fed on hay. The browsing rate and cover-percentage of plant species were studied in June and September of each sample year. The assessment was based on 20 ×20 m-grids. We used a 4-level rating scale with 0: no browsing, 1: top browsing (ca 7%), 2: half browsing (ca 50%), and 3: complete browsing (ca 90-95%). We assessed the cover percentage of a single plant species at each browsing level in a grid cell and calculated the mean browsing level per species and grid. Subsequently, we transformed the browsing values into selection indices after Jacobs (1974), a method that is based on the relation of single species rate of browsing to the total rate of browsing per grid. In addition, we determined animal weights and reproductive performances. All test animals were weighed only once, at the end of each growing season, as the mouflon and some of the fallow deer were extremely shy and fearful. The escape distance was assessed visually by counting the fencing posts. We verified significant deviations of the selection index from zero by means of the one-sample t-test using the software IBM SPSS Statistics v. 22. The used coefficient of variation in percent (CV%) is the standard deviation related to the arithmetic mean.

Results and discussion

Although fallow deer favoured high-quality forage plants, they did not refuse weed plants such as *Urtica dioica* L. (Figure 1). In spring and summer, sheep and mouflon also favoured *Phalaris arundinacea* L., a species that is typical of nutrient-rich drained fen grasslands in north-east German lowlands. Sheep and mouflon browsed on *Cirsium arvense* (L.) Scop; however, the extent of browsing was not great enough to prevent the spread of this weed species. In contrast, the low percentage of *C. arvense* on paddocks with fallow deer was nearly unchanged after two years.

In summer 2014, to obtain a better establishment of these findings, we brought an additional group of fallow deer to a weedy paddock containing 10% *U. dioica* as well as 10% *C. arvense*. After three weeks, the mean browsing level of *U. dioica* was 45% and that of *C. arvense* was 30%. Four weeks later, the browsing level was 60% for *U. dioica* and 57% for *C. arvense* (under conditions without forage shortage). An example is shown in Figure 2. The variability of mean browsing rates per grid in Table 1 illustrates that the involvement of fallow deer resulted in more homogeneous grazing.

The results of the animal-specific characteristics shown in Table 2 provide conclusions about the site adaptation of each species. With few exceptions, the measured values are in the species- and breed-specific normal ranges. We conclude that fallow deer and Skudde are suited for grazing on fen sites. This is

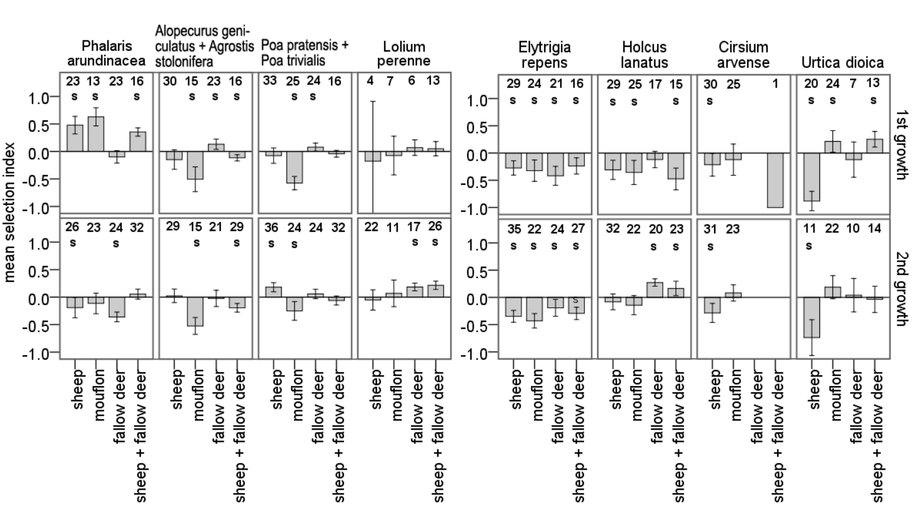

Figure 1. Forage selection of the main and weed species across study years. Values in the upper parts are the numbers of analysed grids containing the respective species. The letter 's' denotes significant deviation of the mean from zero (*P*<0.05).
Values >0: forage preference, values <0: forage avoidance, values =0: mean browsing rate.

Figure 2. *Urtica dioica* browsed by fallow deer.

Table 1. Variability of browsing rates between the grids of a paddock (CV% average over years and growths).

Animal species	CV%
Sheep	51.9
Mouflon	47.8
Fallow deer	31.3
Sheep + fallow deer	33.3

Table 2. Results of animal-specific characteristics (standard deviation in brackets).

	Fallow deer	Mouflon	Sheep (Skudde)
Weight (male)	78 kg (6.0)	49 kg (10.6)	44 kg (7.8)
Weight (female)	53 kg (6.6)	33 kg (11.5)	34 kg (13.1)
Offspring per litter	1.1 (52.6)	1.2 (54.6)	1.6 (42.8)
Survival rate (after 4 month)	81.1%	58.3%	93.8%
Escape distance	40-50 m	60-80 m	10-20 m

clear from their weight development, the number of offspring per litter and the loss rate. In contrast, mouflon is unsuitable for grazing on fen sites. The high lamb losses reflected this. Furthermore, parasite susceptibility and claw problems occurred. The high escape distance of fallow deer and mouflon hinder their health management.

Conclusions

Fallow deer and the Skudde breed of sheep are suited for grazing on shallow-undulated fen grassland sites. The results of forage selection indicate that they are fitting partners for mixed grazing. In contrast, the European mouflon is inappropriate for fen grassland sites.

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EuroDairy – a European thematic network for dairy farming

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Abstract

EuroDairy is a Horizon 2020 Thematic Network to connect dairy farmers wishing to improve the economic, social and environmental performance of their farms, and to provide a more sustainable future for their families. EuroDairy encompasses 40% of dairy farmers, 45% of cows and 60% of EU milk production, drawn from emerging as well as mature milk-producing regions of Europe. The network focusses on four key issues affecting future sustainability: Socio-economic resilience, Resource efficiency, Animal care and Biodiversity. The project follows the interactive model set out by the European Innovation Partnership for Agricultural productivity and Sustainability (EIP-AGRI), putting farmers at the centre of practice-based innovation, adapting and developing new and existing scientific knowledge to produce implementable solutions, which can then be shared across the network. Groups of farmers coming together in the regions (as 'Operational groups') are linked, so that good ideas can be captured and exchanged. Pilot farmers operating excellent levels of physical and financial performance, demonstrate best practice, and push boundaries in the application of new knowledge. EuroDairy facilitates intensive interactions, so that innovations identified in one country or region can be shared with another, via a range of media and easily accessible end-user materials.

Keywords: dairy farming, farm network, resource efficiency, communication tools, EIP-AGRI

Introduction

The European Innovation Partnership on Agricultural Productivity and Sustainability (EIP-AGRI) aims to foster competitive and sustainable farming and forestry that 'achieves more, and better, from less'. It contributes to ensuring a steady supply of food, feed and biomaterials, while respecting the need to work in harmony with the essential natural resources on which farming depends (EIP-AGRI, 2016). The European Rural Development Policy and EU's research and innovation programme Horizon 2020 are mechanisms for funding agricultural innovation projects. Horizon 2020 funds multi-actor projects and thematic networks involving partners from different EU countries. At the national and regional level, EIP-AGRI embraces an 'interactive innovation model' which brings together specific actors (e.g. farmers, advisors, researchers, businesses, etc.) to work together in so-called multi-actor 'Operational groups', in order to find solutions to specific problems or to exploit concrete opportunities. Operational groups are funded by Rural Development programmes, as part of CAP's second pillar budget (Measure 16 – Cooperation).

EuroDairy (www.eurodairy.eu) is one of the early H2020 thematic networks, established to support dairy farmers to improve the economic, social and environmental performance of their farms, and to provide a more sustainable future for their families. EuroDairy connects partners in 14 European countries, encompassing 40% of dairy farmers, 45% of cows and 60% of EU milk production, drawn from emerging as well as mature milk producing regions of Europe. Other H2020 thematic networks include Hennovation (www.hennovation.eu), which started in 2015, with a focus on welfare issues in laying hens. A thematic network on sustainable productivity of grasslands in Europe (Inno4Grass) started early 2017.

Focus on key topics

EuroDairy concentrates on key topics affecting EU dairy farming following the cessation of the milk quota system. It focusses on four key issues affecting future sustainability: Socio-economic resilience, resource efficiency, animal care and biodiversity, aiming to deliver implementable solutions through effective knowledge exchange and increased uptake of innovation and best practice. The challenge is to adopt innovative communication strategies and tools which involve and 'speak to' end-users. The focus is not on research, but on building bridges between research and farmers, business and advisory services. The specific goals are:

- accelerate the uptake of best practice through knowledge exchange;
- capture and stimulate further innovative practices to provide solutions or overcome barriers to effective implementation;
- synthesise scientific and practice-based knowledge;
- produce end-user material;
- disseminate widely to European dairy farmers;
- identify end-user requirements for further R&D.

Within the four topic area, EuroDairy promotes the formation, and cross border connection of over 40 operational groups. In each partner country, a network of innovating pilot farmers (120 in total) has been established, to demonstrate the translation of knowledge and techniques into locally-adapted, best-practice.

Resource efficiency

More efficient use of resources (e.g. feed, fertiliser, fossil fuel, land and labour) is one of the biggest challenges for dairy farming in Europe, directly affecting competitiveness, social acceptability and environmental sustainability (Aarts *et al.*, 2015; Elsaesser *et al.*, 2015). Through the application of best practice, and practice-based innovation, EuroDairy aims to improve the efficiency of dairy farms in the use of resources, in order to increase technical performance, improve profitability and reduce environmentally damaging emissions to air and water. Depending on the quality of management applied, only 20-40% (N) and 30-90% (P) in farm inputs are exported as milk and meat (Dairyman, 2014). Dairy farms have to operate within EU regulations, notably the Nitrates Directive, Water Framework Directive, and National Emission Ceilings Directive. How well these regulations can be met, and conversely, the latitude available to farm within these constraints, are directly related to farmers ability to manage N and P efficiently. Therefore, improving N and P use efficiency is an eco-efficiency priority, and a prerequisite for sustainability of dairy farming. Due to regional differences in the regulatory environment and in approaches to resource management, much can be learned by exchanging information between regions.

Farm N and P balances (farm purchases and exports) will be quantified on the pilot farms, using agreed standard methodologies adapted from Dairyman (Dairyman, 2013). Optimizing the efficient use of home-produced feed (Laidlaw and Šebek, 2012) is an important component of improving resource efficiency on dairy farms. Purchased forages, and particularly concentrates, not only increase cost, but result in the importation of additional nutrients onto the farm system, which subsequently need to be managed if adverse impacts on the environment are to be avoided. The introduction of a permitting system to limit phosphorous loading on dairy farms in the Netherlands is one example of an increasingly challenging regulatory environment and a potent driver for more efficient management practice on dairy farms.

Communication activities

There are around 1 million dairy farmers in Europe – over 400,000 in those countries participating in EuroDairy. They represent a diverse population of potential end-users differing in demographics, formal agricultural education, business skills, linguistic abilities, preferred communication channels, willingness to adapt to face future challenges, and receptiveness to new and innovative ideas. A study by DairyCo in 2013 segmented Great Britain's dairy farmers into 'Monetisers' (motivated by financial drivers), 'Legacy' (motivated by the desire to leave a viable business to the next generation), 'Diversifiers' (seeking to expand business interests beyond dairy farming), 'Settled' (not looking to change from their current level of performance) and 'Exit' (planning to leave the industry within the next five years). Not only do these segments differ in how they are motivated (which determines how knowledge exchange is best targeted) but also in how they acquire and consume information.

Influencing such a large community brings greater challenges than in some other agricultural sectors, which are smaller and more vertically integrated. To influence maximum numbers of a very diverse stakeholder community, EuroDairy has developed and implemented a comprehensive and multi-layered communication plan. It combines tried and tested approaches with innovative web-based tools and social media. The communications engine within EuroDairy is an 'open working lab' (http://eurodairy.eu/the-eurodairy-network). This collaborative tool is multi-actor centred, highly participatory and open sourced. Harnessing the 'wisdom of crowds' (dairy farmers, advisors and scientists) in this way is expected to be an effective and innovative way to capture new and creative ideas from dairy farm practice, and aid the production and refinement of end-user materials.

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Milk production and cow behaviour in an automatic milking system with night-time pasture access

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Abstract

In Scandinavia, pasture for dairy herds with automatic milking (AM) is frequently offered purely for exercise and recreation, rather than as a feed-source. In the present study, cows in an AM-system with 12 h nightly outdoor-access from summer solstice until mid-September were offered either fresh production pasture (treatment *P*; ≥15 kg dry matter (DM) cow^{-1} nightly, combined with 6 kg DM grass silage daytime) or exercise pasture (treatment E; <1 kg DM cow^{-1} combined with *ad libitum* silage allowance day and night). Treatment showed a significant effect on milk yield (P:31.3, E:33.0 kg, P=0.05), and a tendency for milking frequency (P:2.25, E: 2.37 milkings × day^{-1}, P=0.06). Group P spent more time outdoors than E, 4.0 and 3.2 h, respectively (P<0.001). Cows in P grazed approximately 2.5 h throughout the season, while E grazed less overall, 0.6 h (P<0.001) and decreased their time spent grazing over the season (1.0 to 0.3 h). In conclusion, night-time pasture is poorly exploited by cows, irrespective of the quantity of both of pasture and silage that are available.

Keywords: dairy cows, automatic milking, grazing, restricted grazing, supplements

Introduction

Restriction of grazing time may offer the advantages of both pasture and indoor feeding for milk producers. Comparisons between cows on production and exercise pasture in an automatic milking system (AM) have been reported with daytime grazing (Spörndly *et al.*, 2015) and with morning and evening access to pasture (Kismul *et al.*, 2016). Charlton *et al.* (2013) found that cows showed a higher motivation to go to the pasture area at night-time compared to daytime, thus suggesting night-time grazing as a potential strategy for improved exploitation of the pasture resources. A comparison between production pasture and exercise pasture was performed in a management system with AM where grazing was restricted to night-time, testing the following hypotheses: (1) cows on production-pasture spend more time outdoors and graze more than cows on exercise-pasture; (2) milk production of cows on production pasture combined with restricted silage allowance (6 kg dry matter cow^{-1}) is similar to that of cows on exercise pasture combined with *ad libitum* silage in the barn; and (3) Voluntary grazing time decreases as the season progresses, regardless of grazing system.

Materials and methods

During a 12-week grazing trial, 41 cows of the Swedish Holstein (37%) and Swedish Red (63%) breed were blocked with regard to breed and parity, and thereafter randomly assigned to two treatment groups. Both groups had 12 h night-time grazing (18-06 h) during which cows in both groups were allowed to move freely between barn and pasture, while they were restricted indoors during the day. Drinking water, silage, and concentrates were provided indoors for both groups. One treatment group (production pasture; P) was given access to new production pasture at an allowance of ≥15 kg dry matter (DM) cow^{-1} each night and 6 kg DM silage in the daytime, while the other group (exercise pasture; E) was given access to the same exercise pasture each night throughout the experiment with *ad libitum* access to silage both day and night. A group of non-experimental cows spread evenly across the two treatments were also included in the herd giving a total of 57-60 cows in the herd during the entire experimental period to mimic typical AM farming with regard to cow numbers. The exercise and production pasture areas comprised approximately 0.5 and 6 ha, respectively.

Commercial concentrates were fed to both groups according to each animal's milk production before the experimental start, adjusted every fortnight assuming standardised weekly drops in milk yield (MY) after peak lactation (-0.125 and -0.33 kg milk for primi-, and multiparous cows, respectively) according to the Scandinavian feeding system NorFor. Milk yield, milking frequency (MF), and the individual intakes of silage and concentrates were registered automatically. Silage and pasture were sampled every weekday and pooled weekly (pasture) or every second week (silage) for analysis of nutritive value. The behaviour of all cows was observed for a full outdoor-access period (12 h) on 6 occasions over the experimental period. Location (walkway or pasture/paddock), position (standing or lying), and activity (grazing or other) were registered every 15 min for each individual cow in the outdoor area.

Pasture height was measured daily in the production pasture area. These measurements in combination with established regressions between sward height and herbage mass from the same pasture, were used to calculate daily pasture allowance for the P group. The E pasture was mowed at the lowest possible height prior to experiment start and on two subsequent occasions, leaving virtually no grass for the cows to graze at any time.

The data were analysed in a mixed model using SAS (ver. 9.4; SAS Institute Inc.). For analysing the behaviour data, the model contained the fixed variables treatment, parity, breed, season (observation occasion 1-6), and interaction between treatment and season. Animal ID was included as a repeated subject. In the analysis of the averages for MY and MF over the experimental weeks the same fixed factors mentioned above were used, but without the factor season and including the covariate, i.e. MY and MF, respectively, before experimental start. The results are reported as least square means with standard error.

Results and discussion

The nutrient contents of the different feed sources, as well as the intakes of silage and concentrates in each treatment group, are presented in Table 1. Cows in E had significantly higher MY (Table 2). There were, however, no significant differences in MF. The cows in P spent more time outdoors and grazing while those in E had a higher outdoor laying time (Table 2). As the season progressed, the cows in P maintained similar grazing hours while cows in E lost motivation for grazing as the nights became darker, decreasing

Table 1. Nutrient content of feeds, experimental mean with standard deviation in parenthesis and intake of silage and concentrates for cows on production (P) and exercise (E) pasture, least square means ± standard error of mean.

	Roughages		Concentrates[3]	
	Silage[1]	P-pasture[2]	Basal conc.	Protein conc.
Nutrient content of feeds				
Dry matter (DM), g kg^{-1}	356 (54.6)		880	880
Metabolisable energy, MJ kg^{-1} DM	11.3 (0.44)	10.3 (0.41)	13.5	14.4
Crude protein, g kg^{-1} DM	126 (10.0)	167 (22.0)	170	280
Neutral detergent fibre, g kg^{-1} DM	448 (17.0)	406 (22.1)	270	250
Intake cow^{-1} day^{-1}				
Consumption P, kg DM	5.9±0.28		7.9±0.06	2.1±0.03
Consumption E, kg DM	14.5±0.30		7.9±0.06	2.1±0.03

[1] NH_4-N, % of total N: 6.4. Acids, g/kg DM: lactic acid 75.9, acetic acid 11.8, propionic acid 1.2, butyric acid 0.5, formic acid 1.44.

[2] Exercise pasture was not sampled for chemical analysis as there was virtually no grass available to cows on this pasture.

[3] Tabulated values from manufacturer ('Komplett Fiber 170' and Konkret Mega 28'; Lantmännen, Sweden).

Table 2. Production and behaviour data (least square mean ± standard error) for cows on production or exercise pasture, based on experimental averages.

	Production	Exercise	Significance
Milk yield[1], kg	31.3±0.64	33.0±0.44	P=0.05
Milking frequency[1], n day^{-1}	2.3±0.05	2.4±0.05	P=0.06
Outdoor time[2], h	4.0±0.14	3.2±0.15	P=0.0001
Grazing time[2], h	2.5±0.07	0.6±0.07	P<0.0001
Lying outdoors[2], h	0.4±0.07	1.0±0.07	P<0.0001

[1] Based on daily recordings from the automatic milking system over 84 days.

[2] Based on 6 observation studies.

their grazing time from 1.1 h night^{-1} on 28 June to 0.4 h on 10 September (P<0.0001). The results from the present experiment differ considerably from the results presented in two previous experiments with a similar design but where daytime grazing (Spörndly *et al.*, 2015) or morning and evening grazing (Kismul *et al.*, 2016) were practised instead of night grazing. In those experiments, no significant difference in milk yield was observed between cows on production and exercise pasture, in contrast to the results from the night-grazing experiment presented here. It is probable that the difference in milk production was at least partly due to the comparatively short time spent grazing in the night grazing system, but the lower average content of metabolisable energy in the pasture, compared with the silage, may also have contributed to the results. Although night-time grazing has been suggested as a strategy to avoid heat stress in dairy cows, the higher milk yield obtained for cows on E indicates that this treatment may give better production results in AM systems where grazing is restricted to night-time.

In conclusion, the results support the hypothesis that cows on production pasture spend more time outdoors and graze longer hours than cows on exercise pasture. The data showed significantly lower milk yield for the cows on the production pasture with restricted silage access compared with the cows on the exercise pasture and *ad libitum* silage access. Thus the results do not support the second hypothesis of similar milk production in the two pasture systems studies. Finally, there was a difference between treatments with regard to the effect of season, where cows on the exercise pasture spent significantly less time grazing as the season progressed, while no such effect was observed for the cows on the production pasture.

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Effect of low input alpine grazing systems on fatty acid profile and coagulation properties of milk

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Abstract

The effects of two alpine farming systems on the local breed's milk were investigated in the Aosta Valley (AV). The traditional system, in which cows are sheltered in barns, even during summer, i.e. with restricted time at pasture, was compared with full-time grazing where cows were milked at pasture. Two groups of eight multiparous Aosta Red Pied (ARP) cows were selected from two herds and managed under these two conditions and monitored during spring and in two alpine transhumance periods. Grass was complemented with concentrate at 1-3 kg/cow/day. Milk yield (MY), composition, fatty acid (FA) profile and cheese-making properties were determined. The MY was not affected by the system, but decreased as the season progressed (P<0.001). The system did not affect milk composition or cheese-making properties (P>0.05), but it did affect the milk FA profile during first grazing period (group × time, P<0.001). Accordingly, milk from the traditional system had a higher proportion of mono-unsaturated FA whereas milk from full-time grazing had a higher proportion of n-3 FA. This indicates that ARP cows respond differently to the system in their milk fat quality. This response could have been the result of the cows ingesting forage of different composition and is still under investigation.

Keywords: Aosta Red Pied, fatty acid profile, alpine grazing, open-air system

Introduction

Mountainous areas are marginal environments in which intensive milk production is prohibited. Agriculture is maintained through extensive breeding and differentiated products. In the Aosta Valley (AV), Fontina DOP (semi-hard cheese from raw milk) is traditionally produced from the milk of three local cow breeds including Aosta Red Pied (ARP), which graze high alpine pastures in summer. However, high alpine farms are being abandoned. A possibly more cost-efficient system, based on keeping the cows always outdoors, and which is possible only with the availability of mobile milking parlours, was compared with the traditional system of the AV where the cows leave the barn twice a day for a total of 6 h/d for grazing. The effects of the farming system were investigated to assess if milk yield (MY), and its quality and coagulation properties (MCP), would be impaired by adopting the outdoor system.

Materials and methods

Two groups of eight multiparous ARP cows (123±38 d and 110±32 d in milk, respectively) coming from (1) a traditional grazing management system with milking and resting time in the barn (R) and (2) an open-air grazing management system with mobile milking machine (O) were studied. Cows were sheltered in the barn throughout the winter and moved to alpine pastures in June. The R-cows grazed 6 h d^{-1} and were milked and rested in the barn during the remaining time. In O-system cows remained outside for 24 h d^{-1} where they were milked with a mobile milking parlour. Concentrate (around 3 kg cow^{-1} d^{-1} in lowland and 1 kg cow^{-1} d^{-1} on the alp) was distributed at milking time to both groups. The experiment lasted from April to August 2015, with three sampling sessions representing the lowland barn (LB) and two levels of alpine pastures. In LB, cows were fed *ad libitum* with hay in the barn during the night and fresh grass during the day. In the alpine pastures, cows grazed on the low alp (AL, 1,600 m a.s.l.) then the high alp (AH, >2,000 m a.s.l.). Four replicates of 10 cm wide and 5 m long strips of grass were collected during the 4 d of milk sampling and botanical composition was investigated using the point-quadrat approach (Daget and Poissonet, 1971). MY was monitored with a Lactocorder® (WMB AG, Balgach, Switzerland) during four milkings (every month, one week separating two paired milkings). Evening and morning milk samples were pooled, in order to analyse fat content and somatic cells count (SCC). Coagulation time (r), strengthening time (K_{20}), and curd firmness (A_{30}) were measured using Formagraph (Foss Electric A/S). FA profile of milk samples stored at -20 °C were analysed, as described by Leiber *et al.* (2005). The FA investigated were groups of polyunsaturated (PUFA), monounsaturated (MUFA), and omega 3 (n-3) FA. Data were analysed using the MIXED procedure of SAS (version 9.1 Inst. Inc., Cary, NC, USA). Effects of the farming system (O or R, 'group'), the period (LB, AL, or AH, 'time') and their interaction were integrated as fixed factors. Time was considered as repeated factor, with cows nested within group as subject. Significance was fixed for $P<0.05$.

Results and discussion

The MY decreased with time whereas fat content increased (Table 1, $P<0.001$), coinciding with the increase in the stage of lactation of the cows and vegetation maturity. The exposure to various constraints for R and O-cows could explain the absence of difference in MY, even if R-cows were supposed to rest more. There was a general increase of SCC in milk following stage of lactation (from 129×10^3 in LB to 191×10^3 cells/ml in AL; $P<0.01$). After the cows arrived in AL, SCC increased by 15% for R-cows and by 61% for O-cows. We can therefore assume that staying on the alpine pastures did affect the cows' health status, regardless of the system. Between LB and AH an overall decrease in saturated FA (at least -10% for both systems, $P<0.05$) and increase in unsaturated FA was noticeable ($P<0.001$). However, differences between systems occurred only on AL. The SFA proportion decreased by 4% and 12% in the milk of O- and R-cows, respectively ($P<0.05$) whereas MUFA proportions increased by these percentages ($P<0.001$). In contrast, the n-3 FA proportion showed a higher increase (52% compared to LB; $P<0.001$) in the milk of the O-cows. Milk protein content (data not shown) was not significantly affected either by the alpine farming system or by time.

O-pastures that were grazed during AL time showed a greater abundance in *Poaceae* (38.0 versus 22.6%) and *Fabaceae* (14.9 against 10.7%), whereas R-pastures had a higher abundance in *Apiaceae* (19.2 versus 9.1%) and *Asteraceae* (25.2 against 8.8%). Global concentration of polyphenols may have been affected, as

Table 1. Milk yield and milk composition of Aosta Red Pied cows.[1]

	Group	Time			SEM	*P*-value		
		Lowland	Low alp	High alp		Group	Time	Group×Time
Milk yield (kg/milking)	R	7.69^{a}	6.25^{bc}	4.75^{d}	0.433	0.978	<0.001	0.003
	O	7.30^{ab}	6.78^{ab}	4.66^{cd}				
SCC (10^3/ml milk)	R	118^{b}	139^{a}	162^{a}	49.1	0.268	0.006	0.137
	O	126^{b}	214^{a}	265^{a}				
Fat (%)	R	3.16^{c}	3.69^{ab}	3.73^{ab}	0.193	0.626	<0.001	<0.001
	O	3.34^{bc}	3.32^{bc}	4.24^{a}				
Saturated FA (% of fat)	R	66.8^{a}	59.5^{c}	57.4^{c}	0.99	0.250	<0.001	<0.001
	O	66.0^{a}	63.2^{b}	58.6^{c}				
MUFA (% of fat)	R	28.7^{b}	34.6^{a}	36.1^{a}	0.64	0.117	<0.001	<0.001
	O	29.3^{b}	30.4^{b}	34.9^{a}				
PUFA (% of fat)	R	4.51^{c}	5.87^{b}	6.56^{a}	0.252	0.449	<0.001	0.207
	O	4.66^{c}	6.36^{ab}	6.56^{ab}				
n-3 FA (% of fat)	R	0.93^{c}	1.09^{b}	1.32^{a}	0.058	0.097	<0.001	<0.001
	O	0.89^{c}	1.35^{a}	1.41^{a}				

[1] O = open air management system; R = barn system; FA = fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; SEM = standard error of the mean.

pastures rich in dicotyledons (especially *Apiaceae*) tend to have a higher content of secondary compounds than pastures rich in *Poaceae* (Bugaud *et al.*, 2011). These plant metabolites have an inhibitory effect on ruminal biohydrogenation, leading to a higher proportion of unsaturated FA in milk (Khiaosa-ard *et al.*, 2011). Botanical composition here seems to have affected the MUFA proportion more than that of PUFA. The exact reason of this is hard to find as the effect of a mixture of plant secondary compounds is not well known yet, and may depend on their chemical composition, their complex interaction with components of the diet and a possible microbial adaptation of the ruminal flora to polyphenols (Morales and Ungerfeld, 2015).

Conclusions

The present study showed that ARP cows are robust enough to adapt to open-air alpine farming conditions. The main differences found in FA profile seem to be due to differences in botanical composition of the pastures and not to the system. The breed's ability to adapt to the open-air kind of system could allow breeders to use more marginal lands without stables.

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Does land fragmentation currently limit grazing in dairy farms in Lower Saxony, Germany?

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Abstract

A higher share of grazing is frequently viewed as a tool to tackle several challenges on dairy farms: reduced feed costs, reduced environmental impacts, improved animal welfare and better matching with consumer expectations. However, it is unknown whether an increased share of grazing by dairy cattle is a feasible and efficient option given the existing structure of dairy farms. Several aspects influence the ability of a dairy farm to integrate grazing into their feeding regime. One of them is the availability of consolidated grassland in sufficient proximity to the barn or the milking parlour. We analysed the aspect of land fragmentation for dairy farms in Lower Saxony, Germany, using different indices based on IACS (Integrated Administration and Control System) data. We link these indices to the development of different farm characteristics, for instance total area of arable land, percentage of grassland, or number of dairy cows, over a time span of five years. The aim is to determine the possible effect of land fragmentation on decisions regarding herd size and grassland percentage indicating a more grass-based or a more crop-based feeding system.

Keywords: land fragmentation, dairy farms, grazing, Lower Saxony, IACS data

Introduction

Many studies focus on the possible advantages of grazing on dairy farms. They discuss the possible reduction of forage costs, the pros and cons with regard to environmental impacts, the effects on animal welfare and health, or the compliance with consumer expectations. However, whether stabilizing or even increasing the percentage of grazing is a feasible and sensible option for a dairy farm depends also on its ability to integrate grazing into the feeding regime. The ability of individual farms is influenced by several aspects with regard to the existing and possible future structure of the farm. One important factor is the availability of consolidated grassland in a sufficient proximity to the barn or the milking parlour (Kiefer *et al.*, 2014). This paper presents preliminary results from an analysis of the role of land fragmentation with respect to the percentage of pasture on dairy farms in Lower Saxony, a region in Germany with intensive agriculture and a high density of dairy production.

Materials and methods

This analysis is based on IACS (Integrated Administration and Control System) data for Lower Saxony, Germany. Available data were alphanumeric data on farm and parcel level (e.g. animal numbers, size and use of agricultural parcels) as well as the geospatial feature layers representing the IACS reference parcels for five years (2010-2014). In Lower Saxony, LPIS (Land Parcel Identification System) reference parcels are physical blocks that are defined as continuous agricultural areas delimited by physical borders like streets, tracks or streams. However, one physical block may contain the agricultural parcels of several farms. This can cause a fuzziness regarding the spatial location of a specific parcel whose extent depends on the number of parcels within the physical block. We imported the IACS data in a PostgreSQL/PostGIS data base and derived farm characteristics like dairy herd size, utilised agricultural area, total grazing area (as a sum of pastures and grassland used both for grazing and mowing), or total forage area based on the harmonised alphanumeric data.

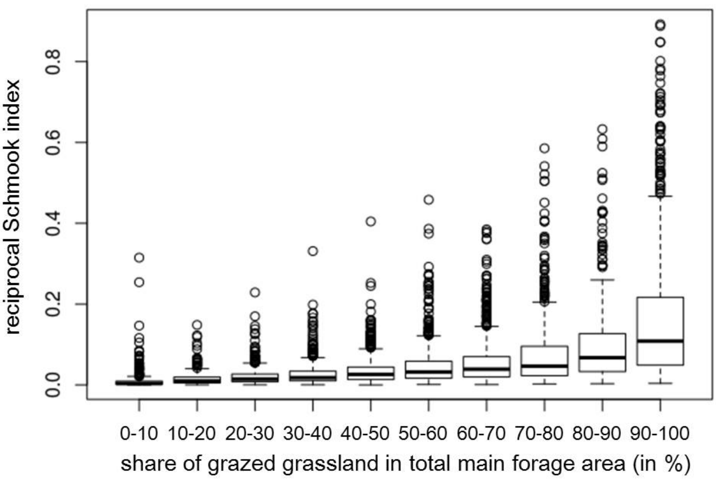
We calculated different indices to describe land fragmentation. The presented results focus on a fragmentation index suggested by Schmook (1976). The Schmook index was originally defined as the ratio between the area of a circumscribing polygon and the farm size. A convex hull wrapping the farm's physical blocks approximates the circumscribing polygon. The farm size was derived from the alphanumeric data. Deviating from Schmook's proposal, we calculated according to Sikk and Maasikamäe (2015) the reciprocal of the ratio. This index is equivalent to the share of the convex hull covered by farm parcels and ranges from 0 to 1. Lower values indicate a higher fragmentation and values near 1 are typical for a farm with a consolidated plot structure. However, a distinct threshold for consolidation cannot be defined for this index. In the results, the index will be referred to as reciprocal Schmook index. We calculated the index for the agricultural farm area in total as well as separately for the grassland and the cropland farm area.

We used the R functions *boxplot* (with default settings) and *cor.test* for the statistical analysis.

Results and discussion

The results presented focus on a selection of farms for the year 2013. The sample includes farms with at least 50 dairy cows and an agricultural area greater than 5 ha consisting of more than one parcel (n=6,204). The minimum herd size ensures a certain relevance of dairy production for the farm.

Figure 1 shows that farms with a higher share of grazed grassland tend to be slightly more consolidated (Spearman's rank correlation *rho*=0.58, P<0.001). The median reciprocal Schmook index increases from 0.004 for the farms with less than 10% of pastures in the main forage area to 0.108 for the class with the highest share of pastoral land. This means that in the first case, e.g. 10 ha pastures would be dispersed over an area of 2,727 ha, while in the latter the 10 ha would be dispersed over 92 ha. The majority of dairy cows are kept on farms with fairly scattered grassland (for 85% of the dairy cattle the reciprocal Schmook index for all their grassland is below 0.10 and for 51% of the dairy cattle the respective index is even below 0.03). The percentages are very similar if the calculations are based on the farm's agricultural area. For better understanding, Figure 1 includes the number of dairy cows per percentage class (x-axis) together with the share in the total number of cows in the selection.



x-axis	dairy cows	% of total
0-10	39 442	5.9
10-20	35 839	5.4
20-30	52 280	7.8
30-40	73 973	11.1
40-50	92 322	13.8
50-60	85 738	12.8
60-70	93 304	13.9
70-80	73 707	11.0
80-90	51 562	7.7
90-100	70 808	10.6

Figure 1. Boxplot of the reciprocal Schmook index for grassland in dependence of the farms' share of grazed grassland in the main forage area for 2013.

There are uncertainties in the results that derive from the inherent limitations of the IACS data. The location fuzziness of individual parcels impacts on the correct calculation of the convex hull as well as the distance calculation. As the distance calculation would also be affected by the unknown location of the farmstead, barn or milk parlour, we decided to focus on the reciprocal Schmook index rather than on distance based fragmentation indices. With regard to the identification of pasture, it is possible that the code for the use of the grassland is not always correct. Furthermore, the data do not allow any differentiation between pastures for dairy cows and other livestock (e.g. heifers). Because the convex hull simply wraps the included polygons, complex polygon shapes are represented poorly, and even a single small but distant plot can strongly influence the results. Therefore, the reciprocal Schmook index has some caveats.

Conclusions

The preliminary results indicate a pronounced fragmentation of the grassland for the majority of Lower Saxonian dairy farms. Furthermore, dairy farms with consolidated grassland parcels will more probably adopt grazing than farms where available grassland is scattered over a larger area. The correlation test shows that increased grazing is often accompanied by a consolidation of the farm's grassland. Whether the high percentage of farms with low reciprocal Schmook indices is really a result of general grassland fragmentation or is biased by the index method needs further investigation.

A more in-depth analysis of the available data should be able to shed light on more specific aspects of the relation between land fragmentation and grazing. Different fragmentation indexes, e.g. excluding 'outliers' or with distance-based approaches can provide new insights.

Further analysis could also include the influence of barriers like streets, settlements or rivers on the assessment of land fragmentation, the exploration of individual grassland parcels regarding their use as pastures depending on their location and proximity to other grassland parcels, the influence of the dairy herd size and a more detailed investigation of the possible impact of fragmentation on changes in pasture area over time.

Acknowledgements

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Impact of grazing practices on farm self-sufficiency, milk and economic performances of three automatized farms

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Abstract

The dairy sector is facing serious economic difficulties linked to low milk price and volatility of feedstuff price. In this context, reducing farm inputs is necessary. Optimization of use of grazed, ensiled or dried grass could be a key strategy to improve self-sufficiency and thus to decrease feeding costs. Yet, the practice of grazing is disappearing due to several factors, including increased size of dairy herds and development of automation. However, combining grazing and automatic milking systems (AMS) is possible. Three Walloon dairy farms equipped with an AMS were monitored to assess their grazing practices, proportion of grass in the cows' diet, both at barn and on pasture, and the economic advantages linked to grass use in 2015. These farms practised various grazing strategies including full-grass system (FG), day and night grass allocation (DNG), and rotational grazing (RG) completed with a partial mixed ration. The effects of grazing on milk yield (MY) were also evaluated. Grazing reduced the daily feeding costs per cow in all systems with variable impact due to grazing management. The most pronounced decline was observed in FG with a severe drop in MY. Conversely, the decrease in MY was less marked in the other farms.

Keywords: automatic milking system, feeding costs, self-sufficiency, grazing management

Introduction

In Wallonia, pasture provides an essential part of dairy cows' diet in winter as in summer. Its use is considered beneficial from several points of view including its positive impacts on farm economy and environment. However, the increased automation of dairy farms and the increased size of herds have driven many farmers to stop grazing. Yet it is possible for farms equipped with an AMS to keep grazing through adopting different grazing-management strategies. Three Walloon dairy herds with contrasting grazing practices were followed up in 2015 and the impact of grass use on milk yield (MY), milk composition and feeding costs at barn and during the grazing period have been evaluated. The aim of this study was to point out advantages and disadvantages of the grazing management options to help in a decision-making process.

Materials and methods

A total of three Walloon dairy herds of Holstein cows with different grazing management practices were followed up in 2015. The herds were visited regularly to collect data on cows' diet, grazing practices, milk production and composition. The percentage of grazed pasture was determined following the method described by Lessire *et al.* (2015). Finally financial data were gathered. Winter and grazing time (WT – GT) data were compared in each herd by t-test to assess the effect of grazing on the selected parameters. Feeding costs were calculated for WT-GT. The effect of grazing was estimated by calculating the costs of feeding at barn and multiplying them by 153 days of grazing, and by the number of cows, and comparing with the costs of feeding during GT multiplied by 153 d and by the number of cows. The MY during the whole grazing time was thus compared with MY recorded at barn during the same period of time for an identical number of cows. The difference in revenue of milk sales was calculated and related to the financial gain obtained by adapting the cows' diet. The milk revenue was evaluated by using the average price paid for milk delivery in 2015, i.e. 25.82€ 100 kg^{-1} milk.

Results and discussion

The main characteristics of the three dairy herds are summarized in Table 1. Herd 1 (H1) was equipped with a mobile robot moved to pastures during GT. During this period, the cows' diet consisted of grass plus concentrates supplied at milking by the AMS (full grazing – FG). At barn, the cows' diet was composed of roughage (12.4 kg dry matter (DM)), cereals, by-products and soybean + canola meal. Concentrate supply (TMR +robot) averaged 5.2 kg DM cow^{-1} d^{-1}.

In H2 and H3, time spent by the cows in pastures was restricted. In H2, paddocks were divided into night and day blocks (DNG) and the change from paddock occurred when cows came back to barn (at 18:00 pm). They received a partial mixed ration (PMR) composed of roughage, by-product and concentrate for a total of 10 to 12 kg DM. Grazed grass was estimated at 50% of the diet. During WT, the ration was adapted from the one received during the summer with an average of 4.6 kg DM concentrate (TMR + robot).

In Herd 3, rotational grazing (RG) was organized with cows coming back to the barn at 6 pm. They then received a PMR composed of roughage, brewers' grain and concentrates (1.6 kg DM) for a total of 12.8 to 15.8 kg DM, fluctuating with grass availability. Cows left the barn for grazing at 8 am after being milked. Grazed grass was estimated at 34% of the total diet. During WT, PMR was adapted to provide 23 kg DM and concentrate supply (TMR + robot) reached to 7.3 kg. Data collected from the herds are shown on Table 2.

Table 1. Main characteristics of the three selected herds.[1]

	H1		H2		H3	
Automatic milking system	1 – mobile		1		2	
Herd size (number of cows)	52 ± 5		71 ± 3		105 ± 7	
Annual milk yield	427,354 kg		561,375 kg		1,095,338 kg	
Pastures available	22.11 ha		24.21 ha		35.75 ha	
Grazing strategies	FG		DNG		RG	
% of grass in cows' diet[2]	Barn 36%	Grazing 88%	Barn 51%	Grazing 50%	Barn 44%	Grazing 57%

[1] H = herd. FG = full grazing; DNG = day and night grazing; RG = rotational grazing.

[2] % of grass in cow's diet is calculated by taking into account grazed, ensiled or dried grass – April and October being transition periods are not taken into consideration.

Table 2. Parameters collected in the three herds during winter and grazing time.[1]

	H1		H2		H3	
	WT	GT	WT	GT	WT	GT
Days in milk	171±20***	199±19	212±11***	217± 5	192±9***	209±4
Milk yield (kg cow^{-1} d^{-1})	26.2±4.5***	18.4±2.2	22.3±0.9***	20.9±1.5	29.7±1.0***	27.9±0.7
Fat %	3.96±0.09***	3.86±0.21	4.18±0.10***	4.02±0.13	4.08±0.06***	3.96±0.12
Protein %	3.34±0.07***	3.24±0.13	3.44±0.06***	3.48±0.07	3.40±0.05***	3.37±0.07
Concentrate provided by the robot (kg cow^{-1} d^{-1})	4.3±0.6***	2.6±0.2	3.3±0.2***	2.9±0.3	4.2±0.2***	3.5±0.5
Feeding costs (€ cow^{-1} d^{-1})	3.89	1.73	3.61	3.01	4.59	3.61
Feeding costs (€ 100 kg^{-1} milk)	15.4	9.0	15.7	13.9	15.4	12.8

[1] H = herd; WT = winter time; GT = grazing time; *** = values within herds statistically different P<0.01; * = value statistically different at P<0.05; ns = non-significant.

For all herds, MY and feeding costs decreased during GT in proportion depending on the chosen strategy. Milk composition was slightly altered with a significant drop in fat percentage in all herds and variable impact on protein percentage.

The FG system caused a huge reduction in feeding costs (difference between WT and GT = 2.16 € cow^{-1} d^{-1}) correlated with an important drop in MY by 7.8 kg cow^{-1} d^{-1}. It should be noted that the end of the grazing season coincided with the end of lactation for most cows as calvings were grouped in winter. The gain in feeding costs over the GT averaged 17,185 €. Milk production over the GT was 62,057 kg lower than estimated during WT. The milk revenue was thus 16,023 € lower. The benefit of FG was thus 1,162 €, i.e. 22.3 € cow^{-1}.

In the other herds, the decrease in MY was less pronounced (H2: delta: 1.4 kg milk cow^{-1} d^{-1} – H3: 1.8 kg milk cow^{-1} d^{-1}). Feeding costs in H2 were lowered at barn and during the grazing season by maximizing the use of silages and by-products in the cows' diet, which promoted self-sufficiency. The MY was moderate (Mean MY: 21.6±1.6 kg cow^{-1} d^{-1}) with a decrease by 0.4 kg during GT. Feeding costs per 100 kg milk were higher than in other herds and dropped less sharply during GT. The gain in feeding costs over the GT averaged 6,517 €. Milk production over the GT was 15,208 kg lower than estimated during WT. The milk revenue was thus 3,927 € lower. The benefit of DNG was thus 2.590 €, i.e. 36.5 € cow^{-1}.

The third herd showed a high milk production level even at grazing. This was linked to a high rate of concentrate supply inducing higher feeding costs cow^{-1} d^{-1}. The gain in feeding costs over the GT averaged 15,743 €. Milk production over GT was 28,917 kg lower than estimated during WT and the milk revenue was 7,466 € lower. The benefit of RG was thus 8,277 €, i.e. 80.3 € cow^{-1}.

Conclusions

Combining AMS and grazing is possible and provides an economic advantage. Full grazing system based on 88% grass is a viable strategy but affords the smallest economic gain. This result has to be confirmed as the decrease in MY at grazing may also be due to calving strategy. In RG, high concentrate level allows the decline in MY during GT to be limited. Herd 2 was less dependent on concentrate supply but lower MY recorded both at barn and during GT increased the costs 100 kg^{-1} milk. Even at a low milk price, this strategy was less efficient.

Acknowledgements

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Growth rate of Norwegian White sheep grazing an abandoned island

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Abstract

Along the Norwegian coast, the numerous islands represent potential pastures for summer grazing. Former structural changes led to abandoned islands, which for different reasons (e.g. predators in mountainous areas) today are becoming interesting to re-open. In an experiment conducted from 2012 to 2014, three islands were grazed with ewes and their lambs, which were weighed repeatedly during summer. In this paper, we present data from the island of Sandvaer (66.3°N 12.7°E). The island is 52 ha and held seven ewes, all with twin lambs. Results show that the lambs grew well on the island pasture. We found that lambs grew best in the year when grazing on the island started early (P<0.05). Carcass weight (P<0.05) and fat content (P<0.05) was also affected by start of island grazing. We concluded that sheep should be let out early for high growth rate but collected correspondingly early to prevent too high a fat content. Island pastures such as on Sandvaer may indeed provide valuable and accessible grazing resources for future sheep farming.

Keywords: Norway, lamb, weight gain, fat score

Introduction

A political goal in Norway (Royal Proposition 11 (2016-2017)) is to strengthen pasture-based livestock production, a system often related to good animal health and welfare. Abandoned areas with encroachment of scrubs and forest is a threat to both biodiversity, productivity and outdoor activities. The number of sheep farms is declining while at the same time flock size is increasing. In addition, during the last decades, farmers in some mountainous areas have experienced losses of sheep during summer due to predation by large carnivores. A search for alternative areas is thus relevant. The national project 'Sheep in motion' looked into utilization, management and performance of ewes utilizing different kind of pastures during summer. One kind of pasture was on islands along the Norwegian coast. Thousands of islands, ranging from a few m^2 to several km^2 dominate Helgeland, a region located in Nordland County. We followed ewes with their lambs at three different islands for three years, 2012 to 2014. Lamb performance was the main aim of the investigation. In this paper we present results from the island of Sandvaer during the three years.

Materials and methods

Sandvaer is an island located at 66.3°N 12.7°E in Nordland County, Norway. The island is 52 ha and dominated by low and tall herb meadow, with some abandoned cultivated pastures. In 2012, 2013 and 2014, seven Norwegian White ewes with lambs (13, 13 and 14, respectively each year) grazed the island. Lambs were born indoors at the farm, on the island of Onoey. After lambing, ewes and lambs grazed a permanent pasture close to the farm before a one-hour boat transport to Sandvaer. Lambs were weighed at birth, when released to the island, twice during summer and when collected in early autumn (Table 1). Average daily weight gain was calculated for periods between each weighing. Total weight gain was calculated for the island grazing period ('on farm', 'early summer', 'mid-summer', 'late summer' and 'total', Table 1 and Figure 1). Mean lamb age at release was 22, 38 and 22 days, respectively for the three years. In 2012 and 2013, the animals were released at Sandvaer in June. By then the vegetation was high and maturing and we recommended the farmer to get the sheep onto the pastures earlier, as was done in May

2014 (Table 1). In total, 26 lambs were slaughtered during September (9, 4 and 13 respectively for the years) and carcass weight, conformation score (EUROP, 1-15, 1=P-, 15=E+) and fat score (EUROP, 1-15; 1=no fat (-1), 15=very fat (5+)) were measured. Data were managed and analysed using SAS v.9.2. For the summer growth rate a mixed linear model, growth = release_weight + sex + year + ewe + e, was used, where growth is individual lamb weight increase (g) per day between release and collecting from the island, relelease_weight is lamb weight (g) at release, sex is ewe or ram, and year is 2012, 2013 or 2014. Ewe is the random effect of ID of the individual ewe and e is the residual error. Corrected (least square) means were estimated for year and pair-wise comparisons made. Carcass traits, with fewer observations (n= 26), and often without repeats within ewe, were analysed using t-tests.

Results and discussion

The fixed effects of the model significantly affected lamb growth: release weight (P=0.03), sex (P<0.01) and year (P<0.01); the significances and relative importance of these two effects should, however, be interpreted with some caution as they are strongly correlated (male lambs will in general be heavier than female lambs at release). Pair-wise comparisons between lsmeans for year (Figure 1) revealed that 2014 (381±(standard error) 12) was different (P<0.01) from 2013 (296±12), as was the difference between 2012 (330±12) and 2014 (P<0.01). Descriptive statistics for all periods are shown in Figure 1. Growth in 2012 and 2013 differed on a P=0.06 level. T-tests showed that on a 95% level, the carcass weights of 2014 (24.7±0.72 kg) were higher than in 2012 (21.6±0.96 kg); there was no significant difference between 2013 (24.7±0.90) and either 2012 or 2014. We found no differences in conformation score between years, while fat score differed significantly (P<0.05) with lambs being fatter in 2014 (7.23±1.54 (standard deviation)) than in 2012 (4.33±0.71); 2013 (6.0±0.82) did not differ from the other years.

The year with the best lamb growth, 2014, differed from the two previous years in its earlier (2-4 weeks) timing of release. The 2014 lambs were born between 1.5 and 2 weeks earlier than lambs released to the island in 2012 and 2013. The lambs with the poorest daily weight gain grazed Sandvaer in 2013; in this year the lambs were older at release (2 weeks) compared to 2012 and 2014. We have not included potentially important (Nielsen *et al.*, 2013, 2014) weather conditions in our analyses; this may be rectified later to explain some of the differences. In this case, however, age and date at release to the island might explain most of the differences between years. Natural vegetation develops during the summer (with highest forage quality in late spring and early summer); quality then declines towards autumn (e.g.

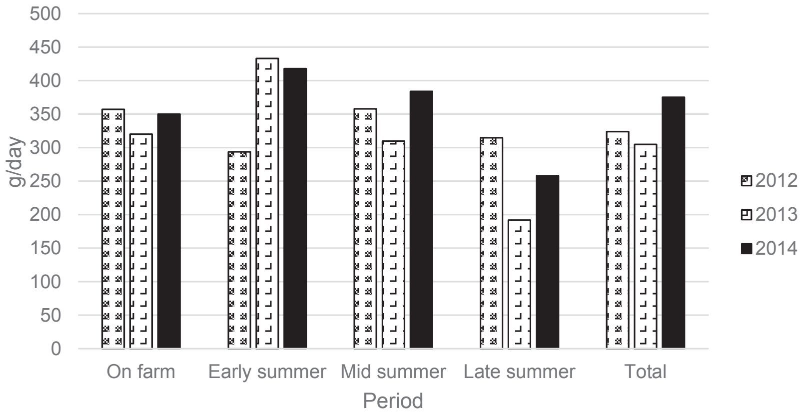

Figure 1. Descriptive average daily weight gain (g day^{-1}) from release to collecting of lambs grazing on Sandvaer in 2012, 2013, 2014. Periods: On farm = birth to release; early summer = release to Summer 1; mid-summer = summer 1 to summer 2; late summer = summer 2 to collecting; total = release to collecting.

Gustavsson and Martinsson, 2001). The release of the sheep in 2013 occurred 2 and 4 weeks later than in 2012 and 2014, respectively. We expect pasture forage in 2013 to be at a later developmental stage thus having lower nutritional value. This may have influenced the growth rate of the lambs. The lambs were collected at the same day each year (Table 1). However, at the second summer weighing in 2014, the lambs were 49 kg, and Norwegian White sheep are mature and ready for slaughter at a weight between 42 and 45 kg. The lambs were thus big enough for slaughter, and extending the grazing probably caused the 2014-lambs to be the fattest. When lambs are mature and ready for slaughter, fat deposition occurs largely at the expense of muscle, which can cause fattier lambs.

Conclusions

Island pastures may provide excellent grazing that leads to fast growth of lambs. In this study, we conclude that sheep should be released to the pasture early in the season to benefit from the high nutritional value of the plant species. At the same time, lambs should be collected when mature and ready for slaughter in order to prevent high fat scores. Using island pastures for grazing demands a dynamic management with plant nutritional level being the keyword.

Table 1. Average birth dates for lambs released to Sandvaer, date of release, date of weighing summer 1 and summer 2 and when collected from the island for 2012, 2013 and 2014.

	Birth	Release	Summer 1	Summer 2	Collecting
2012	May 11	June 3	June 21	July 17	September 8
2013	May 8	June 16	July 16	August 5	September 9
2014	April 28	May 21	July 12	August 17	September 8

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The effect of defoliation frequency on the yield of grass clover swards with and without the inclusion of a variety of herbs and birdsfoot trefoil

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Abstract

The inclusion of legumes and herbs in pastures can increase productivity and biodiversity in forage production. Herbs may also have positive impacts on the nutritive value of pastures and they are known to be beneficial to animal health. In the present study, a grass-clover sward that also contained herbs (chicory, ribwort plantain, sheeps burnet and caraway) and birdsfoot trefoil was compared with a site-specific simple grass-clover sward during two repetitive, non-consecutive years (2014 and 2016). Growth dynamics, yield and forage quality were examined under various defoliation frequencies. This paper, however, is limited to the effects on herbage yield. Independent of harvesting frequency (pasture simulation with 3- or 4-weekly rotation or 6-7-weekly silage cuts), swards established from both seeds mixtures had comparable yields except for one case in 2016, where under 3-weekly defoliation the multi-species mixture outyielded the simple one. In 2014, increased harvesting frequency led to reduced dry matter yields, with an average difference of 249 g dry matter m^{-2} between the highest and the lowest defoliation frequency. The seasonal distribution of growth rates varied between species and seed mixtures and their deferring growth patterns balanced variations in forage production.

Keywords: multi-species swards, grazing, herbs, organic farming

Introduction

In rotational grazing systems, pastures are divided into several paddocks to be grazed successively and a rest period of several days in between allows each paddock to accumulate new herbage. To ensure adequate feed supply for grazing animals, the number of paddocks in rotation is adjusted, depending on the actual rate of herbage growth. The length of the rest period, as associated with the climate, frequency and intensity of defoliation, has effects on several relevant parameters, for instance yield and forage quality (e.g. Chestnutt *et al.*, 1977). Another factor influencing herbage growth and quality is the species composition. Whilst the use of legumes, for example white clover, reduces nitrogen fertilizer needs (Clark and Harris, 1996), herbs improve the nutritional value of the feed (e.g. Pirhofer-Walzl *et al.*, 2011). Additionally, mixed swards can provide a higher herbage production if plants with different functional traits are combined and, if deep-rooting species are included, they can be better adapted to dry conditions than simple pasture mixtures (Huegenin-Elie *et al.*, 2014). In this study, a multi-species sward and a grass-clover sward were compared in terms of their herbage accumulation as affected by defoliation frequency under organic farming conditions.

Materials and methods

The trial was conducted at Kiel University's experimental farm for organic agriculture 'Lindhof' in northern Germany during two separate years (2014 and 2016). The average temperature at the site is 8.8 °C and the total average annual precipitation is 769 mm. Whilst the average temperature during the experimental years was almost the same (10 °C in 2014 and 9.9 °C in 2016), the precipitation sum was clearly higher in 2014 with 866 mm compared with 716 mm in 2016. The swards were established in 2013 and 2015 respectively as understoreys in spelt. The experimental layout was a split plot design with two experimental factors, (1) seed mixture (main plot) and (2) defoliation frequency (split plot) in 4 replications. The factors and their corresponding levels are listed in Tables 1 and 2. The seed mixtures used were a simple grass-clover and a multi-species mixture with a 50% share of herbs and birdsfoot trefoil. The three defoliation frequencies were a 3-weekly system, a 4-weekly system and a system with 4 cuts, each after 6-7 weeks. The plots were subdivided to obtain a system of plot series according to Corrall and Fenlon (1978). Sampling was done weekly by hand-clipping of 0.25 m^2 at a 5 cm stubble height. The samples were hand-separated and oven-dried at 58 °C for 48 h to determine DM contents. A linear mixed model with the factors seed mixture, defoliation frequency and year was used for statistical analysis.

Results and discussion

The results showed that the partial replacement of perennial ryegrass, red clover and white clover by herbs and birdsfoot trefoil did not reduce the total herbage yield under various defoliation frequencies (Figure 1). In 2014, seed mixture 2 yielded higher (P=0.003) than mixture 1 at 3-weekly defoliation. At lower defoliation frequencies, as well as in 2014, the effect was not significant. An effect of the lower per-species sowing rate in seed mixture 2 was reflected in the yields of legumes and perennial ryegrass under the rotational defoliation systems in 2014 only and not in the silage cut system.

However, the gap in the total production was closed by the group of herbs, primarily by chicory and ribwort plantain. Caraway and sheeps burnet, as small plants, contributed little to total yield. The hypothesis of a negative impact on the yield by a high defoliation frequency was confirmed in 2014. Decisive for the differences in total yield was the yield of red clover, as it showed the largest variations between the different defoliation frequencies. In 2016, growth of red clover was unexpectedly low and differences between defoliation frequencies turned out smaller, and did not lead to significant differences in total herbage yield.

Table 1. Defoliation frequencies.

Abbr.	Defoliation frequency	No. of cuts
3W	3-weekly	11 cuts
4W	4-weekly	8 cuts
4C	6-7-weekly	4 cuts

Table 2. Composition and sowing rates of the seed mixtures.

Species		Cultivar	Sowing rate (kg ha^{-1})	
			Mixture 1	Mixture 2
Perennial ryegrass	*Lolium perenne*	Delphi	20	10
Red clover	*Trifolium pratense*	Atlantis	6	3
White clover	*Trifolium repens*	Vyoscan	3	1.5
Birdsfoot trefoil	*Lotus corniculatus*	Lotanova		5
Chicory	*Cichorium intybus*	Spadona		2
Sheeps burnet	*Sanguisorba minor*	Burnet		2
Ribwort plantain	*Plantago lanceolata*	'native'		1.5
Caraway	*Carum carvi*	Volhouden		2
Sum			29	27

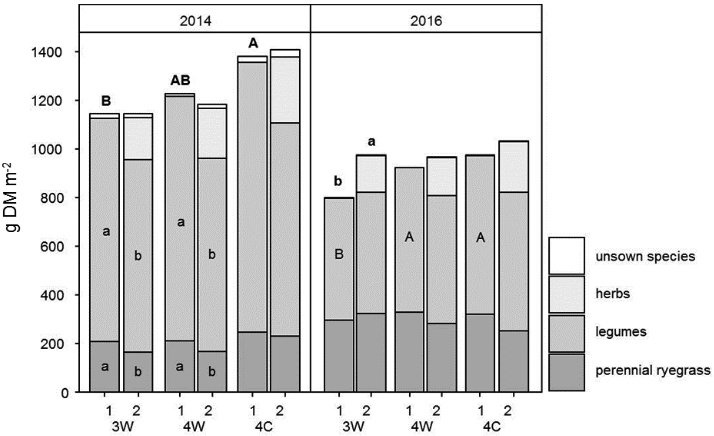

Figure 1. Dry matter yield (g DM m^{-2}) of total herbage, perennial ryegrass, legumes, herbs and unsown species in all cutting systems (3W, 4W, 4C) and seed mixtures (1, 2). Capital letters indicate significant differences between defoliation frequencies, small letters between seed mixtures, bold letters refer to total herbage (P<0.05). Differences between years are not indicated. Dead material was omitted as its share was negligible.

Conclusions

A higher species diversity in pastures can be achieved by reducing the sowing rate of perennial ryegrass, red clover and white clover in favour of herbs and alternative legumes without causing any detrimental effects on the DM yield. However, for final recommendation the longer-term stability, as well as the effect of the different species composition on forage quality, need to be evaluated.

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Changes in protein and energy value of five non-leguminous forb species during the primary growth

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Abstract

Non-leguminous forbs are important constituents of extensive grasslands. Information on their nutritive value is scarce. The present investigation gives a survey on the changes in protein concentration and energy value during the primary growth. Composition and energy value of common yarrow, ribwort plantain, dandelion, broad-leaved dock and hedge bedstraw sampled from two locations were investigated at four sampling dates in 2011. At comparable sampling dates the highest value of crude protein (CP) concentration was found in broad-leaved dock (166 to 271 g kg^{-1} dry matter (DM)) whereas the lowest values were found for common yarrow (70 to 164 g kg^{-1} DM). The highest metabolisable energy (ME) concentration was found in dandelion (9.66 to 11.30 MJ kg^{-1} DM) and the lowest in broad-leaved dock (7.79 to 9.77 MJ kg^{-1} DM). Based on a linear regression model the highest rate of CP and ME decline was obtained in broad-leaved dock (-4.14 g CP kg^{-1} DM per day, R^2=0.92; -0.054 MJ ME kg^{-1} DM per day, R^2=0.67). The lowest rate of CP and ME concentration decline was found in dandelion (-1.09 g CP kg^{-1} DM per day, R^2=0.41) and in ribwort plantain (-0.026 MJ ME kg^{-1} DM per day, R^2=0.41). It was concluded that five species of non-leguminous forbs differ both in concentrations of CP and ME, as well as in rate of their decline during the growth.

Keywords: non-leguminous forbs, crude protein, metabolisable energy

Introduction

In addition to legumes, common yarrow (*Achillea millefolium* L.), hedge bedstraw (*Galium mollugo* L.), ribwort plantain (*Plantago lanceolata* L.), broad-leaved dock (*Rumex obtusifolius* L.) and dandelion (*Taraxacum officinale* Web.) are among the most frequently occurring dicot species in Slovenian semi-natural grasslands. The above-mentioned non-leguminous forbs (NLF) may increase forage quality in terms of mineral composition (Kuusela and Hytii, 2001) and crude protein (CP) concentration (Hofmann and Isselstein, 2005). They may also have a beneficial effect on forage intake and animal health (Derrick *et al.*, 1993). However, they may be considered undesirable in grass swards due to their negative impact on herbage yield. Generally, the nutritive value of grassland forage declines with advancing maturity. Some plant species mature rapidly and, as a result, a decrease in their nutritional value is substantially higher than in species that mature rather slowly and are consequently characterised by having a high nutritive value over an extended period. The aim of the present study was to quantify the differences in protein and energy value of five NLF species and to identify possible differences in the rate of decline of their nutritive value during the plant's ageing.

Materials and methods

The experiment was carried out in spring of 2011 on two semi-natural meadows at Blatna Brezovica (45°97' N; 14°33' E, 194 m a.s.l., in central Slovenia) and Murski Črnci (46°37' N; 16°6' E, 156 m a.s.l. in eastern Slovenia). Both locations were characterised by a *Arrhenatherum elatioris* habitat type. Both meadows are usually used for extensive traditional hay making with cuts taken in mid- to late May and in July or early August. In autumn there is a third cut; at location Blatna Brezovica this is on a regular basis and at location Murski Črnci occasionally, if the weather conditions are favourable. About 1 kg of plant material of common yarrow, ribwort plantain, dandelion, broad-leaved dock and hedge bedstraw were sampled at four sampling dates: (1) 25-28 April; (2) 5-8 May; (3) 12-18 May; and (4) 18-24 May. Fresh plant samples (40 in total) were dried in a forced convection oven at 60 °C and ground through a 1 mm sieve. Dry matter (DM) concentration was determined by drying at 103 °C for 48 h. CP was determined by the Kjeldahl method (ISO 5983-2, 2005). Metabolisable energy (ME) concentration was assessed on the basis of the gas volume which was produced during the 24 h incubation of samples with rumen liquor *in vitro* using regression equations suggested by Menke and Steingass (1987). For creating linear regression equations, the General Linear Models package SAS, was used.

Results and discussion

Mean CP and ME differed among species (Table 1) and were higher or in the range of concentrations typical for forage grasses (115 g kg^{-1} DM and 5-11 MJ kg^{-1} DM for CP and ME, respectively; Minson, 1990). In agreement with Bohner (2001), the highest CP concentrations (166-271 g kg^{-1} DM) were found in broad-leaved dock at the vegetative growth stage, whereas dandelion had the highest ME concentration. The mean ME concentrations of dandelion (9.66-11.30 MJ kg^{-1} DM) and hedge bedstraw at early sampling date (10.37 MJ kg^{-1} DM) were high enough to meet the recommendations for lactating dairy cows. Obtained values (>11 MJ ME kg^{-1} DM) in dandelion are comparable to values for cocksfoot (*Dactylis glomerata* L.) or perennial ryegrass (*Lolium perenne* L.) (Mills and Moot, 2010). On the other hand, considerably lower CP and ME concentrations were found in common yarrow (70-164 g kg^{-1} DM) and broad-leaved dock (7.79-9.77 MJ kg^{-1} DM).

With advanced maturity the CP and ME concentrations declined in herbage of all the investigated plant species. The majority of linear equations for prediction of CP and ME concentrations on the basis of sampling date were statistically significant (P<0.01 or 0.001), R^2 being in the range from 0.88 to 0.98. The exceptions were equations for prediction of CP in dandelion (R^2=0.41; not significant (NS)) and ME in broad-leaved dock (R^2=0.67; NS) and ribwort plantain (R^2=0.41; NS). The regression coefficients b, which indicate the rate of CP or ME decline during the plant ageing are presented in Table 1. The CP concentration of ribwort plantain and broad-leaved dock decreased at higher rate (-4.14 and -3.44 g CP kg^{-1} DM per day, respectively) than in other NLF (-1.09 to -2.73 g CP kg^{-1} DM per day). The decreasing rates are higher than those reported for grass species by Minson (1990). The rate of energy value decline was, to some extent, in accordance with the results for CP. Broad-leaved dock, for instance, expressed the highest rate of decline in both parameters, while dandelion was in both parameters among the species with the lowest declining rate. However, there were also exceptions. Ribwort plantain, which expressed very slow ME concentration, was characterised by a rapid CP decline.

Table 1. The concentrations of CP and ME in herbage of five non-leguminous forbs and parameters (a,b) of linear regression of the above variates upon plant age (days) at cutting.[1,2]

Plant species	Crude protein (g kg^{-1} DM)			Metabolisable energy (MJ kg^{-1} DM)		
	Mean (Range)	Intercept (a)	Decreasing rate (b) [3]	Mean (Range)	Intercept (a)	Decreasing rate (b) [3]
Dandelion	129 (106-169)	170^a	-1.09^a	10.59 (9.66-11.30)	11.73^c	-0.032
Common yarrow	121 (70-164)	233^a	-2.73^{ab}	9.14 (7.91-10.11)	11.72^c	-0.064
Hedge bedstraw	140 (102-176)	236^a	-2.39^a	9.76 (8.86-10.37)	11.63^b	-0.047
Broad-leaved dock	214 (166-271)	358^b	-4.14^b	9.02 (7.79-9.77)	11.09^a	-0.052
Ribwort plantain	130 (90-183)	280^{ab}	-3.44^b	9.54 (9.33-9.81)	10.48^a	-0.026
Significance (*P*-value)		0.000	0.0118		0.0000	0.4412

[1] y = a+bx; where x means number of days after 1 April.

[2] Different superscripts within a column indicate statistically significant differences between intercepts or slopes.

[3] Expressed in g (crude protein) or MJ (metabolisable energy) day^{-1}.

Conclusions

It was concluded that the investigated species of non-leguminous forbs differ both in their CP and ME concentrations, as well as in the rate of their decline during growth.

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Suitability of switchgrass (*Panicum virgatum* L.) as a forage crop in the Mediterranean area

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Abstract

In Mediterranean rainfed cropping systems, drought-resistant crops can increase yield and availability of forage during the summer period. In North America, switchgrass (*Panicum virgatum* L.) has been used for decades as pasture and fodder. In Europe, switchgrass has been investigated mainly for its potential as an energy crop. The overall aim of the present study was to analyse the suitability of switchgrass as a forage crop in a Mediterranean environment. A field trial was carried out in Central Italy (Pisa) to evaluate the productivity and nutritive value in mature stands of two switchgrass varieties (Alamo and Blackwell). Alamo reached the maturity for hay harvest (boot stage) in August, about one-month delay with respect to Blackwell. At this stage, the biomass was 13.3 and 7.5 t ha^{-1} of dry matter (DM) in Alamo and Blackwell, respectively. Both varieties produced a summer regrowth harvested in autumn. Nutritive value declined during the growing season due to the increase of fibre and the reduction of protein content. Saponin content significantly differed between varieties and according to the growth stage, ranging from 1.8 to 4.5 mg g^{-1} DM. This study provides useful knowledge to favour the introduction of perennial grasses as forage crops in the Mediterranean, leading to several environmental benefits when compared with the annual species that currently cover half of the forage cropland in Tuscany.

Keywords: switchgrass, rainfed systems, yield, nutritive value, saponins

Introduction

In the Mediterranean environment, extensive and low-input livestock systems need of a sustainable intensification of forage production to increase their economic sustainability, especially in marginal areas. In lowlands, grassland production is limited by climatic conditions that are characterized by an irregular distribution of precipitation during the year; abundant and stormy rainfall in autumn and spring are followed by severe drought condition during the summer, when rainfall is scarce and evapotranspiration is usually high. In this context, the introduction of drought-resistant crops can represent a win-win strategy to improve the forage yield during the summer period (Gherbin *et al.*, 2007). Warm-season grasses have been used for decades as pasture and fodder in North America. During the last decades, switchgrass (*Panicum virgatum* L.) has been investigated in European environment exclusively for its potential as an energy crop in rainfed systems. Recent studies reported that in low-input systems switchgrass was able to increase carbon sequestration and biomass production compared with that of annual crops (Ashworth *et al.*, 2015; Guretzky *et al.*, 2011). However, few studies have evaluated the biomass quality of switchgrass as a forage crop according to the development stage, harvest time and management system (Guretzky *et al.*, 2011). Moreover, although the potential presence of antinutritional compounds such as saponins in fresh switchgrass herbage has been documented (Lee *et al.*, 2009), knowledge about the main variation factors affecting the concentration of saponins in switchgrass is still limited.

The aim of the present study was to assess in a Mediterranean environment (1) the response of two mature stands of switchgrass varieties (Alamo and Blackwell) to a double harvesting system, and (2) to evaluate their suitability as forage crops considering the variation of main qualitative traits (fibre, crude protein and saponin content) during the growing season.

Material and methods

The experiment was conducted in 2015 at the Centre of Agro-environmental Research (CiRAA), Pisa, Central Italy, on five-year-old stands of two switchgrass varieties: Alamo (AL) and Blackwell (BL). From the first year to 2014, both stands were harvested once per year in winter. The average yield, from 2010 to 2014, was 20 t and 15 t DM ha^{-1} yr^{-1}, for AL and BL, respectively. In 2015, AL and BL were harvested in summer at the boot stage (7 July and 5 August for BL and AL, respectively), and the regrowth was harvested in autumn (5 October). At all cutting dates, the aboveground dry matter yield was determined on sampling areas of 1 m^2, in 4 plots arranged according to a split-plot design. In addition, the biomass for qualitative assessment was collected 5 times, T1 (11 May), T2 (4 June), T3 (22 June), T4 (7 July), T5 (5 August, only for AL), from sprouting to the summer harvest, while the summer regrowth was collected only at harvest T6 (5 October). In all biomass samples, dry matter, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), crude protein (CP), ash and crude fat (CF) contents were determined by the Van Soest method (1991) and AOAC method (1990). Saponins were extracted according to Lee *et al.* (2009) and the total content was determined by spectrophotometric analysis, with Diosgenin as the reference standard. Data were analysed by a general linear model with switchgrass variety and harvest time as well as their interaction as fixed factors.

Results and discussion

In 2015, the total rainfall during the switchgrass growing cycle, from April to early October, was 280 mm. From April to July the average air temperature increased from 13 to 26 °C, then decreased to 20 °C at the end of September. The sprouting of BL occurred in the first week of April, while new sprouts of AL emerged about 10 days later. BL reached maturity for hay harvest (boot stage) in the first week of July (T4), about one-month before that of AL (T5). At this stage, the above-ground dry matter yield was 7.5 and 13.3 t ha^{-1} in BL and AL, respectively. Similar yields were reported by Alexopoulou *et al.* (2015) for a wintertime single-harvest management. After the first harvest, both varieties produced a summer regrowth, which at the harvest in October (T6) yielded 2.1 and 2.9 t ha^{-1} DM, in BL and AL respectively. The different phenology, observed between the two varieties, could allow a better distribution of the stocking rates during summer period. However, to graze switchgrass during the full growing season, a rotational grazing would be advisable (Burns *et al.*, 2011).

All analysed factors and their interactions affected significantly the quality traits (Table 1). During the growing season, a decreasing nutritive value of forage was observed in both varieties. Crude protein content declined from 10.8 to 2.4 g 100 g^{-1} DM (T1-T5) and from 9.0 to 2.8 g 100 g^{-1} DM (T1-T4) in AL and BL, respectively, while a slight increase of values was recorded at the regrowth (T6). In AL and BL, the NDF content increased from 65.8 and 69.5 to 77.2 and 73.9 g 100g^{-1} DM from T1 to T6. In both varieties, during biomass accumulation, the ADF and ADL content nearly doubled; conversely, CF and ash content declined from T1 to boot stage. The content of CP was significantly higher in AL samples than in BL at T1 and T2. At T1, CF and ash content of BL was higher than AL; conversely, NDF and ADF content was significantly higher in AL. Similarly, at T2 AL samples were significantly higher in CP and ash content, and lower in NDF and ADF than BL. These differences recorded in the first part of the growing season may be due to the delay of the sprouting time between the two varieties. In the regrowth biomass (T6), only the NDF content significantly differed between the two varieties, being higher in AL than BL (57.6 vs 55.5). The switchgrass average CP content at T6 was 3.5 g 100 g^{-1} DM. A higher content of calculated non-structural carbohydrate (NSC) and CF was observed at T6 in both varieties.

Saponin content ranged from 1.8 to 4.5 and from 2.2 to 3.8 mg g^{-1} DM at T2 and T6, in BL and AL, respectively. Significant differences were recorded between varieties at T2, T4 and T6. Similar contents were observed by Lee *et al.* (2009) in other varieties of switchgrass in North America. Although the use of late-summer (T6) regrowth as fresh forage resource would be of interest in rainfed systems,

Table 1. Qualitative trait means and standard error (g 100g^{-1} of dry matter) of switchgrass, from sprouting to summer harvest (T1-T5) and at second harvest in October (T6).[1,2]

Harvest	V	CP	NDF	ADF	ADL	CF	Ash	NSC	TS[3]
T1	AL	10.8±0.4a	65.8±1.2b	21.3±2.7b	3.0±0.5	1.6±0.1a	7.3±0.2a	14.6±0.8	2.6±0.1
	BL	9.0±0.2b	69.5±0.6a	28.7±0.4a	3.3±0.4	1.3±0.1b	6.0±0.2b	14.3±0.4	2.4±0.1
T2	AL	6.7±0.4a	70.8±0.4	29.7±0.5b	2.8±0.2b	1.4±0.1	6.8±0.5a	14.3±0.6	2.2±0.1a
	BL	4.9±0.4b	72.5±0.6	35.9±1.0a	3.9±0.3a	1.2±0.1	6.0±0.2b	15.4±0.4	1.8±0.1b
T3	AL	3.6±0.1	72.2±0.2	37.6±0.5	3.8±0.2b	1.1±0.1b	5.7±0.1a	17.5±0.2	2.5±0.3
	BL	3.7±0.3	72.7±0.3	37.4±1.1	5.1±0.1a	1.4±0.3a	4.9±0.3b	17.3±0.6	2.9±0.1
T4	AL	3.0±0.1	73.7±0.9	39.0±1.5	5.8±0.5	0.9±0.1	4.2±0.1	18.1±0.8	2.2±0.1b
	BL	2.8±0.1	73.9±0.1	38.6±1.0	6.2±0.3	0.8±0.1	4.2±0.1	18.3±0.4	2.7±0.1a
T5	AL	2.4±0.0	77.2±0.8	42.8±3.1	7.9±0.4	1.8±0.1	3.2±0.1	15.6±0.8	2.7±0.1
	BL	-	-	-	-	-	-	-	-
T6	AL	3.3±0.3	57.6±1.1a	30.2±0.6	1.7±0.1	2.0±0.1	6.6±0.1	30.5±0.6	3.8±0.3b
	BL	3.8±0.1	55.5±0.7b	29.1±0.7	1.6±0.1	2.2±0.1	6.9±0.2	31.6±0.5	4.5±0.2a

[1] NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; CP = crude protein; CF = crude fat; NSC = non-structural carbohydrates; TS = total saponin.
[2] For each harvest dates and trait different letters indicate significant difference for Tukey's test (P<0.05) between varieties (V).
[3] mg g^{-1}.

the high content of saponins in switchgrass regrowth suggests the need to avoid grazing, especially by small ruminants (Lee *et al.*, 2009). Further studies are needed to investigate the effect on animal health of saponins in the fresh biomass of switchgrass. In conclusion, in several parts of Mediterranean region, annual forage crops are currently used to produce high quality fodder or pasture, but their use is associated with increasing feeding costs, risk of soil erosion, and loss of soil organic matter due to annual soil preparation and sowing. This study provides useful knowledge to promote the introduction of a perennial species in crop rotation because of its capacity to guarantee satisfactory yield levels and, at the same time, to conserve soil fertility.

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Semi-natural grasslands on the Aran Islands, Ireland: ecologically rich, economically poor

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Abstract

AranLIFE is an EU co-funded LIFE project working with farmers in Ireland on the management of semi-natural grassland habitats dependent on agriculture. The farming system, predominantly spring-calving beef cows, has a low herd size, low stocking rate but with a high associated labour input. The poor economic returns and below average policy support payments are resulting in changes in management practices, including a cessation of farming, sub-optimal grazing rates and intensification. These changes have a negative impact on the overall condition of the grassland habitat. AranLIFE is working with 67 farmers to identify the range of management types and associated biodiversity on the farms. Forage analysis indicates that the nutrient content of the grasslands does not always meet the requirements of the grazing animal. This limits the type of suitable livestock systems, with the production of weanling calves and/or store cattle being the main choice. If the farming systems that created these desired habitats are not economically viable, then what are the future prospects of these and similar semi-natural rich grasslands and what support mechanisms are required to ensure their survival?

Keywords: grasslands, AranLIFE, forage quality, policy, grazing, high nature value

Introduction

The three Aran Islands are situated off the west coast of Ireland and contain a mixture of rare European farmed habitat types, classed as priority habitats under the European Habitats Directive. These include Orchid-rich Calcareous grassland (6210), Limestone pavement (8240) and Machair (21AO). Due to the presence of these habitats, over 75% of the total land area is designated as a Special Area of Conservation (SAC). The AranLIFE project (2014-2017), an EU co-funded LIFE Nature project, is working with 67 farmers on the islands. It seeks to develop and demonstrate the best conservation management practices of local farmers. The project includes investigation of the ecological, agricultural and financial context and what policy instruments are required to maintain grassland communities. This work consisted of three main objectives, determining the broad grassland types across the islands, measuring their annual net production and estimating the feeding value of the grasslands. We discuss whether these ecologically rich grasslands can compete in the present policy climate (e.g. Greening under the Common Agricultural Policy) where there is limited differentiation between permanent grasslands that are semi-natural, and intensively managed grassland monocultures.

Materials and methods

A botanical survey of grasslands was carried during 2014 and 2015 based on 77 relevés (4 m^2). Data collection methods followed national survey methodologies (O'Neill *et al.,* 2013). All relevés and species were ordinated using Detrended Correspondence Analysis (DCA) using PC-Ord. Classification of the data to establish community types was carried out using two-way indicator species analysis (TWINSPAN). To determine forage quality, samples were collected every two months on 50 sites from March 2015 to March 2016. Samples were analysed for ash, crude protein (CP) (N ×6.25), dry matter, acid detergent fibre (ADF) and neutral detergent fibre (NDF) following Van Soest *et al.* (1991). Mineral analysis for Ca, P, Mg, Mn, Cu, Fe, Na, Zn and K was determined using inductively coupled plasma-optical emission spectroscopy (ICP-OES) whilst the method for the Heavy metals (Pb, Mo, Se and Co) was Inductively Coupled Plasma – Mass Spectrometry (ICP-MS). Primary production was quantified on nine representative sites using movable cages (McNaughton *et al.*, 1996). At each site, three metal exclusion cages of 1×1×0.4 m were located within representative grassland community types. During each sampling period, gross and residual vegetation was clipped to 3 cm within 0.5×0.5 m^2. Samples were oven-dried to constant weight (60 °C for 48 h) to determine dry matter yield. Cages were moved according to grazing frequency. The Mann Whitney U test was used to test for a significant difference in measured variables between grassland groups.

Results and discussion

Ordination analysis identified four main groups based on the associated vegetation. The grassland communities could be divided into two dominant categories, semi-natural grasslands (SNG) with a high number of species indicative of good quality calcareous grassland (38 relevés), and semi-improved grasslands (SIG) with a lower number of calcareous grassland species and an increase in species more associated with agricultural improvement, specifically nutrient enrichment (39 relevés). When this breakdown of SIG and SNG was analysed for dry matter yield, SIG had yields ranging from 4,136-7,350 kg dry matter $(DM)^{-1}$ yr^{-1} compared with SNG which had yields of 507-2,756 kg DM^{-1} yr^{-1} (Table 1). In terms of minerals, for both grassland categories the forage was deficient in macro- and micro-nutrients, specifically phosphorus, selenium, copper and cobalt (NRC, 2000). These deficiencies were reflected in blood samples taken from the grazing livestock. Phosphorus levels in the SIG were greater than in the SNG. Using crude protein as an indicator of forage feeding value, the SIG showed higher levels than the SNG (Table 1). Furthermore, crude protein values for SIG were above the recommended level for livestock (12% recommendation for good animal performance (NRC, 2000)), but this was not so for the SNG.

Based on this work, the SNG were lower yielding and of poorer feeding value than grasslands with a higher level of agricultural improvement. The poor feeding value also limits the type of stock reared, which is reflected in the predominant enterprise being beef cows with their calves sold as store cattle for finishing in other areas. The nutrition of spring-calving beef cows generally involves feed restriction and mobilisation of body reserves during the winter, and recovery of body reserves during the subsequent grazing season (Murphy *et al.*, 2008).

The CAP policy measures are generally inadequate to (a) pay the Aran farmers a sufficient rate to be economically viable and (b) compete with incentives to convert and intensify wildlife habitats. At present, food production is the main opportunity for the farmers on the Aran Islands to increase their income on existing land, either through improving efficiency and/or increasing output. Existing payments through Pillar 1 are equally available to both intensive agricultural fields and semi-natural grasslands, with no distinction in payment rates. Due to the historic nature of subsidies and low stocking rates associated with

Table 1. Species number per relevé and forage analysis variables (mean ± standard error of the mean) for semi-improved and semi-natural grasslands surveyed on the Aran Islands.[1,2]

Grassland	Species no. per 4 m^2 relevé	Yield (kg DM ha^{-1} p.a.)	CP (g kg^{-1} DM)	Phosphorus (%)	Selenium (mg kg^{-1})	Cobalt (mg kg^{-1})	Copper (mg kg^{-1})
SIG	23.1±1.0**	5,580±941**	151.3±3.6**	0.27±0.01**	0.09±0.01*	0.05±0.03	7.39±0.44**
SNG	34.6±0.8**	1,760±420**	112.5±2.4**	0.12±0.01**	0.11±0.01*	0.03±0.00	5.39±0.21**

[1] Significance: * $P<0.05$; ** $P<0.01$.

[2] SIG = semi-improved grassland; SNG = semi-natural grassland.

semi-natural grasslands, Pillar 1 payments are lower on the islands, € 100 ha^{-1} compared with € 262 ha^{-1} for the rest of Ireland. Agri-environment payments (Pillar 2) are available to farmers. The present agri-environment programme in Ireland contains grassland conservation options that require the presence of a minimum of three to eight grassland species, and permits a maximum chemical nitrogen application of 40 kg N ha^{-1} per annum and an organic nitrogen limit of 170 kg ha^{-1} (Nitrates Directive limit). In this study, the agriculturally improved grasslands would still meet these criteria, but would not meet favourable condition status under Article 17 of the Habitats Directive reporting. Therefore, a farmer who chooses to maintain the high quality semi-natural grasslands in Ireland will be economically disadvantaged compared with a farmer who chooses to apply fertilisers and intensify production on semi-natural grasslands. Experimental evidence would suggest that a reduction of half of the total number of plant species can be observed with applications between 20 and 50 kg N^{-1} ha^{-1} yr^{-1} (Plantureux *et al.*, 2005).

Conclusions

Intensification of agriculture is limited on the Aran Islands due to their remoteness and designation under the Habitats Directive. Policy incentives and market rewards means that there still is generally an economic advantage to intensification in semi-natural grasslands on the Aran Islands and throughout Ireland that are outside of designated areas for nature protection, as improving grasslands through fertilisation still offers benefits in livestock production terms. The agri-environment schemes directed towards high nature value areas also set a low biodiversity target that is insufficient to save these ecologically rich grasslands that are also economically poor. Results-based agri-environment programmes would offer a much better alternative as the financial payment could be designed to favour the priority grasslands.

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The importance of beef production efficiency in the Sonoran Desert

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Abstract

The study was conducted in Sonora, Mexico, during 2008 and 2016, to determine strategies for calf production: (1) very efficient (95% of calving), (2) average (75% of calving) and (3) deficient (50% of calving); all with early weaning. Evaluated variables were: weaning weight of calves, weight of cows at weaning, weight sale of offspring, weight and body condition of cows at weaning, pregnancy rate and calving interval, production costs of meat and the profitability projection in three stages in an area with capacity for 100 animals in order to contrast the effects of two systems of production efficiency. Evaluated variables were analysed by analysis of variance ($P \leq 0.05$). Financial runs were performed using software for analysis and capital budgeting of projects. We found that, on the same property, a very efficient producer achieves annual income average (Mexico pesos [$]/cow) of $4,464, one of average efficiency $2,952 and one with low efficiency $1,127. High efficiency increases the total monthly income ($27,808) compared with average efficiency ($15,206) and low efficiency ($9,393). We conclude that low efficiency of production generates low incomes which might lead to resource deterioration as the producer increases the number of cattle on the ranch in order to compensate for the loss of production inefficiency.

Keywords: range deterioration, inefficiency, early weaning, profitability, pregnancy rate

Introduction

Intensive cattle production characterized by overgrazing has caused major changes in vegetation, reduced the number of species of better fodder quality thereby benefitting less desirable, invasive and often toxic species (Ibarra *et al.*, 2014). Extensive rangeland areas that were once productive and sustained significant densities of livestock and wildlife are currently in a deteriorated condition with bare soil and erosion problems. According to Heady and Child (1994), overgrazing, prolonged droughts, reduction in the intensity and frequency of natural fires, excessive clearing, immoderate land clearing, and overexploitation of other natural resources are the main problems associated with the loss of vegetation and soil degradation. On the other hand, there are factors related to severe droughts, excessive population growth, excessive transfer of the rural population to the urban areas, overgrazing, low cattle prices and high costs of the main inputs for livestock and maintenance of ranches. Uncertain and unstable markets are factors that aggravate the problem of low production and consequently the low profitability of the ranches, which means that cattle ranching in Mexico is currently experiencing problems (FAO-UNESCO, 2003). Several studies conducted in various countries and environments suggest that early weaning is a good alternative to increase calf production on ranches (McSweeney *et al.*, 1993); however, the profitability as a result of the application of the practice of early weaning as a tool to increase calf production and the profitability of the ranches of Sonora is unknown.

Materials and methods

This comparative study was carried out during the years 2008 and 2016, at Rancho Grande (29° 43' 31.9" N; 111° 15' 5.6" W). The area corresponds to Desert Shrublands (COTECOCA, 1982) and was interseeded with buffel grass (*Cenchrus ciliaris* L.) and re-planted during 2001. Two options for calf production were tested under rangeland conditions during the years 2008 and 2016. The treatments were: (1) early weaning (EW) of offspring at 90 days of age; and (2) normal weaning (NW) at 7 months of age (control). Two groups of 30 cows were randomly selected from a group of 100 calving cows (6-year-old Charbray breed). These cows presented 15 female offspring and 15 male offspring and entered the pastures of 300 ha where they remained under equal conditions for 7 months. The variables evaluated were: (1) weight at weaning of calves, (2) weight of cows at weaning, (3) weight on sale of offspring, (4) weight and body condition of cows at weaning and their percentage of pregnancy and interval between calving, (5) cost of production per kg of meat and (6) projection of profitability for both scenarios for a farm with capacity for 100 cows. The experimental design was completely randomized with two treatments and 15 replicates. Each animal was considered as an experimental unit. All variables were analysed using an analysis of variance ($P \leq 0.05$) with the statistical package COSTAT (2002). Duncan's multiple range test was used for the comparison of means.

Results and discussion

The comparison of the economic analysis showed that, for the year 2016, the total cost per calf produced was similar between treatments and varied from \$17.60 per kg for the normal 'control' group (NW) to \$19.50 per kg for the EW treatment, being EW the most profitable practice for production of meat. Beef cattle production is a common mean for farmers and herders to manage economic gains (Stalker *et al.*, 2007); therefore, the adoption of management practices that increase the profitability of meat production is critical for the sustainability of management in rangelands (Ibarra *et al.*, 2014). Under the EW model, the best gains over time were obtained. These fluctuated from \$2,951 to \$4,464 (pesos) per ha for the financial year 2008 scenario and 2016 (Table 1). The results of this study are similar to those reported by Blanco *et al.* (2009), suggesting that early separation of the calf from the cow reduces forage quantity for feed and increases daily gains as well as pregnancy rates. Other studies show that EW can increase net income, reducing overgrazing and increasing beef production efficiency (McSweeney *et al.*, 1993).

Conclusions

It is necessary to increase the efficiency of production on the ranches, since it is uneconomic to maintain unproductive animals with low rates of calf production on farms when there is a very high cost for the natural resources. The application of technology such as EW enhances body weight, body condition and income per day, as well as improving the indicators of cow pregnancy, thereby increasing all economic indicators. By applying this technology ranchers can earn an additional 2,784.35 pesos per head per year. In addition the cows will produce one calf crop every 12 or 13 months.

Table 1. Livestock characteristics and profitability of calf production under: (1) early Weaning (EW) and (2) untreated control (NW), during 2008 and 2016 at Sonora, Mexico.

Variables	Treatments			
	EW 2008	EW 2016	NW 2008	NW 2016
Total weight gain of offspring (kg)	110.75 a	135.6 a	37.8 b	54.1 b
Length of the test (days)	84	90	88	90
Total cost of calves produced ($ kg^{-1})	14.4	*19.5		*17.6
Calving percentage	95	95	75	75
Number of days open	90	90	240	185
Change in body condition of cows during the period	+0.3	+0.5	-0.5	-0.7
Total return (Mexico peso[$] per calf produced per year cow^{-1})	*11,694	*44,641	*-2,306	*29,518
Profitability ($ ha^{-1})	*1,169	*4,464	*-230	2,951

[a] Means between columns followed by the same literal are similar ($P \geq 0.05$) Duncan's test.

* Values obtained from economic analyses.

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Digestibility and protein utilisation of silages of grass, red clover-grass and maize by rams

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Abstract

The aim was to evaluate differences in *in vivo* digestibility and protein utilisation of silages fed to rams. Grass silage (G) from first cut, red clover-grass silage (RC 75% + G 25%) from third cut without inoculant (RC-G) and with bacterial inoculant (RC-G+I; *L. plantarum, L. buchneri*, 2×10^5 cfu g^{-1}) and maize silage harvested at dough stage (EM) and at dent stage (LM) of maturity were fed to 10 rams in a duplicated 5×5 Latin square, where half of the rams (1 square) received 150 g of rapeseed meal daily. G had higher organic matter digestibility than RC-G and LM (76, 65 and 69%, respectively). Neutral detergent fibre digestibility was highest for G (P<0.001). Nitrogen intake and excretion of urea in urine were greatest for RC-G followed by G and maize. Excretion of purine derivatives in urine was greater for G than for RC-G and EM (4.8 vs 3.4 g d^{-1}; P<0.001), which did not differ. No effects of inoculant in RC-G and of maturity stage in maize were found. Thus, G silage can be used as a sole forage whereas RC-G and maize silages need to be combined to optimize energy and protein utilisation by ruminants.

Keywords: feed evaluation, grass, maize, red clover, ram

Introduction

Silages of red clover-grass (RC-G), grass (G) and maize differ in nutrient contents and in digestibility of organic matter (OMD), which results in differences in intake of these forages by ruminants (Mertens, 2007). Therefore, to ensure that diets fulfil nutritional requirements for specific milk yield and/or growth rate of ruminants, there is a need to investigate nutrient availability and utilisation of individual forages to provide the information needed for combining nutrients and ensuring appropriate concentrate supplementation. The objective of this study was to evaluate differences in *in vivo* apparent digestibility and protein utilisation of these different types of silages, with and without protein supplementation, when fed to rams. The RC-G silage was tested with and without a bacterial inoculant.

Materials and methods

The silages were as follows: G silage [timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* L.) and perennial ryegrass (*Lolium perenne* L.)] from first cut; RC (*Trifolium pratense* L.) with G silage (75% RC with 25% G on dry matter (DM) basis) from third cut, both without a bacterial inoculant (RC-G) and with a bacterial inoculant (RC-G+I) (this was *L. plantarum* DSM 3676, 3677 and *L. buchneri* DSM 13573, dosage of $2{\times}10^5$ cfu/g, Kofasil Duo, Addcon Europe GmbH); and maize (*Zea mays* L.) silage harvested at the dough stage (EM) and at the dent stage (LM) of maturity. Silages were precision chopped and stored in hard-pressed round bales, which were wrapped with 8-10 layers of plastic film. The silages were fed to 10 rams in a duplicated 5×5 Latin square, where half of the rams (1 square) received 120 g of rapeseed meal daily fed separately. The rams were crosses of Swedish Finewool, Dorset and Texel, 9 months old, body weight of 63 (standard deviation (SD) 2.65) kg and a body condition score of 2.85 (SD 0.17) at start of the trial. Each of the 5 periods was 4 weeks long, starting with an adaptation period of 14 days before 7 days of registration of *ad libitum* intake, when the rams were housed in individual pens. During the final 7 days, the rams were fed at 80% of *ad libitum* intake in metabolic cages. After a 3-day adaptation to the restricted feeding, total daily collection of faeces and urine occurred during 4 days. Composited daily samples of feed, orts (when present) and faeces from each period were analysed for nutrient content according to conventional methods. Composited daily urine samples from each period were analysed for contents of total nitrogen (N), urea and purine derivatives (PD; allantoin and uric acid). Data were analysed in PROC MIXED of SAS (ver. 9.3) in a model containing fixed effects of silage, protein supplementation and period and random effect of ram within square. Interactions between silage and protein supplementation and carry-over effects were not significant and, therefore, excluded from the model. When a significant *F*-value at $P \leq 0.05$ occurred, pairwise comparisons between least square (LS) means were done with a Tukey-Kramer adjustment at 5% significance level.

Results and discussion

In vitro OM digestibility was highest in grass and lowest in the RC-G silage, which was related to the higher contents of acid detergent fibre (ADF) and acid detergent lignin in the RC-G silage (Table 1; Mertens, 2007). The neutral detergent fibre (NDF) content was highest for the G silage, whereas the crude protein content was highest for the RC-G silage.

No effects of inoculant in the RC-G silage and of maturity stage in maize were found, when averaged across protein supplementation (Table 2). The G silage had higher *in vivo* OM digestibility than the silages of LM and RC-G, with the lowest OMD for the RC-G silages, which is in line with the results from the *in vitro* OMD. The *in vivo* digestibility of NDF and ADF was higher for the G silage than for the RC-G and maize silages, which only differed between RC-G without inoculant and LM, although the RC-G silages were more lignified ($P<0.001$). *In vivo* crude protein digestibility was higher and the faecal N content was lower for the G and RC-G silages than for both maize silages but RC-G+I only differed from the LM silage. Total N intake and excretion of urea in urine were greatest for RC-G silages followed by the G and maize silages when averaged over protein supplementation. Microbial protein synthesis, measured as daily renal excretion of purine derivatives (allantoin and uric acid), was greater for the G silages than for the RC-G, RC-G+I and EM silages, which did not differ (Table 2; Chen and Ørskov, 2003). Supplementation of rapeseed meal increased crude protein digestibility (62.6 vs 57.5%; $P<0.05$), N intake (35.2 vs 25.6 g day^{-1}; $P<0.001$) and urea excretion (23.7 vs 18.0 g day^{-1}; $P<0.001$) without affecting urinary N excretion (42.3 vs 43.6% of total N intake; $P=0.647$) but decreased faecal N excretion (37.4 vs 42.5% of total N intake; $P<0.05$), when averaged across silage types. No interaction between silage type and protein supplementation was found.

Table 1. Chemical composition of grass silage (G), red clover-grass silage without inoculant (RC-G) or with inoculant (RC-G+I) and maize silage of early (EM) or late maturity (LM). The LS mean and standard deviation (SD) are within parenthesis (n=5).

	G		RC-G		RC-G+I		EM		LM	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, g kg^{-1}	337	(8)	286	(10)	322	(14)	298	(17)	371	(15)
Ash, g kg^{-1} DM	73	(2.2)	103	(3.7)	97	(3.1)	44	(3.6)	49	(1.2)
In vitro ODM, %	88.4	(1.1)	73.9	(3.1)	77.6	(0.8)	85.3	(1.3)	83.9	(1.4)
CP, g kg^{-1} DM	117	(0.8)	170	(4.7)	165	(5.2)	85	(3.6)	88	(3.0)
Starch, g kg^{-1} DM	-		-		-		299	(61.2)	312	(57.8)
NDF, g kg^{-1} DM	507	(16.7)	483	(9.4)	465	(12.1)	407	(19.4)	413	(23.9)
ADF, g kg^{-1} DM	286	(5.2)	360	(4.3)	344	(4.5)	221	(21.9)	216	(16.2)
ADL, g kg-^{1} DM	25	(2.7)	53	(2.5)	50	(1.9)	20	(2.3)	20	(2.5)

[1] ADF = acid detergent fibre; ADL = acid detergent lignin; NDF = neutral detergent fibre; OMD = organic matter digestibility; CP = crude protein; DM = dry matter.

Table 2. Effects of grass silage (G), red clover-grass silage without inoculant (RC-G) or with inoculant (RC-G+I) and maize silage of early (EM) and late maturity (LM) on the *in vivo* digestibility and nitrogen (N) utilisation by rams fed 80% of *ad libitum* intake. LS means and standard error of the LS mean (SEM) when averaged over protein supplementation (n=10).[1,2]

	Experimental diets					SEM	*P*-value
	G	RC-G	RC-G+I	EM	LM		
OMD, %	76.0^{a}	64.9^{c}	65.2^{c}	72.6^{ab}	69.0^{b}	0.94	<0.001
NDF digestibility, %	73.9^{a}	60.2^{b}	59.1^{bc}	58.9^{bc}	53.9^{c}	1.34	<0.001
ADF digestibility, %	73.1^{a}	59.2^{b}	56.4^{bc}	57.7^{bc}	51.6^{c}	1.69	<0.001
CP digestibility, %	65.7^{a}	64.0^{a}	61.8^{ab}	56.3^{bc}	52.4^{c}	1.64	<0.001
Total N intake (g day^{-1})	29.4^{b}	42.6^{a}	39.1^{a}	20.1^{c}	20.9^{c}	1.11	<0.001
N in faeces (% of N intake)	34.3^{c}	36.0^{c}	38.2^{bc}	43.8^{ab}	47.6^{a}	1.64	<0.001
N in urine (% of N intake)	43.3^{ab}	42.2^{ab}	48.9^{a}	38.5^{b}	41.6^{ab}	2.17	<0.01
Urea in urine, g day^{-1}	16.0^{b}	34.3^{a}	33.9^{a}	9.0^{c}	11.0^{c}	0.96	<0.001
Allantoin in urine, mmol day^{-1}	28.0^{a}	19.2^{b}	19.6^{b}	18.6^{b}	24.2^{ab}	1.69	<0.001
Purine derivatives in urine, mmol day^{-1}	30.1^{a}	21.5^{b}	21.7^{b}	20.6^{b}	26.3^{ab}	1.74	<0.001

[1] ADF = acid detergent fibre; ADL = acid detergent lignin; NDF = neutral detergent fibre; OMD = organic matter digestibility; CP = crude protein.

[2] LS means in a row with different superscripts differ significantly at $P \leq 0.05$.

Conclusions

Grass silage can be used as a sole forage whereas RC-G silage and maize silage need to be combined to optimize energy and protein utilisation by ruminants. Protein supplementation with rapeseed meal increased crude protein digestibility but had no effect on the proportion of urinary N excretion. Furthermore, effect of protein supplementation was consistent over silage types. There was no effect of a bacterial inoculant in the RC-G silage and of the maturity stage of the maize silage on nutrient digestibility and protein utilisation by the rams.

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Performance of two herd types: suckler cows vs sheep-plus-goats grazing on partially improved heathlands

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Abstract

This study aimed to compare the performance of two types of herds: a beef cattle herd (Asturiana de los Valles breed, 22-24 suckler cows) and a mixed herd of 150 sheep (Gallega breed) and 75 goats (Cashmere breed) managed on partially improved heathlands, recorded during two years. Grazing season started in late April, and lambs and kids were weaned in summer, whereas calves were weaned in autumn. All offspring were born in winter. Changes in body weight (BW) per Livestock Unit (LU) and body condition score (BCS) were studied. Cows showed more favourable BW changes than sheep and goats, while BCS changes were less propitious in ewes. Calves and lambs achieved greater BW gains per LU than kids. Overall BW gains (dams plus offspring) per hectare were greater in the cattle herd than in the sheep+goats herd, whereas there were no differences in the production per improved pasture area between both herds. Other parameters and externalities should be considered to evaluate profitability and sustainability.

Keywords: beef cattle, small ruminants, meat production, heathland-grassland

Introduction

Several studies have revealed differences between domestic herbivore species in their grazing behaviour and complementarity (Celaya *et al.*, 2008; Ferreira *et al.*, 2013), originating differences in meat productive potential among different types of livestock herds, depending on the available vegetation. Heathland vegetation is characterized by its low nutritive quality, and therefore improved pastures are needed to meet animal requirements and to obtain sustainable grazing systems. This study aimed to compare the productivity of two types of herds, suckler cows vs sheep+goats, managed on partially improved heathlands in less-favoured mountain areas.

Materials and methods

The study was performed during two years (2009 and 2010) in a mountain area (900-1000 m a.s.l.) dominated by heathland vegetation in Asturias (N Spain). Two paddocks (17.9 and 22.3 ha) were established in which 12.3 and 5.5 ha (69 and 25%, respectively) were improved by tilling, fertilizing and sowing perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). In the first paddock, a beef cattle herd (Asturiana de los Valles breed) was managed (22 suckler cows in 2009, 24 in 2010), while in the second paddock a mixed herd of 150 sheep (mostly Gallega breed) and 75 goats (mostly Cashmere) was stocked. Grazing season extended from late April to October-November. Offspring were born during winter and weaned in July-August in the case of lambs and kids, and in November in the case of calves. Animals were periodically weighed whilst dams' body condition score (BCS) was assessed on a scale of 1 to 5. Body weight (BW) changes were calculated per Livestock Unit (1 LU = 0.8 cows = 0.1 ewes = 0.1 goats, with the indices halved for the offspring). Analyses of variance were performed to test the effects of livestock species, sex of the offspring, year, and their interactions. Managed stocking rates and productions per unit land area were also calculated and analysed with a paired *t*-test.

Results and discussion

Mean BW and BCS changes of suckler dams and offspring BW changes per LU are shown in Table 1. The BW changes of dams did not differ between sheep and goats, while in general interactions between herbivore species and year were observed. Overall BW changes per LU were more favourable for cattle than for small ruminants because of greater gains of cows during the first half of the grazing season. Changes in BCS were more negative in ewes than in cows and goats. Comparing these results with those obtained by small ruminants in the same paddock but under mixed grazing together with cattle (Celaya *et al.*, 2007, 2008) or with cattle and horses (Osoro *et al.*, 2017), the BW changes of both sheep and goats were more favourable in the preceding years than in the current study (above 100% and 50% more for dams and offspring, respectively). This suggests a beneficial effect of the concurrent presence of cattle in the paddock, which could be related to the less-selective grazing behaviour of cattle leading to a reduction in senescent plant tissues of lower quality in the sward. Some studies have revealed positive effects of cattle presence on sheep performance (Abaye *et al.*, 1994; Nolan and Connolly, 1989). Offspring BW gains per LU were greater for calves and lambs compared to kids (Table 1). Normally, kids present lower growth rates than lambs under similar conditions (Celaya *et al.*, 2008; Osoro *et al.*, 2017). The male offspring had greater gains than female ones (1,609 vs 1,362 g day^{-1} LU^{-1}; standard error of the mean = 58.1; $P<0.01$).

Regarding the productivities per unit land area, managed stocking rates were similar in both paddocks (1.53 LU ha^{-1}, adding mothers and offspring). However, as a result of the lower percentage of improved pasture area in the sheep+goats paddock, the stocking rates per improved area were 173% higher in this than in the cattle paddock (Table 2). Thus, the sheep+goats paddock supported greater BW per improved area, which is related to the greater ability of sheep and particularly goats to utilize heathland vegetation (Celaya *et al.*, 2007; Ferreira *et al.*, 2013). Nevertheless, BW gains per hectare were greater with cattle than with sheep+goats, thanks to the good BW replenishment of cows during the first half of the grazing season and their slight losses during the second half (sheep and goats only managed to maintain their BW), and above all to the scarce individual daily BW gains found in lambs and kids compared to calves, which stayed grazing with their mothers until the end of the grazing season. Thus, the later calf-

Table 1. Body weight (BW) and body condition score (BCS) changes of suckler cows, sheep and goats, and offspring BW changes grazing on partially improved heathlands.

Type of herd	Single	Mixed		SEM	Significance		
Livestock species (Sp)	Cattle	Sheep	Goat		Sp	Year	Sp × Yr
Dams' initial BW (kg)	444^a	39^b	34^b	3.6	***	***	***
Initial BW per Livestock Unit (kg LU^{-1})	555^a	394^b	343^c	11.5	***	**	**
BW change per LU (g day^{-1} LU^{-1})							
Period 1 (late April to mid-July)	985^a	317^b	217^b	151.1	***	ns	ns
Period 2 (mid-July to October-November)	-56	-100	-110	64.3	ns	ns	**
Overall	278^a	67^b	57^b	37.6	***	ns	***
Dams' initial BCS (scale 1-5)	2.35	2.39	2.38	0.050	ns	***	ns
BCS change (scale 1-5)							
Period 1 (late April-mid-July)	0.31	0.20	0.23	0.054	ns	**	ns
Period 2 (mid-July to October-November)	-0.07^a	-0.22^b	-0.03^a	0.052	***	ns	ns
Overall	0.24^a	-0.02^b	0.20^a	0.062	***	***	ns
Offspring initial BW (kg)	53^a	12^b	12^b	1.7	***	ns	*
Initial BW per LU (kg LU^{-1})	133^c	245^a	231^b	9.7	***	ns	*
BW change per LU (g day LU^{-1})	$1,687^a$	$1,669^a$	$1,100^b$	92.6	***	ns	***

* $P<0.05$; ** $P<0.01$; *** $P<0.001$; ns = non-significant ($P>0.05$). Means with different superscript letters are significantly different ($P<0.05$ with Tukey's test).

weaning period enhanced the absolute BW gains relative to sheep and goats. Calculating the BW gains per improved area, there were no differences in productivity between the two types of herd.

Finally, to assess the profitability of each type of herd, the market prices of each livestock species should be applied to the weaned offspring BW as the saleable product. In addition, the investments in land improvement and winter-feeding costs should be considered in assessing the sustainability of these extensive grazing systems.

Table 2. Stocking rates and productions per unit land area of a cattle herd and a mixed sheep+goats herd grazing on partially improved heathlands.

Per total area	Stocking rate (LU ha^{-1})			Initial bodyweight (kg ha^{-1})			Bodyweight gain (kg ha^{-1})		
	dams	offspring	total	dams	offspring	total	dams	offspring	total
Cattle	1.03	0.51	1.54	573	68	641	54.8	172.0	226.8
Sheep+goats	1.01	0.50	1.51	380	122	503	9.0	68.1	77.1
Significance[1]	ns	ns	ns	ns	*	ns	ns	ns	*
Per improved area									
Cattle	1.50	0.75	2.24	833	98	931	79.7	250.0	329.7
Sheep+goats	4.09	2.05	6.14	1,543	496	2,038	36.5	276.2	312.7
Significance[1]	*	*	*	+	*	+	ns	ns	ns

[1] + $P<0.1$; * $P<0.05$; ns = non-significant ($P>0.1$).

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Influence of robust and production-oriented cattle breeds on pasture vegetation

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Abstract

In recent decades robust cattle breeds, such as Scottish Highland Cattle, have spread beyond their countries of origin and have been used for new applications, such as conservation of marginal pastures in mountain and alpine areas. They differ from production-oriented breeds in terms of robustness, modesty and lower weight. Moreover, they likely have different movement and forage-selection behaviours, which could affect the vegetation. The aim of our study was, therefore, to identify the level of breed-dependent effects of a robust cattle breed on the composition of pasture vegetation. In mountain areas of southern Germany and the Swiss Alps, vegetation samples were taken in 50 paired pasture sites grazed by either Scottish Highland Cattle or other cattle breeds. Irrespective of site conditions, pastures grazed by Scottish Highland Cattle generally had higher plant species richness and less shrub cover than their respective counterparts. Therefore, we conclude robust cattle have the potential to sustain biodiversity and ecosystem services of mountain pastures.

Keywords: cattle, breed, vegetation, grazing, species richness

Introduction

Low-intensity grasslands are often rich in biodiversity and provide society with recreational value, as well as specialized animal-based products. These ecosystem services are endangered by both intensification and abandonment (Pornaro *et al.*, 2013). Pasturing by robust breeds is often advocated as an attractive alternative for conservation and even improvement of extensively used grasslands (Zehnder *et al.*, 2016). Robust breeds are generally smaller and less heavy than production-oriented breeds and grow more slowly. Scottish Highland Cattle, for example, have daily weight gains of 0.6-0.7 kg d^{-1} (Berry *et al.*, 2002), which is substantially lower than that of common cattle breeds with 1.1-1.4 kg d^{-1} (Mutterkuh Schweiz, 2015). This suggests that the diets of robust breeds are less demanding of forage and that they harm vegetation less by trampling because of low weight and comparatively large claws (Nuss *et al.*, 2014). In contrast to more theoretical considerations, the few detailed comparisons between cattle breeds currently available reported only marginal differences in diet selection (Dumont *et al.*, 2007) and effects on sward structure (Scimone *et al.*, 2007). Because the number of sites was very limited in these studies, the present study was designed to investigate differences in vegetation of pastures grazed by production-oriented breeds and robust breeds across a large gradient of environmental conditions.

Material and methods

Scottish Highland Cattle were chosen as the model robust breed because they show typical characteristics, are widely spread and commonly reared as pure breeds in separate herds. Across the Swiss Alps and mountain areas in southern Germany, 25 pairs of pastures were selected that had either been grazed for at least five years by suckler cows and beef cattle of Scottish Highland Cattle or a production-oriented breed. Great care was taken to ensure that both parts of each pair were similar in terms of abiotic factors (e.g. soil type, altitude, inclination) and management. In each pasture, abundance and cover of vascular plant species were recorded in three plots of 5×5 m, that is 150 plots in total. One plot was located in a steep area with little signs of grazing activity indicating low grazing intensity, one in a moderately steep area and the third in a flat part of the pasture highly frequented by cattle. For all available species, grazing and trampling indicator values were extracted from Klotz (2002). Differences between plots grazed by Scottish Highland Cattle and other breeds were tested by paired *t*-tests.

Results and discussion

Since the study covered pastures from lowlands to subalpine level, there was a large variation in data; however, the quality was not diminished because of the paired structure of the experiment. Despite the wide gradient of site conditions, vegetation of the Scottish Highland Cattle pastures was significantly ($P<0.05$) more species-rich than vegetation of pastures of other breeds (Figure 1A). However, there was no significant difference in nutrient indicator values of vegetation, indicating similar grazing intensity within pairs. Plants growing in plots grazed moderately by Scottish Highland Cattle tended ($P<0.09$) to have a lower grazing tolerance than plants in plots grazed by production-oriented breeds (Figure 1B). Less trampling by Scottish Highland Cattle was also indicated ($P<0.07$) by a lower proportion of vegetation-free ground in moderate and intensive plots.

Despite a less strong grazing effect on vegetation by Scottish Highland Cattle, generally a lower cover of woody species was found on their pastures compared with pastures of production-oriented breeds (Figure 2). Since woody species were mainly found in steeper plots with low grazing intensity, the difference indicates a distinct foraging behaviour of Scottish Highland Cattle, with a higher preference of woody species and steeper slopes.

Conclusions

Scottish Highland Cattle have a positive influence on plant species richness of pasture vegetation on both extensively and intensively grazed sites. Pasture vegetation grazed by Scottish Highland Cattle is characterised by smaller shares of woody species as well as grazing indicator species. Hence, Scottish Highland Cattle have a higher potential to sustain species-rich extensive grassland and to protect it from shrub encroachment.

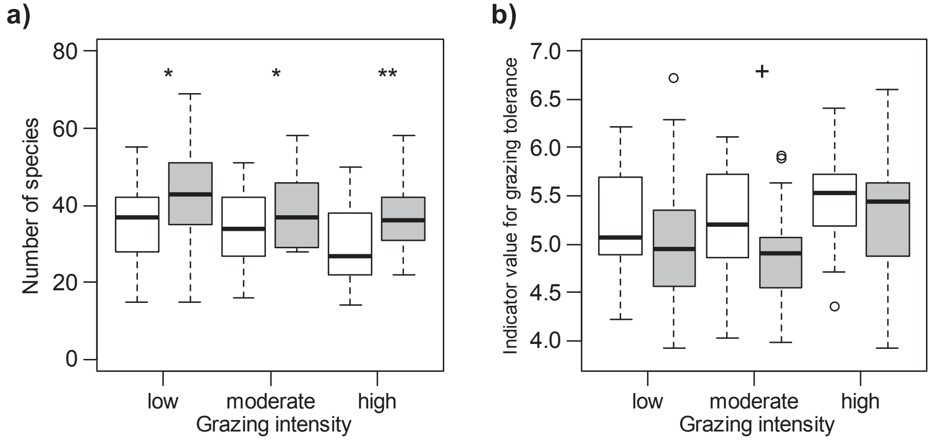

Figure 1. Number of plant species (A) and cover weighted mean of the indicator values for grazing tolerance (B) in plots of 5×5 m (n=150) grazed by Scottish Highland Cattle (grey boxes) or by a production-oriented breed (white boxes) at low, moderate and high intensity. Indicator values for grazing tolerance range between 1 (no tolerance) and 9 (high tolerance). Bold horizontal lines show median values, boxes the 50% quartile range, whiskers 1.5 of the interquartile range and circles the outliers. Pairwise significant differences are shown as ** $P<0.01$, * $P<0.05$, + $P<0.1$.

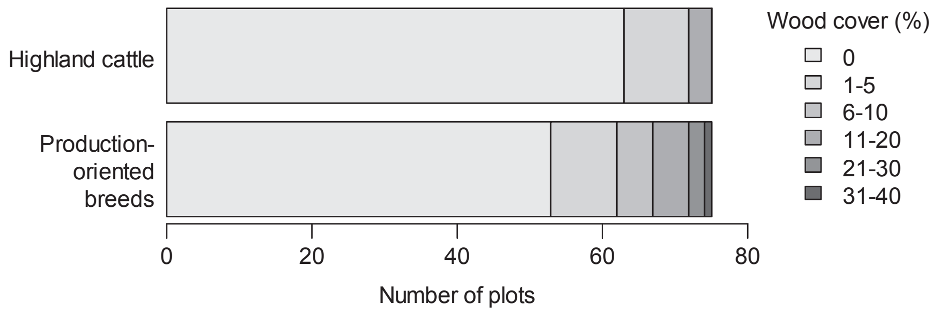

Figure 2. Frequency of plots with different cover of woody species in pastures grazed by Scottish Highland Cattle and production-oriented breeds.

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Effect of irrigation and N fertilization on the botanical composition of mountain meadows

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Abstract

Because of recurrent drought events in the last fifteen years, grassland irrigation has gained relevance in the mountainous areas of the southern slopes of the Alps. Besides the beneficial effects on forage yield, the effect of the irrigation on the botanical composition of grassland represents a relevant issue for grassland management, but experimental evidence about this topic is limited. A data set describing the botanical composition of permanent meadows at the first cut at 220 environments in South Tyrol (NE Italy), encompassing a broad range of environmental conditions and management intensities, was used to explore the effect of irrigation on the most common grassland species by means of mixed models taking into account the design effects, N input and the occurrence of irrigation. For about one-third of the species a significant (*Dactylis glomerata*, *Lolium perenne*) or marginally significant (*Poa trivialis*, *Rumex acetosa*) effect of irrigation on their yield proportion could be ascertained. Specifically designed research is required to provide more precise information about this topic.

Keywords: irrigation, meadows, mountain environment, botanical composition

Introduction

The use of irrigation in mountain grassland has increased on the southern side of the Alps. This is due to repeated, pronounced drought events during the growing season, which have occurred in the last fifteen years. The effects of irrigation on forage yield and forage quality are well documented (Andrey *et al.*, 2014; Calame *et al.*, 1992; Dumont *et al.*, 2015), but to the best of our knowledge there is, so far, only limited experimental evidence on how irrigation affects the botanical composition of meadows at a species level. In order to gain further insight into this topic, a statistical analysis of a data set describing the vegetation of irrigated and non-irrigated mountain permanent meadows in South Tyrol (NE Italy) was performed.

Materials and methods

The botanical composition of permanent meadows encompassing a broad range of environmental conditions and management intensities (altitude 666 to 1,593 m a.s.l., cutting frequency two to five cuts per year, average rainfall 809.4 mm year^{-1}, mean yearly temperature 7.3 °C) was described at 220 environments (meant as the combination of site × year) in South Tyrol. Data were collected between 2003 and 2015; 36 sites were surveyed at least once. The botanical composition was described in terms of yield proportion of each species, in percent, by means of visual assessment 3 to 4 weeks after the meadows attained a sward height of 15 cm. Irrigation was available at 47% of the environments. Information about the management of the meadow was provided by the farmers. N input was computed starting from the amount of liquid manure, slurry (in m^3 ha^{-1}) and farmyard manure (in t ha^{-1}) applied during the whole year. The values provided in classes (<7, 7-15, >15 for liquid manure; <15, 15-30, >30 for slurry; <150, 150-250, >250 for farmyard manure) by the farmers for each application were converted into numerical values (respectively 5, 11.5, 17.5 for liquid manure; 12.5, 22.5, 32.5 for slurry; 125, 200, 275 for farmyard manure). The N input in kg ha^{-1} was obtained by means of average total N contents of the respective manure type (unpublished data of the Agricultural Chemistry laboratory of the Research Centre for Agriculture and Forestry Laimburg), mineralisation rate and application losses computed according to Baumgarten *et al.* (2006) and amounts ranged between 21 and 286 kg ha^{-1} year^{-1}. The effect of irrigation on the yield proportion of 14 common meadow species was investigated by means of mixed models accounting for the design effects (random effects experimental site, sampling area within the experimental site, year), the relationship between the dependent variables and the N input and the presence of irrigation (yes/no). The effect of N input was modelled by means of a polynomial regression, using the Akaike Information Criteria as a criterion to decide whether to include the quadratic term as well. Data were transformed if necessary to achieve normal distribution of the residuals and variance homogeneity. A probability of $P<0.05$ was considered to be significant; P-values between 0.05 and 0.07 were regarded as marginally significant.

Results and discussion

Only two of the 14 analysed species were found to be significantly affected by irrigation (Table 1). The yield proportion of *Dactylis glomerata* was slightly decreased by irrigation, whereas *Lolium perenne* seemed to be advantaged by irrigation. Also, the legumes as a species group, increased if irrigation was provided. The results for *D. glomerata* are in partial accordance with those of Troxler *et al.* (1992), who found contrasting effects of irrigation depending on the investigation site. The positive effect of irrigation on the proportion of legumes has also been reported elsewhere (Andrey *et al.*, 2014; Troxler *et al.*, 1992). The effect observed on *L. perenne* is in accordance with expectations, as this species has a limited tolerance to dry conditions. For an additional two species (*Poa trivialis*, *Rumex acetosa*), marginally significant effects of the irrigation were detected as well. The slight increase of the yield proportion of both species following irrigation is also in accordance with the results of Troxler *et al.* (1992).

Significant effects of the N input were found for four species. In accordance with expectations, *Alopecurus pratensis* and *P. trivialis* were positively affected by fertilisation, whilst *Trisetum flavescens* and *Trifolium*

Table 1. *P*-values of effects of irrigation and of N input (linear and quadratic term) on the yield proportion of 14 common mountain meadow species and of grasses, forbs and legumes, and yield proportion depending on irrigation. Significant effects are in bold, marginally significant effects are in italics. In case of data analysis with transformed data, back-transformed marginal means are shown for yield proportion.

Species[1]	*P*-values of effects			Yield proportion (%)	
	Irrigation	N input	N input^2	Not irrigated	Irrigated
Alopecurus pratensis#	0.478	**0.036**		3.47	2.31
*Arrhenatherum elatius**	0.941	0.235		0.24	0.22
Dactylis glomerata#	**0.041**	0.656		8.79	6.46
Elymus repens†	0.279	0.136	*0.062*	0.06	0.25
Festuca pratensis‡	0.706	0.861		0.45	0.38
Lolium perenne†	**0.028**	*0.065*		0.36	2.81
*Phleum pratense**	0.769	0.405		1.31	1.07
Poa pratensis#	0.805	0.102		3.77	1.44
Poa trivialis#	*0.069*	**0.026**		3.58	5.52
Trisetum flavescens#	0.460	**0.003**	0.094	4.40	3.07
Trifolium repens#	0.389	**0.042**	**0.044**	6.00	7.03
Carum carvi†	0.073	*0.063*		0.46	0.12
Rumex acetosa†	*0.055*	0.189	0.114	0.51	1.27
Taraxacum officinale#	0.310	0.159	0.095	8.72	7.60
Grasses	0.494	0.326		60.27	60.27
Legumes#	**0.047**	0.180		8.20	10.45
Forbs#	0.304	0.421		29.13	26.16

[1] Data transformation: #square root, *cubic root, †fourth root, ‡fifth root.

repens were negatively affected. Marginally significant effects of N input were detected for *Elymus repens*, *Lolium perenne*, *Carum carvi* and *Rumex acetosa*. All in all, significance or marginal significance of fertilisation was detected for about two-thirds of the species, whilst less than one-third of the species was found to respond to irrigation. This may also be due to the non-availability of quantitative information concerning irrigation and precipitation at the single environments.

Conclusions

The present findings provide evidence for the effect of irrigation on few grassland species, which are common in mountain meadows. All in all, the differences between irrigated and non-irrigated sites seem to be small. However, as irrigation is likely to have been provided by the farmers at the drier sites, and because of the limited information available to quantify water availability for the plants at a single environment, specifically designed research is required to provide more precise information about this topic.

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Effect of slope and altitude on the costs of forage production in mountain areas

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Abstract

Mountain grassland, if site-specific managed, is an important source of ecosystem services. However, by increasing altitude and slope, climatic constraints and limitation to the use of machinery are expected to increase the costs of forage production, increasing in turn the risk of abandonment. In order to assess the effect of altitude and slope on the production costs for the mountain meadows in South Tyrol, a three-year study was locally conducted on about 100 fields with an altitude ranging between 1,300 and 2,100 m a.s.l. and a slope of between 3 and 86%. Costs of machinery and personnel, as well as an estimate of the forage yield, were assessed. The costs per hectare were found to depend on the slope only, while both the altitude and the slope affected the costs per unit of weight.

Keywords: forage production costs, mountain agriculture, natural constraints, slope, altitude

Introduction

Mountain grassland, if site-specific managed, provides multiple ecosystem services, such as biodiversity, landscape diversity and soil protection from water erosion, of which the whole of society takes advantage. Its maintenance requires a site-specific agricultural use, which is ensured by the local farmers producing forage for their own livestock. However, according to the current socioeconomic changes, mountain grassland is endangered by abandonment because of natural constraints such as climate harshness and unfavourable topography, requiring great efforts of the farmers to manage their farms. Public supporting measures (i.e. rural development programmes) specifically aim, amongst others, at reducing the profitability gap of mountain farms. For this task, reliable figures of the production cost are required. This paper focuses on the effects of two main natural constraints (altitude and slope) on the costs of forage production in the mountain region of South Tyrol (Italy).

Materials and methods

A three-year survey was conducted from 2011 to 2013 on 19 farms in the Puster valley. Data were collected in 100 to 109 selected grassland fields, depending on the observation year. Of these, 95.9% were meadows and 4.1% pastures. Their altitude ranged between 808 and 2,084 m a.s.l., their slope between 3 and 86%. The fields were almost exclusively fertilized with the farm's own organic manure at a mean load of 1.8 livestock units ha^{-1}. The farmers, who participated on a voluntary basis with the assistance of technical personnel, recorded over the whole investigation period the labour times, the machines and devices used, the personnel involved and all other occurring costs for each agricultural operation related to forage production. All in all, data concerning 11,640 operations were recorded. As the operations that at least partially involved contractors accounted for only 4.5% of the total number, the field work was found to be mostly done by farmers and other unpaid family members and/or neighbours. Its economic cost was estimated according to an opportunity cost approach (AAEA, 2000), making reference to the wages of the local contractors association (Maschinenring Südtirol). The unpaid labour of farmers and other operators with an agricultural training was evaluated at the rate of skilled workers, and that of people without an agricultural training at the rate of semi-skilled workers. Half of this rate was used for the labour of children aged less than 16 and elderly people of more than 65-years old. The costs of machinery were computed according to Gazzarin (2011). The forage production was estimated according to a volumetric approach. The volume of the transporting vehicles and of the forage bales were computed for each farm through interviews, whilst the number of transports and a rough estimate of the moisture state of the harvested forage were recorded for each operation by the farmers. The specific weight of silage bales was determined according to Resch *et al.* (2009), those of the other kinds of forage were based on some measurements made during the survey. Further details on data collection and computation are available in Peratoner *et al.* (2013, 2015). The production costs per hectare and per forage weight unit were analysed as functions of altitude and slope by means of mixed models. A first grade polynomial was imposed for slope, as higher order polynomials yielded implausible results. The farm was considered as a random factor, the year as a repeated factor with the field as a subject. The full model was optimised using a stepwise backward selection, until no further improvement of the Akaike Information Criterion (AIC) was achieved. The predictive accuracy of the final model was assessed by a five-fold cross-validation (Hawkins *et al.*, 2003). Both dependent variables were log-transformed prior to analysis to achieve normality of residuals and variance homogeneity.

Results and discussion

Results of the statistical analyses (Table 1) show that neither the altitude nor its interaction with slope affected the production costs per hectare, whereas unit costs per hectare increased with increasing slope (Figure 1). This is in accordance with a previous analysis of these kinds of costs related to the first observation year (Peratoner *et al.*, 2013). Slope provides a constraint to mechanisation, which results in an increase of labour input, or an increase of the use of costly machinery. The analysis of the production costs per unit of forage weight shows, in contrast, an apparent effect of both the slope and altitude, but not of their interaction (Table 1). The effect of altitude can be explained by the decrease of forage yield with increasing altitude. The effect of altitude seems to become stronger above about 1,400 m s.l.m. (Figure 1). At high values of both slope and altitude the predicted costs amount to about a four-fold increase relative to the costs of the more favourable areas.

The prediction accuracy of both cost types shows, however, that the statistical model explains the variability of the costs only to a partial extent, suggesting that other factors, presumably related to management strategies and choices, as well as farmers' skills, play a role in determining the production costs. A further source of uncertainty in the case of production costs per weight unit probably arises from the approximate method of estimation of forage yield.

Table 1. Effect of slope and altitude on the production costs of forage per hectare and per forage weight unit (dry matter).[1]

Effect	*P*-values for production costs (€ ha^{-1})		*P*-values for production costs (€ per 100 kg dry matter)	
	FM	RM	FM	RM
Slope (S)	**0.002**	**0.006**	**0.018**	**0.04**
Altitude (A)	0.493	-	**0.015**	**0.013**
A × A	0.342	-	0.081	**0.023**
S × A	0.155	-	0.607	-
S × A × A	0.292	-	0.966	-

[1] *P*-values are shown for the full model (FM) and the stepwise backward-reduced model (RM). *P*-values of significant effects at an α-level of 5% are in bold.

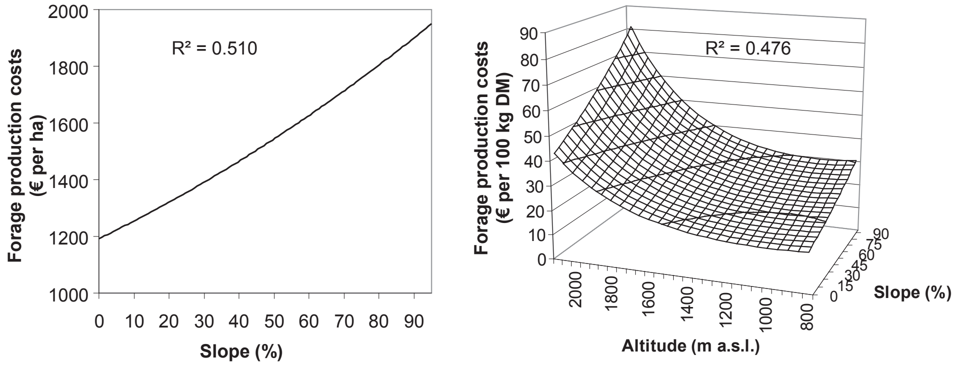

Figure 1. Predicted costs of forage production per hectare and per forage weight unit depending on slope and altitude. Prediction accuracy is expressed as squared Pearson correlation coefficient (R^2) between observed and predicted values according to a 5-fold cross validation.

Conclusions

Only the slope affects the production costs per hectare, whilst both slope and altitude greatly increase the production costs per unit of forage weight. The present findings provide reference values for the effect of altitude and slope on the production costs of forage in the mountain environment of South Tyrol. They are relevant for quantifying public payments in order to ensure the long-term provision of the ecosystem services provided by mountain grassland farmers, who are rational economic agents.

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Ecological intensification of beef grazing systems

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Abstract

Ecological intensification is the production of more agricultural product and ecosystem services relying on ecological processes and biodiversity while reducing external inputs. Recent intensification of beef grazing systems in South America based on increased use of grain in diets, and therefore more fertilizers, pesticides, and fossil energy, have produced negative environmental impacts. The aim of this study was to identify ecological intensification strategies for grazing beef systems modelling case studies from Uruguay. Production and environmental indicators were assessed for cow-calf beef farms and finishing beef farms, using a partial life cycle assessment. Intensification trajectories graphs were used to identify ecological or input-based intensification strategies. Cow-calf farms which optimized the stocking rate and forage production achieved higher beef productivity and lower greenhouse gas emissions per kg and per ha. Finishing beef farms based on native grasslands or improved pastures achieved improved environmental performance in terms of energy and nutrients, although higher emissions. Sustainable intensification of grazing systems relied on efficient utilization of pastures rather than increased use of inputs.

Keywords: grassland, grazing management, sustainability, cow-calf, finishing

Introduction

In the face of global climate change problems, there is a call for 'ecological intensification' of agriculture: the production of more product and ecosystem services through the utilization of ecological processes and biodiversity, attaining greater resource use efficiency and reducing external inputs. There is a need for understanding which management practices and strategies fulfil the ecological intensification requisites, and are not just a greener version of conventional intensification based on external inputs (Tittonell, 2014). Recent intensification of grazing beef systems in South America, based on increased use of grain in diets, and therefore more fertilizers, pesticides and fuel, have increased soil erosion, water pollution, and fossil energy use, while diminishing biodiversity (Modernel *et al.*, 2016; Picasso *et al.*, 2014). The aim of this study was to identify practical strategies for ecological intensification of beef grazing systems modelling case studies from Uruguay.

Materials and methods

Production and environmental indicators were assessed for cow-calf beef farms and finishing beef farms, using a partial life cycle assessment (from cradle to gate), for a range of farms representing the diversity of beef systems in the country. For the cow-calf systems, we used four examples from a typology based on previous surveys of 20 farms based on forage resources, grazing management, reproductive indicators, beef productivity and carbon footprint described in Becoña *et al.* (2014): traditional (low productivity on overgrazed grasslands), improved grazing (higher productivity based on reducing stocking rate), 'carbon smart' (minimum carbon footprint through improved pastures), and input intensive (highest productivity with high stocking rates on improved pastures). For the finishing systems, we modelled four combinations of backgrounding (i.e. stocker, based on grasslands or sown grass-legume pastures) and finishing (based on pastures or feedlot) characterized in terms of animal productivity, feed intake, carbon footprint, energy consumption and nutrient efficiency described in Modernel *et al.* (2013). The methodology for the calculations is based on IPCC 2007 tier 2 equations and described in detail in the previous publications. These real cases provided a wide range of technological strategies, productivities and environmental performances for beef production (Table 1). We developed 'intensification trajectory' graphs (Figure 1) plotting carbon footprint (on the Y axis) versus beef productivity (the X axis) and adding other production and environmental variables as area-sized bubbles in the same plot.

Results and discussion

The graphical analysis (Figure 1) enabled identification of 'ecological intensification trajectories' for farms that increased productivity and reduced environmental impacts (black arrows), and 'input intensification trajectories' for farms that increased productivity based on external inputs (white arrows). For cow-calf systems, reducing stocking rate from traditional overgrazed systems, and increasing forage productivity were management practices that sustained ecological intensification, increasing productivity and reducing carbon footprint per unit of beef and per unit of area. Further intensification based on increased inputs for sown pastures, although increased productivity, also increased emissions. For finishing systems, the increase in productivity linearly increased fossil energy use and nitrogen inefficiency, while reducing GHG emissions per unit of beef (but not always per unit of land).

Conclusions

Sustainable intensification of grazing systems relied on efficient utilization of pastures rather than only increasing use of inputs. Ecological intensification trajectories made efficient use of grasslands and pastures, optimized grazing intensity, and supported high forage productivity.

Table 1. Production and environmental indicators for case study beef systems.

Variable and units[1]	Cow-calf systems				Backgrounding-finishing systems			
	Traditional	Improved grazing	Carbon smart	Input intensified	Grasslands-Grasslands	Grasslands-Pasture	Pasture-Pasture	Pasture-Feedlot
Grasslands[2] (%)	95	93	66	88	100	73	17	19
Pastures[2,4] (%)	5	7	34	12	0	25	75	38
Grain[2] (%)	.	.	.	.	0	2	8	43
Intake (kg DM.an^{-1}.d^{-1})	.	.	.	.	10.8	9.4	8.1	9.3
Weight gain (kg.an^{-1}.d^{-1})	.	.	.	.	0.4	0.5	0.7	0.9
Feed prod.[3] (kg DM.ha^{-1})	3,845	4,196	4,423	4,654	3,765	3,666	2,889	3,070
Stocking rate (LU ha^{-1})	0.81	0.72	0.81	0.86	0.95	1.08	1.06	1.59
Weaning rate (%)	61	82	72	85	.	.	.	.
Productivity[5] (kg LW ha^{-1})	73	89	125	141	143	194	250	299
GHG (kg CO_2 eq.kgLW^{-1})	29	21	16	18	29	22	16	12
GHG (kg CO_2 eq.ha^{-1})	2,061	1,817	1,931	2,551	4,095	4,329	4,071	3,524
Energy[6] (MJ kg LW^{-1})	.	.	.	.	0.0	4.9	11.8	17.3
Nitrogen inefficiency[7]	.	.	.	.	0.4	2.4	4.3	4.8

[1] DM = dry matter, an = animal (steer), d = day, LU = livestock units, GHG = greenhouse gas emissions (methane was 75% in cow-calf, 50-70% in finishing). [2] Percent of land area for cow-calf, and % of diet DM intake for finishing systems. [3] Forage in cow-calf and forage and grain in finishing systems. [4] Mainly grasslands fertilized and over-seeded with legumes in cow-calf, and sown grass-legume pastures for finishing systems. [5] Livestock productivity (LW=livestock live weight gain). [6] Fossil energy use by farm per unit of product. [7] N Input/output ratio.

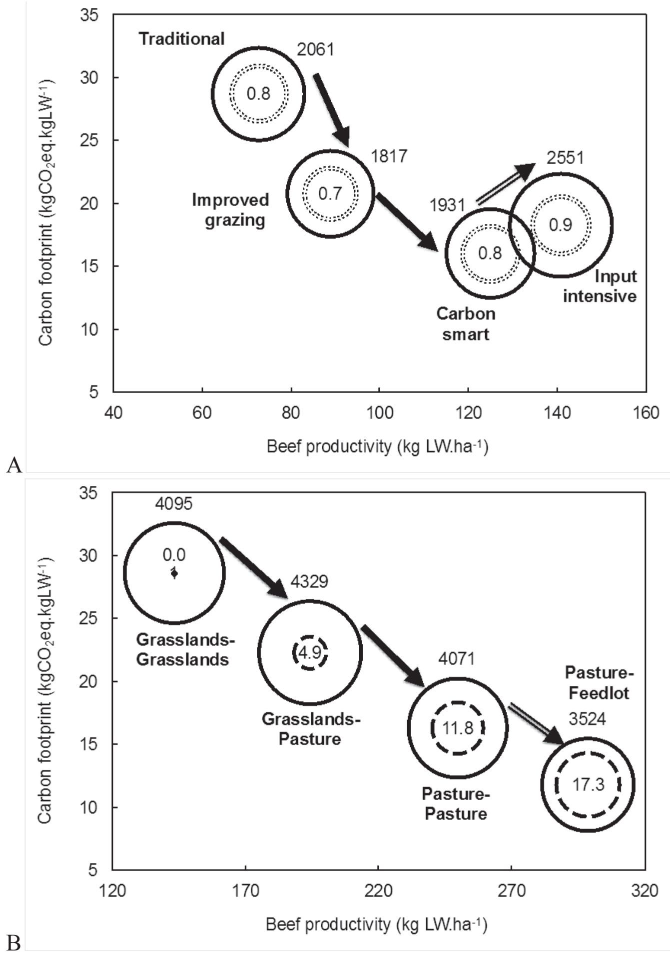

Figure 1. Intensification trajectory graphs for cow calf (A) and finishing (B) systems. Area of continuous-border bubbles represents GHG emissions per unit of land (values outside the bubble in kg CO_2eq ha^{-1}). Area of dotted-border bubbles represents stocking rate in cow calf systems (values in centre of bubble, in LU ha^{-1}). Area of dashed-border bubbles represents fossil energy consumption in finishing systems (values in centre in MJ kg^{-1}).

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Animal activity and welfare of suckler cows grazing during hot and cold days on fen grassland in northeast Germany

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Abstract

In east Germany, the number of heat days (>30°C) will be three to four times higher by the end of this century compared with 1961-1990. Such high temperatures are far above the temperature optimum for cattle and can cause depressions of health, fertility and performance. In a grazing experiment suckler cows were equipped with collars and ruminal transmitters, measuring the GPS position and different physiological data of the animals over the grazing season. Characteristics of animal behaviour were described and compared to the weather conditions. During hot days, the heart rate of the grazing cows increased. They decreased moving over the pasture and changed their activity rhythm. The cows were able to limit a temperature increase of the body surface and reduced the rumen temperature presumably by increasing their water consumption.

Keywords: heart rate, rumen temperature, water consumption, GPS collar, ruminal transmitter

Introduction

Gömann *et al.* (2015) prognosticate a triplication or a quadruplication of the number of heat days (>30°C) for east Germany by the end of this century compared with 1961-1990. Cattle have a rather low temperature optimum and heat can lead to depressions of health, fertility and performance. For a better understanding of the interactions of weather, site conditions and animal performance on pastures we carried out a grazing experiment. The objective of this experiment was to measure and analyse behavioural and physiological data of grazing cows. This will be the basis for the optimization of pasture systems that ensure high animal husbandry efficiency and animal welfare with reference to climate change. The paper provides results of the initial experimental season.

Materials and methods

The grazing experiment was conducted at Paulinenaue, northeast Germany (52°68′N, 12°72′E; 28.5-29.5 m a.s.l.; mean annual temperature 9.2 °C; mean annual precipitation 534 mm, Eutric Histosol) from June to September 2016. Two suckler cows of the herd were equipped with a GPS Plus Collar (Vectronic-aerospace, Berlin). Each device consists of a collar and a ruminal transmitter, measuring the positions by GPS to describe moving distances (MD) and velocity, locomotion activity on the X and Y axis accumulated over a sampling interval (LA), temperature in the reticulum (Tr), temperature of the body surface at the collar (Tc) as well as the heart rate (HR) and HR counting (HRc). HR data were filtered and only recorded when the animal rested and had very low LA (Signer *et al.* 2010). The activity and physiological data, GPS and the weather data were recorded in 3, 5 and 15 min intervals, respectively. The GPS and weather data were transformed by interpolation into 3-min intervals of the animal data. The data were evaluated for each cow during periods of each three cold and three hot days in June, August and September. A 'cold day' was characterized by maximum air temperatures (Tmax) of about 15 to 20 °C (mean air temperature Tmean=17.6 °C) and a 'hot day' by Tmax of about 25 to 35 °C (Tmean=29.3 °C). The daily comparison of the animal and weather data covered the time from 10:00 to 18:00 p.m.

Results and discussion

Animal data in the course of a cold day (22 Sept 2016) and a hot day (12 Sept 2016) are shown in Figure 1 as examples for cow No. 10. The cows adapted their behaviour. They reduced moving over the pasture and changed their activity rhythm on hot days.

The distribution of periods with high and low LA has completely changed on a hot day and is characterized by longer continuous rest periods compared with a cold day. But there was no significant difference in the average daily LA. In contrast to a cold day, very low velocities of the cow´s movement were reported on a hot day, where the MD values significantly decreased (Table 1). Compared with the cold days, Tc increased significantly by about 10 °C, but did not exceed a difference of about 3 °C to Tmean.

In contrast to a cold day, periods of very slow rebound of Tr, e.g. between 14:20 and 15:20 h, were reported as well as a significant decrease of Tr on a hot day. The remarkably slow rebound of Tr was only found when the position of the cows was very close to the watering point.

When the cows grazed during hot days, their heart rate increased significantly (Table 2). In comparison with the cold days, less HRc were registered during the hot days, indicating greater difficulties for the cows to come to rest.

Table 1. Average animal temperatures and behavioural data on cold and hot days (significant differences cold vs hot days are indicated by different letters; $P<0.05$).

Cow	Cold days				Hot days			
	Tr (°C)	Tc (°C)	LA (%)	MD (m)	Tr (°C)	Tc (°C)	LA (%)	MD (m)
7	36.30a	21.81a	47.33a	2,005a	36.09a	31.71b	51.11a	1,771a
10	36.38a	21.26a	44.75a	2,148a	33.84b	31.66b	43.01a	1,968a
Average	36.34a	21.55a	46.11a	2,073a	34.96b	31.69b	47.06a	1,870b

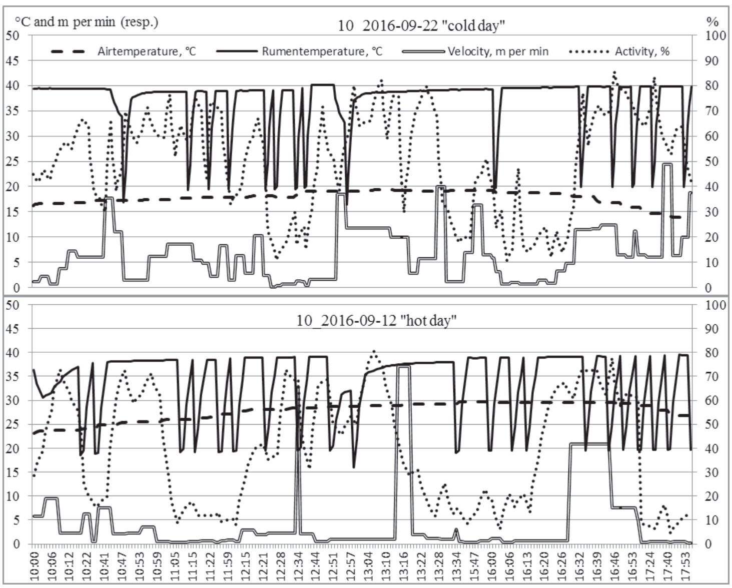

Figure 1. Course of various parameters of cow no. 10 on a cold and a hot day.

Table 2. Heart rate (HR) numbers and HR counting during cold vs hot days (significant differences cold vs hot days are indicated by different letters; $P<0.05$).

Cow	Cold days		Hot days	
	HR (min^{-1})	HRc (n)	HR (min^{-1})	HRc (n)
7	64.20a	10.89a	66.85b	12.78a
10	62.45a	24.22a	64.72a	16.00a
Average	63.33a	18.00a	65.78b	14.00a

Conclusions

By changing their behaviour during grazing, our suckler cows were able to adapt to high temperatures. Although we did not measure it directly, we assume that particularly high water consumption was the precondition for that result. This underlines the importance of an adequate water management on pastures in our region. The summer months of 2016 were comparatively cool and each of the heat periods lasted for only three days and was preceded and followed by cooler periods. If such heat periods last longer, as is not uncommon for our region, a larger heat stress could be expected. The reported data on the hot and cold days showed a similar reaction of the two cows to the different temperature situations. But particularly in terms of HR and HRc, there are some differences between cows. Therefore, the study should be extended over longer heat periods, involving larger samples of cows.

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Partial grazing of natural pasture reduces the cost of feeding supplementation and improves features of meat from bulls

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Abstract

Ten Sardo-Bruna young bulls were managed, after weaning, on two different systems: 5 bulls (Graz) grazed natural pasture during the daylight (for 5-6 h d^{-1}), with a predominance of annual grasses and legumes, and confined during the night and supplemented with concentrate and hay; 5 bulls (Con) were permanently confined and fed with concentrate and hay. Animals were slaughtered at 500-550 kg of live weight. Live and carcass weight, days of fattening and daily weight gain were measured. Dry matter, crude protein, fat content, fatty acid composition and lipid oxidation, in raw and cooked samples of the *Longissimus dorsi* were determined. Live and carcass weight were not different between groups, but Con showed higher daily growth (1,384 g d^{-1} vs 1,027 g d^{-1}) and shorter fattening period (202 vs 240 days); conversely, Graz bulls showed higher cold dressing (53.2 vs 51.2%) and lower drip loss (1.8 vs 2.2%) and lower cost of supplementation (368 vs 450 € $head^{-1}$). Regarding meat quality, differences were found in fat and protein content between groups: meat of grazing animals tended to have higher short chain fatty acids, poly unsaturated fatty acids (PUFA)n3 and conjugated linoleic acid content and lower PUFA n6, n6/n3 ratio and lipid oxidation, both in raw and cooked meat, compared with meat of confined bulls.

Keywords: management, meat quality, cost supplementation

Introduction

In Italy beef meat production has great importance both for the number of animals slaughtered and for the economic value generated by the industry (SMEA, 2014). The most common breeding system provides animals in confinement and fattening in the stable from 6-8 months of age up to slaughter, which in the case of males can take place at 16-18 months of age (Gottardo and Cozzi, 2005; SCAHAW, 2001). The feeding system is exclusively based on the use of stored feed and the main forage source is represented by corn silage on irrigated farms, and hay and/or straw on non-irrigated farms. Sardinia ranks as the sixth region in Italy for cattle stock and has about 80% of its cattle population aimed at meat production (http://statistiche.izs.it). Nevertheless, it has a limited number of fattening farms due to high feed costs, so a high number of weaned calves are sold to fattening centres in northern and central Italy (https://www.vetinfo.sanita.it). Taking into account the features of cattle farms in Sardinia, the new regional policies on animal welfare and of the demands of consumers, the aim of the work was to evaluate the effects of grazing of fattening bulls, on their productive performance, meat quality and the economic aspects of this farming system.

Materials and methods

Ten Sardo-Bruna bulls, after weaning (average of 8.5 months of age), were finished on two different management systems: 5 bulls (group Graz) grazed natural pasture (with a predominance of annual grasses and legumes) during the daylight (5-6 h d^{-1}), and were housed during the night. A mixture of barley-corn meal, a complete mixed feed and meadow hay were used as supplementation, and administered before and after grazing; the other 5 bulls (group Con) were permanently confined and fed with a mixture of barley-corn-peas meal, a complete mixed feed and meadow hay, and administered twice per day. Once they had reached a suitable weight for the breed, about 500-550 kg live weight, bulls were slaughtered. Live weight and daily growth were monitored monthly; fattening days to reach final body weight and costs of supplementation were also considered. Cold carcass weight was measured and dressing percentage on cold carcass weight and associated carcass drip loss during chilling were calculated. Five days after slaughtering and storage of carcass at -4 °C, one sample of *M. Longissimus dorsi,* between the 5^{th} and 7^{th} rib, was taken from each animal. Dry matter, crude protein, fat content, fatty acid composition and fatty acid oxidation were determined on the day after collection (Day 0) and after 7 days of freezing storage at -20 °C (Day 7). Fatty acids composition of fat from muscle samples was determined by gas chromatography (Nudda *et al.*, 2013). Fatty acid oxidation was assessed by determining secondary products of fatty acid oxidation such as 2-thiobarbituric acid-reactive substances (TBARs), as described by Nudda *et al.* (2013). Data were analysed using one way ANOVA procedure to evaluate differences between management systems; statistical significance was accepted at P<0.05 and statistical trends at P<0.10.

Results and discussion

Live body weight at slaughtering and carcass weight were not different between groups, because this was set as the target. Con animals showed significantly higher average daily growth (1,332 vs 1,027 g d^{-1}; P=0.014) and shorter fattening period (202 vs 244 days; P=0.029) than Graz animals (Table 1). However, the use of the pasture, even for a few hours a day, reduced the amount of concentrates and hay administered to animals and the costs of food supplementation (368 vs 450 € $head^{-1}$). Moreover, Graz animals tended to have higher cold dressing percentage (P=0.065) and lower drip loss (P=0.084) than Con group (Table 1).

Regarding meat quality, the management systems did not affect the fat and protein content in raw meat. The moisture content was higher and the protein content lower in cooked meat of Graz than Con group (Table 2), probably due to a higher water-holding capacity in this meat. The meat of the Graz group showed a more favourable n6/n3 ratio and a higher content of conjugated linoleic acid (CLA) both in raw and cooked meat (Table 2). Overall, fatty acid composition of meat in Graz animals showed greater variability than Con animals (Table 2), probably because the bulls were slaughtered at different times: one part, after spending the whole fattening period grazing green pasture; another part, after spending the last two months of fattening period grazing on dry grassland. This may be the reason that we did not detect more significant differences in fatty acid composition.

The fatty acids oxidation (Table 2) was lower for Graz than Con raw meat samples at 0 and after 7 days of freezing storage at -20 °C. Cooked meat of Graz group showed higher stability to oxidation than Con only after 7 days of freezing storage (Table 2). The amounts of TBARs formed with the cooking were below the critical value of 3 mg kg^{-1} at which rancidity is virtually detected (Wong *et al.*, 1995).

Table 1. Animal performance and supplementation cost.

Parameters		Graz	Con	SEM[1]	*P*-value
Initial live weight	(kg)	287	291	10.54	0.766
Final live weight	(kg)	536	560	18.27	0.415
Average Daily Growth	(g d^{-1})	1,027	1,348	64.80	0.014
Fattening period	(days)	244	202	11.26	0.029
Supplementation cost	(€ $head^{-1}$)	368	450	26.71	0.060
Cold carcass weight	(kg)	283	289	10.35	0.732
Drip loss	(%)	1.80	2.17	0.13	0.084
Cold dressing	(%)	52.9	51.2	0.55	0.065

[1] SEM = standard error of the mean.

Table 2. Chemical composition, fatty acid profile and oxidative status in raw and cooked meat of *Longissimus dorsi* muscle.[1]

Parameters		Raw meat				Cooked meat			
		Graz	Con	sem	*P*-value	Graz	Con	sem	*P*-value
Moisture	%	76.02	75.66	0.25	0.339	67.12	64.66	0.51	0.009
Fat	%	1.42	1.50	0.22	0.796	2.86	2.06	0.70	0.442
Protein	%	21.54	21.48	0.35	0.906	28.28	32.06	0.64	0.003
Fatty acid (g/100g FAME)									
SFA	%	48.85	47.29	1.35	0.438	49.84	47.07	1.01	0.088
MUFA	%	35.92	35.77	1.98	0.958	37.08	36.45	1.96	0.827
PUFA n-6	%	12.66	15.05	1.74	0.360	10.71	14.71	1.80	0.155
PUFA n-3	%	1.67	1.13	0.29	0.224	1.43	1.00	0.18	0.127
n6/n3	n.	7.89	14.03	1.13	0.005	7.38	14.89	0.94	0.000
CLA	%	0.34	0.26	0.03	0.104	0.37	0.25	0.03	0.014
TBARs-day 0		0.07	0.23	0.03	0.004	0.52	0.56	0.09	0.743
TBARs-day 7		0.39	1.29	0.31	0.072	1.59	4.41	0.24	0.000

[1] SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; CLA = conjugated linoleic acid; n6/n3 = PUFA n-6/PUFA n-3 ratio; TBARs = 2-thiobarbituric acid reactive substances; sem = standard error of the mean.

Conclusions

Feeding fattening bulls at pasture for a few hours a day reduced the costs of food supplementation, permitted a high cold dressing percentage and lower drip loss. Furthermore, grass-finished diet has been shown to modify fatty acids profile, increasing the CLA content and reducing the n-6/n-3 ratio and the fatty acid oxidation, with a consequent improvement of nutritional quality of the beef. The results show that feeding bulls with grass can have positive effects on the quality of their meat. In addition, an added value should be related to the welfare status of grazing compared with confined animals.

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Feeding behaviour of dairy cattle during summer grazing on mountain pasture

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Abstract

During summer grazing on mountain pasture, a supplement can be offered to dairy cattle in order to maintain milk production and to favour the recovery of body condition. However, supplementation could influence the feeding behaviour of the animals on pasture. This paper reports the preliminary results about the effects of the supplement level on selection behaviour and feeding time of dairy cattle grazing on high mountain pasture. Eight Italian Simmental cows were maintained day and night in a mountain pasture located at 1,520 m a.s.l. and characterized by a *Poion alpinae* alliance (main species: *Festuca pratensis* Huds., *Phleum rhaeticum* (Humphries) Rauschert, *Poa alpina* L., *Trifolium repens* L., *Trifolium pratense* L., *Alchemilla vulgaris* L. and *Ranunculus acris* L.). Cows were milked twice a day and, during milking, the animals received either 1 kg head d^{-1} or 4 kg head d^{-1} of supplement. Samples of available and selected herbage by cows were collected and analysed. An automatic noseband sensor (RumiWatch, ITIN-HOCH, Switzerland) assessed the ruminating and eating time of animals by recording jaw movements. The supplement level did not influence the ruminating and eating time of dairy cows but partially affected their feed selection on pasture.

Keywords: dairy cows, mountain pasture, feeding behaviour, automatic sensors

Introduction

Effective alpine pasture management can be supported by data on feeding behaviour of grazing animals. Moreover, information on the effects of supplementary feeding could further strengthen the effectiveness of management practices on pasture and animals. In fact, it is known that grazing animals may consume a smaller amount of grass when they receive food supplements (Bovolenta *et al.*, 2008). In addition, the walking time spent for grazing, especially in the alpine environment, affects significantly the maintenance requirements of animals. In recent years the development of compact and accurate equipment for continuous monitoring of feeding behaviour (Ruuska *et al.*, 2016) provides reliable data with relative ease. This study aims to evaluate the effect of different levels of supplementation on the feeding behaviour of Simmental cows grazing on an alpine pasture.

Material and methods

The study was conducted in an alpine farm (Malga Montasio, Udine, Italy; 46°24'45''N, 13°25'53''E; 1,500-1,800 m a.s.l.). A herd of 110 Italian Simmental cows was allowed to graze on a pasture located at 1,520 m a.s.l. and characterized by a *Poion alpinae* alliance (main species: *Festuca pratensis* Huds., *Phleum rhaeticum* (Humphries) Rauschert, *Poa alpina* L., *Trifolium repens* L., *Trifolium pratense* L., *Alchemilla vulgaris* L. and *Ranunculus acris* L.). Cows were maintained day and night on pasture. For the experiment, 8 dairy cows of the herd were selected and assigned to two balanced groups. During a 10-day experimental period 4 cows (group High) were supplemented with 4 kg of concentrate $head^{-1}$ d^{-1}. The other 4 cows (group Low) were supplemented with 1 kg of the same concentrate $head^{-1}$ d^{-1}. The supplement was given twice a day during milking. The grazing and ruminating times of the 8 cows in the trial were assessed through an automatic noseband sensor (RumiWatch, ITIN-HOCH, Switzerland) by recording jaw movements continuously. In addition, 8 pedometers were used to record walking times. For 3 consecutive days the composition of available herbage (AH) was estimated by cutting 6 strips of

10 × 0.10 m at 0.04 m stubble height, using electric grass shears (herbage mass: 2,386 kg DM ha^{-1} on average), while the composition of selected herbage (SH) by dairy cows was estimated by a hand-plucking technique. Samples of AH and SH were hand-separated into botanical families. On the sub-samples, oven-dried at 70 °C, a botanical assessment by weight was carried out. The palatability index (PI) was calculated, for each sub-sample, as ratio of the incidence in SH against their occurrence in AH (Carpino *et al.*, 2003). Chemical composition and nutritive value, expressed in forage units for milk (FUM), of AH and SH were analysed and assessed as reported by Bovolenta *et al.* (2014). The effect of the supplement levels on chemical and floristic composition of selected plant species was evaluated statistically with a mixed model that took into account the effect of the supplement level, the day of grazing and the animal nested within treatment as random factor. The effect of the supplement level on grazing behaviour was evaluated with a mixed model for repeated measures that considered the level of supplement as a fixed effect, the day of grazing as repeated measurement and the interaction between these two factors. The interaction was not reported as it was not statistically significant.

Results and discussion

The botanical composition of AH (Table 1) showed a high presence of *Poaceae* (676.6 g kg^{-1} DM), mainly composed by *F. pratensis, Festuca rubra* L., *Helictotrichon pubescens* (Huds.) Pilg., *P. rhaeticum* and *P. alpina*. The *Fabaceae* essentially comprised clovers (*T. pratense* and *T. repens*). The 283.7 g kg^{-1} DM of AH classified in 'Other families' included several species typical of *Poion alpinae* alliance, as *Achillea millefolium* L., *A. vulgaris, Galium anisophyllum* Vill., *Plantago atrata* Hoppe, *Potentilla aurea* L., *R. acris* L. and *Veronica chamaedrys* L. *Poaceae* in SH decreased to 415.5 g kg^{-1} DM compared to AH, with a PI of 0.61 for both groups. Conversely, *Fabaceae* increased and were preferred ($P<0.05$) by animals with high supplement level (98.1 g kg^{-1} DM; PI=2.45) compared with animals with low supplement level (63.2 g kg^{-1} DM; PI=1.58). It is well known that *Fabaceae* have a higher protein content and a greater palatability than *Poaceae*. However, the results of the chemical analysis showed no significant differences between the two experimental groups. The nutritional value of AH was 0.66 FUM kg^{-1} DM. Grazing dairy cows selected the herbage (SH) in such a way as to increase its nutritional value (0.79 FUM kg^{-1} DM on average) compared with AH.

Data concerning feeding behaviour are reported in Table 2. During the day, 561 and 522 min of grazing time, for high and low supplementation, were recorded respectively (no significant difference). Rumination time was 473 min for both groups. These findings are similar to those reported by other authors for Holstein-Friesian cows, grazing day and night (Abrahamse *et al.*, 2008; O'Driscoll *et al.*, 2010). The average bite rate (number of bites min^{-1}) is similar among the experimental groups (72.4

Table 1. Botanical and chemical composition of available (AH) and selected herbage (SH).[1]

	AH	Supplement level (SH)		SEM
		High	Low	
Poaceae (g kg^{-1} DM)	676.6	420.7	410.3	27.03
Fabaceae (g kg^{-1} DM)	39.7	98.1[a]	63.2[b]	8.24
Other families (g kg^{-1} DM)	283.7	481.2	526.5	27.73
Ether extract (g kg^{-1} DM)	26.7	26.0	27.5	0.59
Crude protein (g kg^{-1} DM)	86.0	99.9	99.7	1.36
NDF (g kg^{-1} DM)	585.8	599.6	574.3	8.09
ADF (g kg^{-1} DM)	385.7	322.5	300.8	6.74
ADL (g kg^{-1} DM)	123.8	112.8	118.1	4.06
Nutritive value (FUM kg^{-1} DM)	0.66	0.77	0.81	0.013

[1] SEM = standard error of the mean; DM = dry matter; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; FUM = forage units for milk. [a,b] = $P<0.05$.

Table 2. Feeding behaviour of Simmental cows with high or low level of supplementation.[1]

	Supplement level		SEM
	High	Low	
Grazing time (min day^{-1})	560.5	522.4	13.33
Grazing bite rate (bites min^{-1})	72.6	72.2	1.40
Ruminating time (min day^{-1})	472.6	472.8	13.74
Ruminating bite rate (bites min^{-1})	60.8	60.6	1.17
Walking time (min day^{-1})	93.9	99.9	6.24
Steps count (steps day^{-1})	3,409	3,546	218.7

[1]SEM = standard error of the mean.

bites min^{-1}). These values are higher than those reported in the two aforementioned papers (64.5 and 58.5 bites min^{-1}, respectively). The statistical analysis did not present any significant difference in terms of walking time.

Despite the large gap in the level of supplementation, there were no differences in the feeding behaviour of the two experimental groups. Therefore, it is likely that the animals have consumed the supplement without reducing their herbage intake. In a previous experiment carried out on Brown cows with different levels of supplementation, significant reductions in grazing time and herbage intake were observed (Bovolenta *et al.*, 2005).

Conclusions

Monitoring feeding behaviour of grazing animals, made easier and accurate by modern electronic devices, allows the evaluation of the effect of different management choices or behavioural anomalies of individual cows. In future studies, it seems useful to deepen the understanding of feeding behaviour of different breeds, in particular dual-purpose breeds, given their key role in alpine farming.

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How extensive grassland is seen from a dairy industry perspective

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Abstract

While discussing the protection of high-value grassland management systems, conservationists usually point to the Common Agricultural Policy (CAP). However, besides these, the dairy sector is also a keystone for conservation. In this paper, we outline the point of view of the dairy industry in Germany via an environment analysis. There is a strong market for milk-products from high quality grassland management systems. It is not the CAP, but the retail sector and a distinct lack of clear definitions that prevents the dairy sector from investing in more sustainable milk-products. Therefore, more attention by conservationists should be given to market imbalances, as opposed to the CAP, which is difficult to influence.

Keywords: dairy industry, grassland conservation, milk production

Introduction

The domestic dairy industry is one of the keystones for quantitative and qualitative grassland protection, besides that of consumers, the retail industry, policy-makers and farmers. Milk products are the most important final economic output of grassland systems and it is (or should) therefore also be for the dairies to guide farmers into different economic activities. Despite this fact, there is very little scientific work on dairies and their influence on different ways of producing milk, including animal husbandry, feeding, liquid manure disposal etc. Production, processing, sale and consumption are not part of a framework that is subjected to 'the' market forces. Rather, the individual links of the chain can themselves be understood as forces shaping their own market and its resources. Germany holds the top position within the European milk market with a delivered quantity of 31.38 million tons or 21% of the total milk production volume of the EU-28 (EPRS, 2015). While the quantitative loss of grassland has been halted, the loss of ecologically valuable (extensive) grassland has been dramatic. The goal of this study is to visualize the potential interest of dairies in grassland management that takes nature conservation goals into account.

Material and methods

The literature review for this study has been performed under consideration and adoption of the guidelines of Pullin and Stewart (2006). We have also performed qualitative interviews with dairies of different sizes from regional to national operating companies. The information was then used to perform a SWOT analysis. A SWOT analysis (Strengths, Weaknesses, Opportunities and Threats) enables businesses to assess their status and to plan their strategic development. The external influencing factors as key component of SWOT analysis can be analysed and evaluated from the outside. The proposed goal was evaluating chances for dairies to positively contribute to (qualitative) grassland conservation as long as they can generate income from it.

Results and discussion

From the perspective of the dairies, the opportunities for establishing product lines based on high-value grassland management (preferably including grazing regimes) are counterpoised by considerable risks (Figure 1). The dairies have only limited strategic means to counteract these. The retail industry in Germany wields considerable market power (the five largest retailers generate 80% of the annual revenue) (Bundeskartellamt, 2014).

The current CAP favours intensively managed grassland farm enterprises. In addition, the national implementation in pillar 2 only marginally promotes high-value grassland management. This is in stark opposition to the culturally rooted image of 'free-range-dairy-cattle' as held by consumers. Apart from the purely quantitative (future) procurement problem for milk from free-range pasture feeding, this puts further strain on the image of milk as the 'healthy food' which it is still perceived to be, because consumers associate it with the animals' opportunity to spend time outside. Expert opinions project that pasture feeding will continue to decline (Reijs *et al.,* 2013). As a result, the procurement of milk from grassland management, as envisaged here, will be possible only with long-distance transit. This is especially true when prices for organic milk do not guarantee significant additional revenue.

Consumers' endorsement of high-intensity agriculture is steadily declining (Balmann, 2016). This decline in acceptance of conventionally produced agricultural products is accompanied by a potential entrepreneurial image boost through the marketing of sustainably produced goods such as organic foods from high-value grassland management. In Germany, dairies are so far reluctant to engage in the marketing of milk products from ecologically valuable grassland management with respective quality labelling strategies. While in the neighbouring Netherlands, 80% of fresh milk originates from certified, clearly defined pasture feeding milk production, the respective standards are lacking in Germany (Deutscher Bundestag, 2016). Without these clearly defined provisions, German dairies are primarily dependant on existing certification schemes if they are to bolster credibility and trust in the short term. Inconsistent regulations further consumers' distrust in organic product lines. German consumers are generally discriminating and investments into (expensive) organic product lines are therefore highly risk-prone, if the product quality cannot be guaranteed. Nonetheless, the considerable commercial potential has already been proven in the organic milk product area, as organic milk products are experiencing high and stable demand. Price elasticity on the consumer side is very well established, as organic products cater to values currently in high esteem in society – 11% of Germans buy organic milk products exclusively, 27% regularly and 42% occasionally (Hölscher, 2016). For example, the global-market-oriented dairy

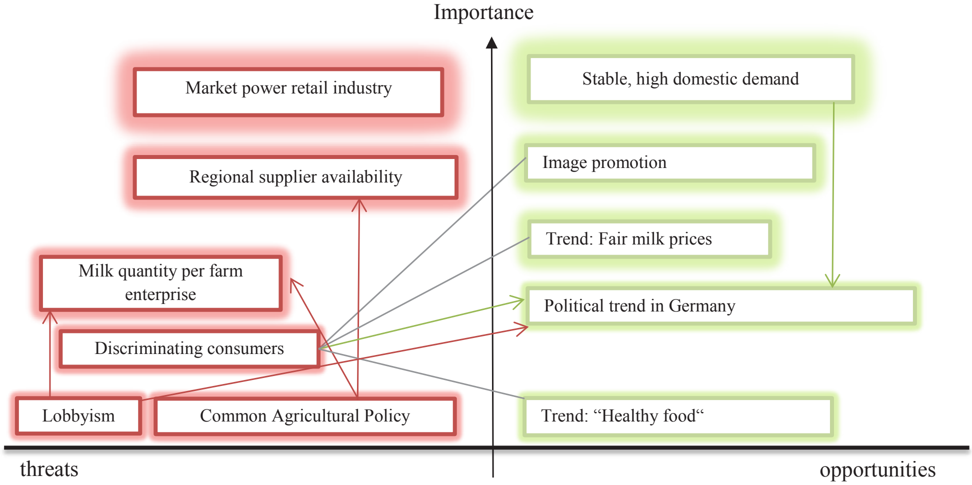

Figure 1. Visualisation of the environment analysis as part of the SWOT-analysis from the dairies' point of view. The dairy sectors aim is 'using milk from dairy farmers with grasslands of higher biodiversity'. Higher Y-values indicate a stronger possible influence on economic decisions.

‘Ammerland’ has been able to expand their pasture feeding milk programme and features long waiting-lists with potential farmers. The ‘Berchtesgadener Land’ dairy has managed to pay their supplying farmers up to 10 cents more than its competitors (Ilchmann, 2016).

At the same time, and in comparison with other European regions, a very powerful lobby (especially the farmers’ association ‘Bauernverband’) favouring intensively managed agriculture and globally oriented dairies still exists in Germany, with strong ties to policymakers. While this lobbying influence has decreased somewhat in recent years, its efficacy is still significantly higher than that of organic farmers. Apart from niche business ventures, it appears that dairies still see stronger facilitation potential in joining these conventional advocacy groups or at least in not opposing them. Surprisingly to us, many dairies appear to barely have any contact with their farmers. Especially large dairies operating at the national scale were generally uninterested in our requests for interviews and about the type of business and the situation of their suppliers.

Our research shows that the CAP, which has merely been applied as a setscrew in the area of grassland conservation, has only little and indirect influence on strategic decisions of dairies regarding a realignment towards milk products from high-value grassland management. It is much rather existing market barriers, such as the concentration of market power with the retailers, that prove to be deciding factors for dairies. In the discussion around Greening and the 2nd pillar of the CAP, the fact that there is already a trend towards products from high-value grassland management is currently largely overlooked. Consequently, enhancing consumers’ awareness should be focused on far more strongly.

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Effects on grassland productivity when converting from a conventional to an organic system

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Abstract

Well-established conventional grass fields on three different soil types were selected to determine the effect on forage yield when converting from a conventional system to an organic based system. Each field (block) was divided into 3 main plots, with different fertilizer treatments: (I) cattle slurry only (organic system), (II) cattle slurry + mineral fertilizer (conventional system) or (III) mineral fertilizer only (mineral system). The organic based system (I) was inferior to both the conventional (II) and the mineral (III) systems in terms of harvested dry matter and nutrient yield, nutrient content and nutrient use efficiency (NUE). It is concluded that organic systems during the first two years of conversion are less productive than conventional systems for grass-fed livestock under Icelandic conditions.

Keywords: Grassland, productivity, conventional, organic

Introduction

Global comprehensive meta-analysis compared relative harvested crop yield of organic and conventional farming systems and concluded that organic crop yields (per area unit) are typically lower than conventional crop yields. Organic systems showed on average 34% lower yields than conventional systems in studies where these systems were most comparable (Seufert *et al.*, 2012). However, it was emphasized that crop yield differences are very contextual and related to sites (environment), crops and products. The smallest difference (5% lower organic crop yield) was in rain-fed legume and perennials on weak-acidic to weak-alkaline soils. Icelandic farming is not comparable to that of other countries in many ways because of its unique juvenile volcanic soils and exceptionally cold growing seasons. There has been interest in increasing the share of organic food production in Iceland, which primarily is based on grass-fed livestock farming. However, no domestic research has been conducted on what effects conversion from conventional farming systems to organic farming systems have on forage (grass) yields and livestock productivity. The aim of this study was to evaluate with experimentation what effects conversion from conventional to organic dairy farming has on forage yield in well-established conventional grass fields.

Materials and methods

Conventional well-established semi-permanent grass fields were selected on three different soil types: (1) dryland brown *andosol* (*Phleum pratense* > *Trifolium pratense*), (2) drained *andic histosol* (*P. pratense* > *Poa pratensis*), and (3) fine sandy *vitrosol* on river delta (*Alopecurus pratensis* > *Deschampsia cespitosa*). These fields were all located within 2 km distance of the meteorological station at Möðruvellir Research Farm (65°46.239'N. 18°15.080'W) in northern Iceland (Table 1).

Each field (block) was divided into three plots of 3-4,000 m^2 each with different fertilizer treatments (2-year means): (I) organic system with cattle slurry only (140 kg total nitrogen (N), 20 kg phosphorus (P) and 127 kg potassium (K) ha^{-1}); (II) conventional system with cattle slurry (70 kg total N, 10 kg P, 63 kg K + mineral fertilizer, 60 kg N, 11 kg P, 0 kg K ha^{-1}) and (III) mineral system with mineral fertilizer only (120 kg N, 16 kg P, 52 kg K ha^{-1}).

Both the cattle slurry (66 g DM kg^{-1} fresh weight, s.e.d. = 2.8) and the mineral fertilizers were applied at the beginning of the growing seasons in early May with ordinary farm equipment. The timing and

Table 1. Meteorological data during the two growing seasons at the experimental site.

Month	Daylight	2 m air temperature (°C)		10 cm soil temp. (°C)		Precipitation (mm)	
	hours 24^{-1}	2013	2014	2013	2014	2013	2014
May	18.5	5.4	7.0	3.0	7.4	26	10
June	21.4	11.1	12.0	10.4	13.8	8	26
July	20.2	11.0	12.5	11.5	14.4	26	61
August	16.6	10.0	10.5	11.2	12.3	9	16
Mean/sum	19.1	9.4	10.5	9.0	12.0	69	113

application methods followed standard procedures in Iceland. The slurry and mineral fertilizers were surface spread with broadcasters. The dry matter (DM) and nutrient content of the slurry was determined by standard laboratory analysis from samples taken *in situ* during application in all fields. The mineral fertilizer for treatment II was a mixed 26.5% N / 5.0% P / 2.0% S fertilizer, and for treatment III a mixed 22.6% N / 3.4% P / 1.0% S fertilizer (content determined by the fertilizer inspection authorities in Iceland).

The fields were harvested in two seasons (2013, 2014) and cut twice per year. Yield determination was made with a plot mower (stubble height of approximately 5 cm) on 4 subsites in each plot. Harvest sites were randomly selected at the beginning of the experiment and fixed with GPS navigation points to secure that the yield determination was repeatedly made at the same position in all cuts and years. The average net harvest area was 4.7 m^2 (±0.4). The herbage mass was weighed on a plate scale (±10 g accuracy) *in situ*. Chopped samples (200-300 g fresh weight) were taken from each plot for DM and nutrient analysis.

The experiment was a randomized block design for analysis of variances (ANOVA) where fields are block nested within years (2013, 2014). All statistical calculations were made with the software JMP Discovery TM to compare system effects on yield and nutrient use efficiency (NUE).

Results and discussions

The meteorological data during the two growing seasons reveal big differences in temperatures and precipitation between 2013 and 2014 (Table 1). The 2014 season was much wetter and warmer than the 2013 season resulting, on average, in 39% higher DM yield in the second year. There were also significant yield differences between soil types with the field on the sandy *vitrisol* giving the lowest yield (4.0 t DM ha^{-1} on average) and the field on the *andic histosol* giving the highest yield (5.8 t DM ha^{-1} on average). However, no significant interactions between treatments and seasons or soil types were observed. Therefore, only main effects of production systems on harvested yield are discussed further here (Table 2).

There were significant differences between all forage production systems when it came to DM-, N- and P-yields, whereas K-yields were similar. The mineral system (III) yielded 36%, 60%, 36% and 10% more DM, N, P and K respectively than the organic system (I) in spite of receiving less total N, P and K from fertilizer application.

DM yield is primarily regulated by N availability for plant growth. On average 46% of the total N in the slurry was organic bound and not directly available for plants, 54% of the slurry-N was present as mineral N (mostly as NH_4^+) which can be taken up by plants. This means that applied mineral N in the organic system (I) was 76 kg ha^{-1} compared with 120 kg N ha^{-1} in the mineral system (III). This explains the yield differences between the systems, which also causes lower P and K yield. The P concentration in

Table 2. The effect on forage production system on total annual yields and NUE. ANOVA least mean squares of two years (seasons) and three soil types.

System	Yield (kg ha^{-1})				Nutrient use efficiency (kg)			
	DM	N	P	K	DM kg N^{-1}	N kg N^{-1}	P kg P^{-1}	K kg K^{-1}
Organic (I)	4,278	82	13	85	30.3	0.6	0.6	0.7
Conventional (II)	5,310	106	16	88	40.9	0.8	0.7	1.4
Mineral (III)	5,806	132	17	94	48.3	1.1	1.1	1.8
Mean	5,131	107	15	89	39.8	0.8	0.8	1.3
Standard Error	160	3.2	0.4	3.6	1.25	0.03	0.02	0.06
P-value	<0.0001	<0.0001	<0.0001	0.2314	<0.0001	<0.0001	<0.0001	<0.0001
Organic/mineral	0.74	0.62	0.73	0.91	0.63	0.53	0.57	0.37

DM was the same in all systems with 0.29%, even though the organic system received 25% more P than the mineral system. This indicates that P in cattle slurry is slightly less available for plant growth than P in regular mineral fertilizers and confirms previous findings from this area (Sveinsson, 2013). The K concentration in the DM was, as expected, significantly higher in the organic system than the mineral system, 2.0 and 1.6% K, respectively. High doses of cattle slurry as applied in this experiment results in excessive K fertilization and luxury K uptake in grasses. NUE in respect to DM, N, P and K was in all cases significantly highest in the mineral system (III). The NUE for total N was 0.6 and 1.1 kg kg^{-1} applied total N for the organic and mineral system, respectively. If the organic N is excluded from the calculation, then the NUE would also reach 1.1 kg in the organic system as it was in the mineral system. The NUE for N, P, K in the mineral system is in all cases >1 but <1 in the organic and conventional system with the exception of K in the conventional system. These systems if continued will presumably lead to different successions of changes in soil properties with time. A long term experiment (28 years) in a grass field on a fresh *histosol* with continuous, yearly application of farm yard manure (FYM) from sheep has shown significant changes in soil properties and yield responses with time compared with mineral fertilizer applications only (Brynjólfsson, 2005).

Conclusions

Organic grassland systems during the first two years of conversion are less productive than conventional systems under Iceland's cool growing conditions.

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Sheep diet and performance in two contrasting heathland pastures during winter

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Abstract

Micro-histological analyses were used to examine sheep diet and performance in two contrasting extensive pastures during winter. Both sites were located in the winter mild oceanic parts of Norway, site A in the south (60°42'N) and site B in the north (66°10'N). Site A consists of well-managed *Calluna*-dominated heathlands with grasslands, mires and small woodlots in between. Site B was a mosaic between grasslands with patches of unmanaged *Empetrum*-dominated heathlands and mires. Sheep breed was the native Norwegian *Ovis brachyura borealis* at both sites. Grazing pressure, in accordance with national conservation recommendations, was moderate at both sites (0.20-0.25 sheep units (ewes incl. lambs) ha^{-1}). At both sites, the ewes changed their diet from herbs and grasses in summer towards woody species during winter, most importantly *Calluna vulgaris* at site A and *Juniperus communis* at site B. The ewes gained weight mainly during autumn, and lost weight during winter. Weight loss was higher at the northern site. The results reveal the need of proper management of heathlands to stimulate the growth and renewal of *C. vulgaris* in winter pastures, as well as to regulate the grazing pressure to ensure animal welfare.

Keywords: coastal heathlands, sheep performance, winter grazing

Introduction

Sheep grazing marginal pastures has been a part of agriculture and small-scale farming systems since the beginning of the Neolithic period, creating landscape values and semi-natural habitats (Hejcman *et al.*, 2013). These habitats are now considered as the main source of biodiversity in agricultural landscapes (Poschlod *et al.*, 2009). To preserve semi-natural habitats, grazing has been maintained or reintroduced after giving environmental grants to manage such species-rich systems. Coastal heathland is a 5,000-year-old semi-natural habitat created through all year round grazing and prescribed, rotational burning when heather (*Calluna vulgaris*) grows old. In Norway as in the rest of Europe, the habitat is now endangered due to land use changes and abandonment (Diemont *et al.,* 2013; Lindgaard and Henriksen, 2011). Rotational burning creates grasslands for summer grazing which gradually develop into heathlands dominated by heather. Winter grazing is important to maintain coastal heathlands, and young heather stands are normally wintergreen under suitable climatic conditions and regarded as an acceptable winter feed (Garmo, 2011). The native Norwegian Old Norse Sheep (ONS), *Ovis brachyura borealis,* are considered as well adapted to climate and environmental conditions in coastal heathlands. As a grazer, ONS is believed to utilize woody species better than the national breed NKS (Norwegian White Sheep). In this paper the results from a study of sheep performance at two contrasting coastal heathland pastures during winter is presented.

Material and methods

The study was performed at the coast of Norway within two coastal heathland pastures enclosed at isolated islands. Site A was located at Lygra (60°42'N, 5°3'E) in the south-western part of the Norwegian heathland region. Mean annual (1961-1990) precipitation and temperature is 2,190 mm and 6.9 °C and mean winter temperature (Dec-Feb) is 0.7 °C. Site B was located at Risvær (66°10'N, 12°37'E) in the north-western part of the heathland region. Mean annual precipitation and temperature is 1,195 mm and 5.5 °C, while mean winter temperature (Dec-Feb) is -0.1 °C. Snow cover is rare at both sites and seldom lasts more than a few days. Both sites have been grazed all year round for several years by a herd of ONS, normally without any supply of winterfeed. Vegetation cover of both islands was interpreted from aerial ortho-photos into a GIS and verified through field inventories. Both pastures are a mixture of heathlands and semi-natural grasslands. Most of the heathlands in site A are managed through regularly prescribed burning with a normal rotation of less than 20 years. Some remaining areas are old and degenerating. At site B there has been no prescribed burning, and the heathlands are dominated by *Empetrum nigrum* and *Juniperus communis*. The productivity and quality of the grasslands at the two sites are comparable. The ewes were followed through three periods for one year (1: Jun-Oct, 2: Oct-Feb, 3: Feb-Jun). Live weight was recorded for all ewes at the beginning of each period, and at the end of the last one. All ewes were treated against intestinal parasites. Daily live weight changes were calculated for each period and adjusted for differences in live weight by calculating the relative weight change (%) for each animal. Normal winter temperature is slightly lower in site B than in A, and this might affect sheep performance. To test for such effect, the same ewes in site A were followed through two consecutive winters (2007-2009) with slightly different weather situations (precipitation differ by 117 mm and temperature by1.4 °C in favour of the first year). In an initial test we found no significant effect on sheep performance (P=0.677) between these two winters, and we concluded that small differences in winter weather situation would not affect the results. The weather situation in the second of these winters is closest to the one at site B (50 mm less precipitation and 1.2 °C higher temperature at site A), and was used to test for sheep performance between sites during winter. Faeces samples were collected and sent to micro-histological analyses for diet composition, following appropriate methods. To study differences in diet selection between the two herds, the outcome was analysed using multivariate ordination methods (Linear Gradient Analysis (RDA)) in Canoco 4.5 (Microcomputer Power. Ithaca, NY, USA). Live weight change was analysed using a general linear model (GLM) in Minitab Inc. 17.2.1.

Results and discussion

We found that ewes at site B had a significantly higher relative live weight loss compared with ewes at site A (P<0.001) for period 2 (Table 1). This implies that an average ewe at site B lost 1.06 kg 30 days^{-1} during winter, while an average ewe at site A lost 0.44 kg 30 days^{-1}. For period 1 we found a significantly higher weight gain for the ewes in site B (P<0.001). For period 3 there was no significant difference between the herds. In the ordination diagram (not presented here) we found a separation of the diet along the first axes, implying a different winter diet of the two herds (Table 2). Both herds had the highest proportion of woody species in the winter diet, at site A this was dominated by *C. vulgaris* while at B this was dominated by *J. communis*. At both sites graminoids were also grazed during winter, but to a much larger extent at site B. The results show that the herds performed well during winter at both sites, but somewhat better at site A, a pasture dominated by managed heathlands. However, the herd at site B performed better through autumn and produced more lambs than the herd at site A, which probably reflects that more grasslands are available at site B and that grassland plants have higher protein and nutrient levels than *Ericales*. Thus, grasses and grassland plants prove to be an important basis for production and welfare for ONS as for other sheep. The fact that sheep at site B mainly fed on grasses and sedges during winter may explain the higher weight loss and raises some concerns. Graminoids wilt during winter and have a low nutrient level comparable to hay harvested after flourishing (Garmo, 2011).

Conclusions

To secure sheep performance and animal welfare, heathland management should be optimized to ensure a proper mixture between *C. vulgaris*-dominated heathlands and grasslands within the pasture. At pastures where heathlands are dominating this can be achieved by intensified prescribed burning and increased

Table 1. Mean ewe live weight (kg) at two different sites and changes in live weight (g/day) and relative live weight (% of live weight/day) over one year.

Site	n	Mean live weight (kg±SD)		Daily live weight change					
				Period 1 (Jun-Oct)		Period 2 (Oct-Feb)		Period 3 (Feb-Jun)	
		Start	End	g	Rel. (%)	g	Rel. (%)	g	Rel. (%)
A	48	35.2±4.2	35.0±3.7	+23^{A}	+0.07^{A}	-16^{A}	-0.04^{A}	-6.0^{A}	–0.01^{A}
B	25	43.6±3.7	45.0±3.4	+72^{B}	+0.13^{B}	-34^{B}	-0.07^{B}	0.0^{A}	+0.01^{A}

[1] Results with different letters are significant different from each other (P<0.05) in a Tukey paired comparison. SD = standard deviation.

Table 2. Availability of vegetation types in pasture (ha ewe^{-1}) and mean proportion (%) of micro-histological fragments from different plant groups in faeces samples originated from a sub selection of ewes from the two sites.

Available in	Site A				Site B			
pasture	Heathland		Grassland		Heathland		Grassland	
(ha ewe^{-1})	0.73		0.09		1.14		0.20	
Proportion in	Woody		Gramineous		Woody		Gramineous	
diet (%)	Total[1]	Dominant[2]	Total	Dominant[2]	Total[1]	Dominant[2]	Total	Dominant[2]
Period 1	38^{A}	*C. vul;* 27	46	*Car* spp.[1]; 24	2^{a}	*Sal* spp.*;* 0.5	77	*F. rub;* 45
Period 2	53^{B}	*C. vul*; 43	40	*Car* spp.; 19	4^{b}	*C. vul;* 2	87	*F. rub;* 61
Period 3	54^{C}	*C. vul;* 30	39	*Car* spp.*;* 35	28^{c}	*J. com;* 27	64	*F. rub;* 29

[1] Sample numbers: A=16, B=13, C=10, a=16, b=11, c=10.

[2] Dominant species: *C. vul* = *Calluna vulgaris*; *Car* spp. = *Carex* spp.; *Sal* = *Salix* spp.; *F. rub* = *Festuca rubra*; *J. com* = *Juniperus communis*.

grazing pressure. This will transform heathlands to grasslands and increase pasture production for a period until *C. vulgaris* recovers. At pastures where grasslands are abundant and heathland quality is poor, reduced grazing is required for a period combined with restoring heathland through prescribed burning.

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Activity and weight gain of free-ranging beef cattle in south-boreal forests of Norway

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Abstract

The Norwegian beef cattle population has grown steadily over the last ten years, resulting in an increased interest in using coniferous forests as summer grazing areas. We attached collars, programmed to take GPS positions and two-dimensional activity measures every 5 min, to free-ranging beef cows grazing in an area of high and low stocking rate in one and two summers, respectively. The cattle were weighed before and after the grazing period. Data from the collars were calibrated with direct observations and classified into the three main activities: grazing, walking and resting. Lactating cows spent more time grazing (on average 38 vs 32%) and less time resting (58 vs 63%) than dry cows. In the area of high stocking rate, the weight gain of calves was only 67% of that of calves in the area with low stocking densities. The weight gain of the cows was positive in the low stocking area in 2015, close to 0 in the same area in 2016, and negative in the high stocking area in 2016. We conclude that production of beef cattle in boreal forests needs careful planning in relation to stocking rates and suitable groups of cattle for grazing in a forested habitat.

Keywords: cattle, forest, activity, grazing, foraging

Introduction

Norwegian forests have traditionally been used as grazing areas for small dairy farms but modern intensified operational systems have resulted in fewer cattle turned out on forest grazing. Today, these forests are managed according to intensive forest management plans and multiple use including livestock grazing, big game hunting and public recreation. A national strategy to intensify domestic beef production has led to a growing number of beef cattle in extensive grazing areas in the productive coniferous forests of south-eastern Norway during summer months. Our project 'Grazing of free-ranging cattle in boreal forests of south-eastern Norway' aims to generate knowledge about habitat selection, grazing behaviour and production of beef cattle in forested multiple-use habitats, in order to diminish possible negative interactions between the ways of use. Performing observational behaviour studies in forested habitats is challenging for several reasons: visibility is often poor due to the rugged terrain and high tree density, and the cattle often turn shy in these habitats. In recent decades, GPS collars with built-in activity sensors have brought new insights into movement ecology and activity of both wild and domestic herbivores. This article summarizes our results from studies on grazing activities of beef cattle in the grazing season of 2015 in an area with low stocking rate achieved by validation of data from activity sensors and GPS positions, and our results from studies on weight gain in cattle in the grazing seasons 2015 and 2016 from two grazing areas with varying stocking rates.

Material and methods

The studies were performed during the summer grazing seasons on two communal areas located in south-eastern Norway, in Stange/Romedal (150 km^2) in 2015 and 2016, and Furnes (100 km^2) in 2016. The grazing habitats mainly consist of large clear-cut areas generated by harvesting of timber. These are characterized by low plant diversity and wavy hair grass (*Avenella flexuosa*) covers up to 60-80% of the areas. The number of grazing livestock varies amongst the two areas; 2,296 sheep and 386 cattle were turned out in Furnes, which was characterized as a high stocking rate. The area of Stange/Romedal was characterized as low stocking rate with 1,200 grazing sheep and 305 cattle.

In the grazing season, 28 adult beef cattle grazing in the low stocking rate area in 2015 and 14 cattle grazing in each area in 2016 were fitted with collars equipped with a GPS-receiver and a two-axis activity sensor (Tellus, Followit AB, Sweden). The test animals which were of different beef cattle breeds, dominated by crossbreeds, Charolais and Hereford were grouped into lactating cows with suckling calves and dry cows. The sensors recorded activity as X ('nodding') and Y ('shaking') values every 5 min during up to 90 s. To calibrate the activity data for the grazing season of 2015, we observed selected cattle at different times of the day throughout the grazing season. During bouts of 30-50 min, we noted the behaviour of the focus animal during the first 90 s of the 5 min interval. The dominant behaviour was classified into 'Grazing', 'Low activity' (standing or lying inactively or while ruminating) and 'High activity' (walking and measurable behaviours classified as 'other') and was used to build a classification tree based on the activity data and distance to the previous 5 min GPS positions. We applied the package 'evtree' (Grubinger *et al.,* 2014) in the software R (R Core Team, 2016). The resulting decision tree was used to predict the behavioural state of all collared cows every 5 min throughout the season. Cattle in the studies were weighed shortly before turn-out to the forest and after housing, the average initial weights of cow groups were 561, 622 and 718 kg for Stange/Romedal in 2015, 2016 and Furnes in 2016, respectively. An independent-samples t-test was conducted to compare activities of dry and lactating beef suckler cows and weight data for all cattle groups.

Results and discussion

Total classification accuracy for the model was 80.4%, where 'Low', 'Grazing' and 'High' activities were classified with the accuracy of 87.3, 75.9 and 58.3%, respectively. We found that the cattle activity was strongly affected by daylight and that the activity level peaked around sunrise and sunset. Long daylight periods in the beginning of the summer resulted in equalization of activity levels throughout the day. The cattle compensated for decreasing hours of daylight in autumn by increasing activity during the day and by expanding hours of rest at night.

An average time budget for the whole grazing season (Table 1) showed that cattle spent 36% of their time grazing, while time spent on 'Low' and 'High' activities was 60 and 4%, respectively. Lactating cows spent on average 86 more minutes per day grazing than dry cows. Conversely, they used on average 72 and 14 min less per day for low and high activity behaviour, respectively (Table 1).

We found weight gain of lactating cows to be affected by seasonal variations. Lactating cows grazing at low stocking rates in 2015 achieved a higher weight gain (Mean (M)=37.3, standard deviation (SD)=15.3) than those grazing in the same area of 2016 (M=-14.0 SD=53.6), t (42)=5.36, P=3.40e-06. This seasonal difference may be explained with interannual climatic variations: the grazing season 2015 was colder and wetter than normal (period 1961-1990), and the season of 2016 was warmer and drier than normal (Norwegian Meteorological Institute 2016). There was no significant difference in weight gain of spring-

Table 1. Diurnal activity (mean ± standard deviation) of free-ranging grazing cattle, grouped into lactating and dry beef suckler cows, in low stocking rate areas of south boreal forests of Norway in summer 2015.

Activity[1]	Lactating cows	Dry cows	Statistics
Grazing (%)	38±2.5	32±3.7	t(6)=2.45, P= 0.023
High (%)	4±0.07	5±0.06	t(8)=2.31, P=0.010
Low (%)	58±0.3	63±0.3	t(6)=2.45, P=0.043

[1] High activity includes walking and other measurable behaviour. Low activity includes lying and staying inactive or while ruminating.

born suckling calves and dry cows. The energy intake of spring-born calves mainly consists of milk, and cows appear to give priority to produce milk for calves instead of depositing body weight when feed resources are scarce. We compared cattle grazing in areas of high and low stocking rates in 2016 and found weight gain of dry beef suckler cows and spring-born suckling calves to be negatively affected by high stocking densities (Table 2).

Conclusions

Farmers may be challenged by large interannual variation in growth rates, potentially triggered by changing climatic conditions. We urge the need for a more careful investigation of factors driving body growth rate, by incorporating habitat selection and breed into growth rate models.

Table 2. Weight gain (mean ± standard deviation) of spring-born suckling calves (g d^{-1}), lactating and dry beef suckler cows (kg) in areas with low (Stange/Romedal) and high (Furnes) stocking rates from the grazing season 2016.

Weight gain	Low stocking rate, 2016	High stocking rate, 2016	Statistics
Spring-born calves, g d^{-1}	932±204	620±292	$t(12)=3.37$, $P=0.005$
Lactating cows, kg	-14.0±53.6	-29.3±29.4	$t(54)=1.45$, $P=0.15$
Dry cows, kg	36.8±32.6	-6.4±34.7	$t(47)=5.06$, $P=6.8e-06$

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Nitrogen cycling in extensive grassland-based animal production systems

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Abstract

Extensive grassland systems are characterized by low external inputs as synthetic fertilizer and concentrates. These extensive systems rely on nitrogen from atmospheric nitrogen deposition, biological nitrogen fixation, mineralization of soil organic matter, and animal manure. An accurate estimate of the magnitude of these nitrogen sources in extensive systems is difficult. On intensive grassland-based farming systems effective measures to improve the nitrogen use efficiency are: improvement of animal feed conversion, more manure nitrogen available for crop production, and more biological nitrogen fixation. For extensive systems the animal component is more manageable than the crop component. So, herd management and animal feeding strategies are important issues and the animal budget sheet is a useful tool for monitoring.

Keywords: sheep, nitrogen surplus, nitrogen use, overgrazing

Introduction

Our planet comprises nearly 5,000 Mha of agricultural land, subdivided into 1,500 Mha arable land and 3,400 Mha grasslands. The natural and low productive grasslands are used for pastoral farming, defined as low external input farming systems. In 1995 the pastoral systems were responsible for 20% of the global beef, sheep and goat meat production. In 2030 the pastoral systems are forecasted to produce 30% more meat, while the mixed and landless farms produce 68% more meat. The distribution of the global grassland area is equal during this period: 83% pastoral farming and 17% mixed and landless farms (Bouwman *et al.*, 2005).

This paper focuses on sheep production in extensive grassland systems. In the Nordic countries sheep are grazed in summertime and are housed in wintertime with supplementary feed. The main income from sheep production in those countries is derived from lamb meat (Ross *et al.*, 2016; Vatn, 2009). In European Mediterranean countries sheep production is more focused on milk production, followed by cheese production (Vagnoni *et al.*, 2015).

Extensive grassland systems are characterized by low external inputs as synthetic fertilizer and concentrates. These systems sometimes suffer from overgrazing which results in decreasing grass yields and lower soil cover with grasses. Overgrazing leads to more sensitivity to soil erosion (both water and wind erosion). In the long term, overgrazing leads to lower carbon storage in the soil due to reduced plant growth.

Materials and methods

Nitrogen cycling in extensive grassland-based animal production systems is discussed using two nitrogen indicators: NUE (nitrogen use efficiency, the ratio between output and input) and N surplus (the difference between input and output). Because of the abundance of data on intensive systems and the scarcity of data on extensive systems, we start with intensive systems before discussing nitrogen cycling in extensive systems.

Data for intensive dairy farming are based on the Dutch Farm Accountancy Data Network and relate to intensive grassland production systems in the Netherlands. The data represent an average farm with 45 ha of grassland, 8 ha of fodder maize, a production of 7,900 kg milk per dairy cow, and using 145 kg synthetic

N fertilizer per hectare of grassland (Aarts *et al.*, 2008). Data for extensive systems are based on sheep production. Data for the animal budget sheet for sheep originate from the Netherlands and relate to one ewe including 1.5 lambs reared per ewe (CBS, 2012). The farm-scale budget for sheep originates from New Zealand and relates to grassland with clover without synthetic fertilizer additions (Ledgard, 2001).

A nutrient budget sheet keeps track of all inputs (I) and outputs (P) from a system and allows calculating the nutrient use efficiency (NUE) and the nutrient surplus. In this paper we use budget sheets for animal production, crop production, and farm-scale production.

The following system boundaries and definitions are used:
- NUE = P/I, for respectively animal, crop, and farm-scale.
- N surplus = I – P, for respectively animal, crop, and farm-scale.

Budget sheet for animal production:
- Animal input I = consumed grass, forage, silage, concentrates.
- Animal product P = milk, meat, wool.

Budget sheet for crop production:
- Crop input I = synthetic fertilizer, animal manure, nitrogen deposition, biological nitrogen fixation (BNF).
- Crop product P = grass, forage, arable crop products.

Budget sheet for farm-scale production:
- Farm input I = purchased animal feedstuffs, synthetic fertilizer, purchased animal manure, nitrogen deposition, biological nitrogen fixation (BNF).
- Farm product P = milk, meat, wool, sold crops, sold animal manure.

This formulation means that farm-grown forages internally used as feed, and farm-produced animal manure internally used as organic fertilizer, enter the animal or crop budget sheets. Only those quantities that are sold to other farms enter the farm-scale budget sheet. Biologically fixed nitrogen by forage legumes is considered as an entry on the budget sheets because when the fixed nitrogen becomes part of the farm soil system it is not distinguishable from synthetic nitrogen fertilizer. It therefore contributes in the same way as synthetic nitrogen fertilizer to nitrogen losses. The nutrient budget sheet does not provide information about the distribution of the surplus on the individual components ammonia, nitrate and nitrous oxide.

Results and discussion

Results for intensive dairy farming and extensive sheep production are presented in Table 1. Data on intensive dairy farming show that NUE for animal production is much lower than for crop production. As a result, NUE on farm-scale is slightly above NUE for animal production. Data on extensive sheep production show about equal NUE for animal production and farm-scale production. A model-based sensitivity analysis of different measures to improve NUE on intensive dairy farming systems in the United States indicated that improvement of animal feed conversion, more manure nitrogen available for crop production, and more biological nitrogen fixation were all very effective (Kohn *et al.*, 1997).

Extensive grassland-based animal production systems rely on nitrogen from atmospheric nitrogen deposition, biological nitrogen fixation, mineralization of soil organic matter, and animal manure (O'Connor, 1983). Even today an accurate estimate of the magnitude of these nitrogen sources in extensive systems is difficult. For extensive systems the animal component is more manageable than the

Table 1. Nitrogen balances for the year 2006 for intensive dairy farming and extensive sheep production. N-input, product and surplus in kg N ha^{-1}, and nutrient use efficiency (NUE) (N-product $N\text{-input}^{-1}$).[1]

Farming system	N budget sheet for	N-input	N-product	N-surplus	NUE
Dairy cattle, intensive	animal production	359	75	284	0.21
Grassland, intensive	crop production	449	265	184	0.59
Fodder maize, intensive	crop production	308	203	105	0.66
Dairy farm, intensive	farm-scale	299	80	218	0.27
Sheep, extensive	animal production	16.5	2.3	14.3	0.14
Sheep farm, extensive	farm-scale	140	18	122	0.13

[1] Sources: dairy cattle, grassland, fodder maize, dairy farm: Aarts *et al.* (2008); sheep animal production: CBS (2012); sheep farm: Ledgard (2001).

crop component. Research on extensive sheep farming in Patagonia showed that grass and animal output both will increase when stocking rates are adaptive, annually adjusted to fluctuations in carrying capacities (Oliva *et al.*, 2012).

Therefore, herd management and animal feeding strategies are important issues and the animal budget sheet is a useful tool for monitoring (Van der Hoek, 2016).

Conclusions

1. The animal component in extensive grassland-based animal production systems is more manageable than the crop component. Therefore, herd management and animal feeding strategies are important issues and the animal budget sheet is a useful tool for monitoring.
2. Adaptive, annually adjusted stocking rates in relation to fluctuations in carrying capacities will increase both crop and animal output.

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Linking empirical and academic knowledge. A case study of silvopastoral Dehesa system

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Abstract

Agrosilvopastoral systems dominated by Evergreen oak (*Quercus* sp.), known as Dehesas in Spain and Montados in Portugal, are very common in the central-western and south-western region of the Iberian Peninsula. These agroecosystems support multiple ecosystem services. However, nowadays drivers of change, as intensification or abandonment, threaten these high value systems. A systematic search of scientific papers in the ISI Web of Science database using 'dehesa', and 'montado' as title keywords was performed for the period 2001-2015. In addition, semi-structured interviews with producers and tenants were carried out in 11 representative estates to identify the key issues and relevant management problems. The main objectives were to determine the available information and detect the principal gaps between the empirical and academic knowledge. There was a steady increase in the number of publications since 2001, more acutely from 2007. The most studied topics in scientific literature were related to biodiversity and the effects of the tree layer. The interviews' most relevant issues related to production system and historical changes. Results show that scientific and management problems are only partially connected, and solutions are usually not associated. For this reason it is important to improve the scientific research transfer to stakeholders and implement socio-ecosystemic methodologies.

Keywords: silvopastoral system, dehesa, montado, local knowledge

Introduction

Dehesas and Montados are traditional agroecosystems in the Central-West and South-West region of the Iberian Peninsula. These silvopastoral systems present multifunctionality, with the integration of extensive animal production and crops, agroforestry uses and hunting, in a landscape that combines scattered trees, shrubs and grasslands. These agroecosystems support different services (provisioning, regulating, cultural). However, the changes of the last decades have caused social, economic, cultural and environmental problems, some of which threaten these High Nature Value systems (Alejano-Monge *et al.*, 2011).

Some drivers of change that are adversely affecting dehesas and montados are phytosanitary diseases, low economic profitability, disappearance of former diversified uses, public policies, globalized food market, etc. (Costa *et al.*, 2006). Most of those drivers are related to management, making the decisions about the diversity of uses a current research challenge in Dehesas and Montados. For these reasons, current research has difficulties in transferring conclusions to managers (Acosta-Naranjo, 2016). New procedures are needed, where methods and data sets must be integrated in a socio-ecosystemic approach (Ferraz-de-Oliveira *et al.*, 2016).

In this context, it is necessary to study in depth the vulnerability of these agroecosystems and the mechanisms that give them stability, but also to improve knowledge, in order to understand the relationships between management and ecosystem functions and services (Gómez-Sal and González-García, 2007).

The main objective of this study was to identify the current knowledge status of the empirical and academic world on different topics, to integrate and detect the principal gaps between them, and help to promote the transfer of scientific knowledge to dehesa social agents.

Materials and methods

A systematic search of scientific papers in the ISI Web of Science database using 'dehesa', 'dehesas', 'montado' and 'montados' as title keywords for the period 2001-2015 was performed. A review was conducted and the findings were classified regarding the year of publication and the main related themes. The historical publication trend and the relative proportion of each theme where calculated. In addition, 11 semi-structured interviews with producers and tenants of representative estates within the Demonstrative Dehesas Network of the Life BioDehesa project were carried out to identify the key issues and relevant management problems. The interviews were analysed subdividing the spoken comments into different themes (historical perspective, physic environment, vegetation, fauna, livestock, conservation and values, territory, economics, and future). For each theme, the length of the spoken comments and the number problems were identified and studied.

Results and discussion

On one hand, the scientific search resulted in 112 publications between 2001 and 2015. These increased steadily, more acutely from 2007 (Figure 1A). The most recurrent themes in the literature were biodiversity (21%), tree effect (13%), regeneration (13%) and climate (13%). On the other hand, in the interviews the lengthiest themes were livestock (28%), historical perspective (23%), vegetation (17%) and physical environment (which included climate) (9%) (Figure 1B). However, focusing on identified problems, the highest appearance was on historical perspective (10 of 11 interviews), followed by vegetation (9), economics (9), fauna (8) and physical environment (7). However, the higher means were registered on vegetation due to regeneration problems, ageing and diseases, followed by economics, because of low profitability, subvention dependency and negative effects of public policies.

Most of the articles about biodiversity (16 of 23) related it to the change of use in the dehesa (grazing, abandonment, loss of traditional uses, etc.), the effect of shrub management and ploughing. These issues were also described by interviewees in the historical perspective, livestock and vegetation topics related to changes during the last decades. In general, the scientific results showed that maintaining an adequate proportion between trees and shrubs cover, and traditional practices are the most suitable uses to sustain biodiversity. Thus, the management has a key role on biodiversity conservation. The stakeholders

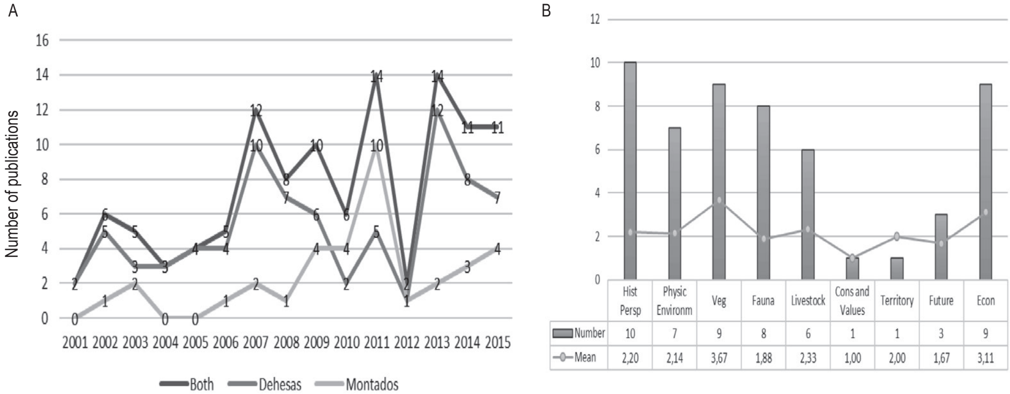

Figure 1. (A) Scientific publication trend and number and (B) mean of problematics in the interviews.

associated biodiversity loss indirectly with socioeconomical processes such as specialisation in the agrofood chain, market prices or CAP (Common Agriculture Policy) as drivers of system transformation.

Regarding the tree effect, the literature showed a positive effect of trees on nutrient availability, essentially on those nutrients which are mobilised by biological mechanisms, as well as on soil carbon fixation, grass and crop productivity, biodiversity and microclimatic regulation. At the same time, the managers considered trees as key elements of dehesas; however, lack of tree regeneration and population decline are sustainability problems nowadays in dehesas. Scientific knowledge analysed both the causes, mitigation measures and social perception of that problem. The causes are diverse, with great influence of the implementation of public policies, loss of traditional activities (like transhumance), and intensification of agroforestry uses. Linking to that, all the respondents agreed that current pasture admissibility coefficient has a negative impact on regeneration actions.

Conclusions

Results show that the problems described in both scientific literature and management are interrelated, although priorities, general context and solutions are not well integrated and associated. Because of that, it is important to improve research transfer to increase management awareness, but also the connection of empiricism to scientific research. For this reason, the development of socio-ecological approaches, where transdisciplinarity and local knowledge are included, are needed in order to preserve those High Nature Value areas, by linking science and practice.

Acknowledgements

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Theme 2.
Sustainable grassland management in high nature value areas

High Nature Value extensive livestock and grasslands: can innovation secure a sustainable future?

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European Forum on Nature Conservation and Pastoralism

Abstract

High Nature Value (HNV) livestock farming is low-intensity in terms of external inputs and uses predominantly semi-natural forage. These systems are found mainly in marginal areas where physical factors, and in some cases social factors, have prevented intensification of land-use. HNV livestock and grasslands are generally in decline in the EU, although consistent data is sorely lacking. HNV-LINK is a Horizon2020 project that seeks to improve the socio-economic and environmental sustainability of HNV farming in 10 Learning Areas, and more widely across the EU, by promoting innovation. HNV-LINK aims to identify the types of innovation that work for HNV farming, to highlight the innovations that are needed but not yet implemented on a significant scale, and to promote the exchange and up-take of good innovation practice in support of HNV livestock farming. Overall, we are finding that the keys to a sustainable future for HNV livestock are found as much in social, institutional and regulatory innovation, as in agronomic or market innovation.

Keywords: High Nature Value livestock and grasslands, sustainability, innovation

Introduction

High Nature Value (HNV) livestock farming and grasslands are generally in decline in the EU, putting at risk a range of social, cultural and environmental values. This decline is due to a combination of factors, including limited economic viability, difficult working conditions, remoteness and unfavourable aspects of policy.

HNV-LINK (http://www.hnvlink.eu) is a Horizon2020 multi-actor networking project that emerged from the EIP-AGRI Focus Group on Sustainable HNV farming (https://ec.europa.eu/eip/agriculture/en/content/FGHNV). The HNV-LINK project seeks to improve the socio-economic and environmental sustainability of HNV farming in ten Learning Areas, and more widely across the EU, by promoting innovation in support of these farming systems and their particular characteristics. The project takes a broad view of innovation, as explained below. Essentially, by working across these ten areas we aim to identify the main challenges to achieving a sustainable future for HNV livestock farming, and the keys for solving these challenges.

The learning areas and HNV farming characteristics

The ten Learning Areas of the project are as follows (Figure 1):

- Dartmoor, Devon, UK
- Sítio de Monfurado, Alentejo, Portugal
- Dalmatian Islands, Croatia
- Eastern Hills of Cluj, Transylvania, Romania
- Western Stara Planina, Bulgaria
- Dalsland, Götaland, Sweden
- The Burren, County Clare, Ireland
- Western Thessalia, Greece
- Causses et Cévennes, Occitanie-Pyrénées Méditerranée, France
- La Vera, Extremadura, Spain

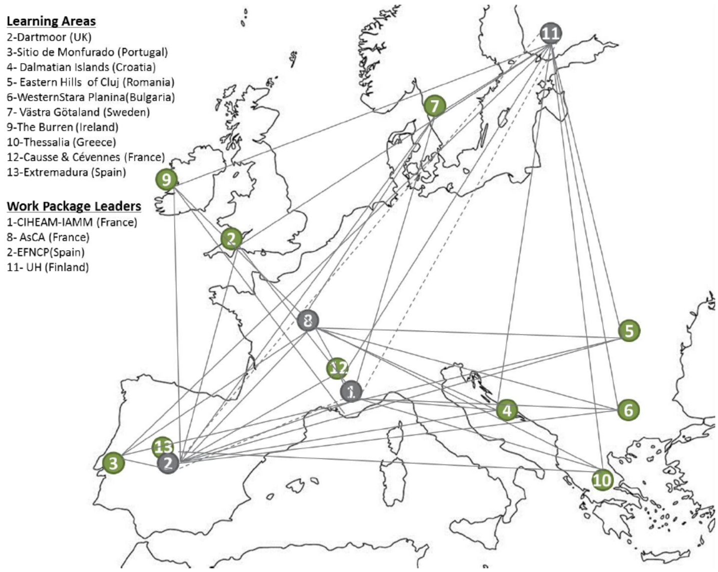

Figure 1. Learning areas of the HNV-link project.

Between them, these areas illustrate many of the common characteristics of HNV livestock farming in Europe, including:

Land

- HNV livestock farming is found mainly on marginal land, typically in uplands, but also on land that is physically disadvantaged in other ways (poor soils, extreme climatic conditions, islands).

Farming system

- The most widespread livestock types in HNV systems are sheep (meat and/or milk depending on the area) and beef cattle. Goat systems dominate certain areas especially in southern Europe), while dairy cattle are associated with HNV systems in very specific areas. Also in local cases, horses are important grazers on some extensive rough pastures. Livestock are mainly hardy breeds and often, but not necessarily, local or rare breeds.
- By definition, HNV livestock systems use predominantly semi-natural forage resources, supplemented to a greater or lesser extent by on-farm or purchased fodder and feeds. Semi-natural forage areas include grasslands with varying proportions of shrubs and trees. Some HNV pastures are predominantly ligneous, especially in extensive goat systems.
- Shepherding is a common feature of HNV livestock farming, especially in sheep and goat systems on extensive, unfenced pastures.
- Seasonal movements of stock are common, especially between uplands and neighbouring lowland areas.
- Very large areas of HNV pastures, including those under private and public ownership, are under common use. In total, there are approximately 10 million ha of farmland in common use in the EU (EUROSTAT, 2017), most of which is probably semi-natural pasture.

Production (private goods)

- HNV livestock systems are generally low yielding, but produce high quality foodstuffs.

- In some cases, HNV systems have developed specialist markets, through combinations of on-farm processing (e.g. cheese, meat products), product differentiation (e.g. products from grasslands, from natural areas), and direct sales.
- However, in many other cases, HNV livestock farms produce young animals that are sold for fattening elsewhere; and/or milk that is sold to the dairy industry, or to cooperatives. In these cases, the product often is not differentiated from the products of intensive systems.

Environmental services (public goods)

- High presence on HNV livestock farms of Natura 2000 habitats and other semi-natural habitats not on Habitats Directive Annex 1, often in complex habitat mosaics, and dependent on continued, balanced use for forage.
- Maintenance of accessible, diverse landscapes.
- In some areas, conservation of water catchments.
- In some areas (especially, but not only, in southern Europe) fire prevention by animals reducing combustible material.

Tendencies in HNV livestock systems and grasslands

At EU level, there is no consistent data or analysis available to show tendencies in HNV livestock and grassland, nor is such information available for most Member States (MS). Only certain countries have established systems for measuring the extent of HNV livestock and/or HNV grasslands, and the methods (with the exception of Germany) are mostly unsuitable for monitoring tendencies and do not have (with the exception of Scotland) separate indicators for monitoring the livestock systems and the grasslands (see Keenleyside *et al.*, 2014). The HNV-LINK project aims to assess the situation within the Learning Areas, as far as existing data allows, supplemented with expert interviews and workshops.

At a farm and local landscape level, the tendency reported in many regions in recent years is to abandon semi-natural pastures (especially the least accessible) and to concentrate stock on more productive land, with increased intensification on this land, for example in Ireland (Kramm *et al.,* 2010), in Spain (Iragui Yoldi *et al.*, 2010) and in Sweden (Jordbruksverket, 2010). Extensive livestock systems have gone into severe decline in some areas, for example in the west of Scotland (McCracken *et al.*, 2011; SAC, 2008).

The parallel process of abandonment and intensification has become recognised widely in EU publications, although it does not always follow the same pattern:

- Abandonment: can take different forms, such as sudden withdrawal of farms/stock from an area, or more gradual process of under-grazing, insufficient management and encroachment of less palatable species. It can affect patches of marginal land locally, or entire landscapes.
- Intensification: can take the form of intensified use of grassland (e.g. reseeding and fertilisation) in pursuit of more production and/or productivity; but often is simply the concentration of stock/production on more accessible/better land, usually with supplementary feeding and sometimes leading locally to overgrazing and loss of nature values.

Initial information suggests that the HNV-LINK Learning Areas find themselves at different stages and with variations in these processes. For example, in Causses et Cévennes there has been some reversal of historic abandonment in recent years as a result of policy interventions to encourage the restoration of pastoral areas; on a smaller scale, this is also the case in the Burren. Dartmoor, on the other hand, has seen a recent period of destocking of upland pastures, largely as a direct objective of agri-environment measures, and now there are signs of potential under-use and abandonment of these pastures in future. Meanwhile in La Vera an on-going process of abandonment has accelerated in recent years and at present shows no signs of reversal.

Similarly, comprehensive data and analysis are sorely lacking on the economic situation of HNV livestock across the EU, and in most MS. The general picture seems to be one of low net incomes, long hours, and heavy dependence on public payments. Again, the situation varies between regions, partly as a result of large differences in the levels of financial support directed to extensive livestock farming through national CAP models.

As an example, Table 1 shows approximate support received from the CAP by a 150 ha suckler-cow and goat farm in central Spain, and compares this with an equivalent situation in Bulgaria and in France. Under each of the five payments considered (Basic+Greening, Coupled payments, Agri-Environment-Climate and Areas with Natural Constraints) the farms in Bulgaria and France can receive much higher payments than in Spain, resulting overall in a massive difference in the economic situation of apparently similar farms.

How to define a sustainable future for HNV farming?

The HNV-LINK project aims to establish an HNV Vision for each LA, which will describe a sustainable situation for HNV farming in the area 20 years from now. The essential requirements for a sustainable future for HNV livestock farming can be summarised as a combination of socio-economic and environmental sustainability:

Socio-economic

- Economic viability generating an acceptable return on labour, whether part-time or full-time.
- Acceptable working conditions, including working hours.
- Access to basic services (schools, health care, internet access…).

Environmental

- Balanced use of pastoral resource (reverse the process of abandonment/intensification).
- Maintenance or improvement of the current range of environmental services – these are not measured at present and their remuneration is patchy and inconsistent.

Table 1. Approximate comparison of support rates available to a 150 ha farm of semi-natural pastures in Spain, Bulgaria and France.

	Spanish example farm1	Equivalent farm in Bulgaria	Equivalent farm in France
Basic + greening per hectare	90 €	150 € + 77 € for first 30 ha	128 € + 95 € for first 52 ha (2019 projected figures)
Agri-environment-climate payments	0	126 € (grazing maintenance of HNV grasslands)	50 € (basic measure for pastoral systems)
Area with natural constraints (150 ha farm)	1.50 €	5.75 €	7,750-12,750 € (approx. range of possible payments)
Suckler cow coupled payment	108 €	114 €	187 € (first 50 cows); 140 € (next 49 cows)
She goat coupled payment	7 €	23 €	15 €

[1] For more information on the example farm in Spain see: http://tinyurl.com/hukc7j6.

What is HNV-LINK actually doing?

2016-2017 in each LA:

- Baseline assessment of HNV livestock farming situation, drivers, tendencies.
- Identify the Business As Usual (BAU) scenario for 20 years hence: if there is no change of path (no innovation) what will be the consequences for HNV farming and associated environmental services?
- Identify an alternative scenario with sustainable HNV livestock farming 20 years hence: how would this look?
- Evaluate the barriers to achieving the sustainable HNV scenario.
- Evaluate the innovation options (solutions) in order to overcome the barriers (identify innovation that is working in the LA, or outside LA, or not existing but needed).

2018 in the LA and beyond:

- Promote and exchange innovative solutions (within the LA, at regional/national levels, across HNV-LINK network).

2019:

- Final conference in Brussels: presentation of recommendations to policy makers and wider stakeholders.

Innovation themes

Innovation has been defined by the OECD as 'the introduction of a product (good or service), process, organization form or marketing, which is new or significantly improved (OECD/Eurostat, 2005)'. Innovation is multidimensional and consequently often difficult to identify and measure. We also keep in mind the context of a given area, and a given time. It is thus a relative notion: the same activity may be regarded as an innovation in one locality or country, but as well-established and conventional in another.

The project is focusing specifically on innovation that leads to the maintenance or improvement of HNV characteristics, even if this is not an explicit objective of the action but rather a side-effect of another objective (e.g. socio-economic viability of HNV farms). There may also be innovations that currently are not applied to support HNV farming and characteristics, but that could usefully be applied and that would be expected to produce a positive result for conserving HNV values if targeted in this way.

HNV-LINK is working with 4 broad innovation themes, informed by and adapted from the work of the EIP-AGRI Focus Group:
- social and institutional innovation;
- regulatory and policy innovation;
- products and markets innovation;
- technical and management innovation.

The boundaries between these themes are fuzzy, and some degree of overlap is expected. From a review of literature and initial results emerging from the Learning Areas, we are expecting these themes to capture the following types of innovation.

Social and institutional innovation

This is in many ways an overarching theme, in the sense that an innovative social and institutional context is likely to generate innovation in the following 3 themes.

Innovations under this theme could include:
- co-operation between HNV farmers, focusing on sustainability;
- co-operation of HNV farmers with other local actors, including NGOs;
- local facilitation and catalysing of farmer groups to actively promote sustainability, targeting HNV systems and farmers;
- mechanisms that facilitate effective participation of HNV farmers and other experts in design of policy and its implementation;
- local level mechanisms for dialogue, cooperation, problem-solving in relation to HNV farming;
- institutions and institutional structures that favour innovation benefitting HNV farming, including co-innovation across different institutional levels (local-national-EU).

The challenge is that in some cases there are key institutions exhibiting a lack of basic innovation in their *modus operandi*. Examples would be environmental and agricultural authorities that fail to integrate policy design or delivery that do not communicate constructively with each other, or do not work with an integrated approach to implementing, e.g. Natura 2000 and the CAP. The result being that they leave it to farmers and other local stakeholders to sort out the inherent tensions between policies on the ground.

Another example could be authorities that do not seek dialogue or collaboration with farmers, but rather impose regulations on them. What this tells us is that without some basic values and communicative skills in key institutions, social and institutional innovation will not take place.

Regulatory and policy innovation

As with the previous theme, the regulatory and policy framework can have the effect of facilitating innovation in the production and market chain, or it can have a powerful blocking effect, or a mixture of both, depending on the design and implementation model of rules and policies.

Policies of particular relevance to the sustainability of HNV livestock systems, and where innovative approaches are often necessary, include:
- CAP Pillar 1 direct payments, including criteria for basic payments and for coupled livestock payments, and eligibility rules for permanent pastures.
- CAP Pillar 2, including a wide range of measures with potential for supporting HNV livestock farming, if used appropriately.
- Birds and habitats directives, establishing Natura 2000, which includes large areas of HNV grasslands and has the potential to facilitate positive initiatives, such as support payments and product differentiation, but in some cases imposes restrictions even on HNV farming activities.
- EU food hygiene regulations, setting common rules for processing and selling of food products, including dairy and meat products. The EU framework allows considerable scope for adaptation of rules to specific circumstances, such as small-scale and artisan production systems, but this requires innovative implementation by national and regional authorities.
- EU food 'quality schemes' for labelling of geographical origin and traditional foods (Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Specialties Guaranteed (TSG), Mountain Food Label), which in some cases include primary production requirements of relevance to HNV farming, but in many cases do not.

Despite common EU regulatory and policy frameworks, the implementation model for each of the above policy areas shows considerable variation from one Member State to another (and in some countries also between regions). Through HNV-LINK we aim to highlight examples of innovative, HNV-friendly policy implementation that potentially can be extended to all HNV livestock areas.

Products and markets innovation

The products and markets theme includes innovations, such as:

- product differentiation that is relevant to HNV farming, such as labelling of livestock products that are produced largely on semi-natural pastures, or in Natura 2000 sites, for example;
- access to markets for HNV farmers, for example producers' and/or marketing cooperatives; short-supply chains such as farmers' markets, local markets, on-farm sales, Internet sales, local fairs, festivals and events, marketing in cooperation with NGO conservation projects; and producer-consumer contracts (Community Supported Agriculture (CSA);
- diversification into innovative products, including on-farm processing, adding value to HNV farming produce;
- innovations in packaging and advertising of HNV products.

Technical and management innovation

This theme refers to technical and management innovations at farming system level, to do with the primary production and management system or the processing of HNV farm products. Sub-themes include:

- pastoral management, such as electric fencing, GPS tracking of animals;
- adapted machinery, such as mowers adapted to the conditions of small-scale HNV grasslands (Transylvania, Romania), devices for removing twigs and branches from pastures (Dalsland, Sweden);
- supplementary feeding systems that facilitate better management of semi-natural forage resources (Burren, Ireland);
- management planning systems and methods for monitoring of results (Monfurado, Portugal);
- integrated diagnostics of pastoral resources, combining ecological and production objectives (Causses et Cévennes, France).

We expect to find several examples of innovation that integrate two of more of the themes, and these may be the most useful innovations to learn from. For example, in Thessaly (Greece) a GPS livestock-monitoring platform has been developed in order to certify extensive sheep farming in mountain and semi-mountain environments. The aim of this innovation is twofold: to certify the extensiveness of the livestock system, differentiating it from more intensive systems and giving added value to the corresponding dairy products; and to understand and facilitate livestock movement, also serving to prevent conflicts between farmers and forestry services by using 'GPS geo-fences' and other technical functions. This innovation example combines the Products/Markets theme, the Technical/Management theme and to some extent the Social/Institutional theme as well.

Conclusions

Under current circumstances, the future for HNV livestock across the EU is far from sustainable. Although in some places, public and/or private initiatives are working towards a sustainable future, in many other places this is not happening. A more consistent response is needed, both to identifying the situation and tendencies of these systems, and the consequences for environmental values; and to identify and promote solutions consistently across all HNV livestock and grassland areas. HNV-LINK aims to help answer these questions for the 10 local areas in the project, and also to point the way towards a more exhaustive EU-level effort in future.

From initial work under the HNV-LINK project and the EIP-AGRI Focus Group on HNV farming, it seems that the key to a sustainable future for HNV livestock and grasslands is to create an innovative policy, regulatory and socio-institutional context, well-adapted to the needs of these systems. Technical and farm management innovations undoubtedly have an important role to play in some situations, but these are more likely to happen, and to be successful on a significant scale, if the policy-institutional context is favourable.

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Evaluating ecosystem services and disservices of livestock agroecosystems for targeted policy design and management

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Abstract

We present a critical analysis of the positive and negative impacts of pasture-based livestock systems on the environment. We use the case of sheep and mixed sheep-crops systems in Mediterranean Spain to illustrate our arguments and results. In the first part of the paper, we enumerate some limitations in the use of the ecosystem services concept when applied to agriculture in general and grassland ecosystems in particular. Next, we present an expert-knowledge assessment of the relationships between agricultural practices and a number of relevant ecosystem services identified in previous research. We introduce a Payment for Ecosystem Services (PES) system based on these relationships that can be applied at the farm level and can accommodate different policy targets. In the second part of the paper, we critically discuss the use of live cycle analysis in grassland-based livestock systems to evaluate ecosystem disservices, using as example the carbon footprint of lamb meat. We briefly discuss the limitations regarding the functional unit, the system boundary, the allocation approach, the availability of data, the consideration of carbon dynamics in soils, the land use management, and other sustainability factors.

Keywords: agricultural practices, expert-knowledge, payments for ecosystem services, carbon footprint, life cycle assessment

Introduction

The conceptual framework of ecosystem services (ES) is now well stablished in the scientific literature. However, there is still little evidence of its utility in environmental policy design, and it is uncertain that the ES framework will change decision-making and improve policy outcomes (Van Wensem *et al.*, 2017). When applied to agroecosystems, some limitations in the usability of the ES concept remain. First, the concept is not assimilated by society at large and farmers in particular, and there is lack of understanding of the different perspectives held by diverse stakeholders (Bernués *et al.*, 2016). Second, most non-provisioning ES constitute public goods, which are very difficult to measure and value, and therefore their incorporation in agri-environmental policies is difficult. As public goods do not have a market price, farmers have little or no economic incentive to produce them and public intervention is required to achieve a desirable level of provision according to societal demands (Cooper *et al.*, 2009). Third, analysing the multiple contributions of ES to human well-being requires diverse perspectives, combining biophysical, socio-cultural and economic tools to uncover different values (Rodríguez-Ortega *et al.*, 2014). Fourth, there are multiple trade-offs between ES at different spatial and temporal scales, typically between provisioning and non-provisioning ES that are widely described in the literature (e.g. Sabatier *et al.* (2014)). Synergies also exist (Bernués *et al.*, 2011) that could be promoted through adequate farming practices that can constitute action levers to maintain a diverse range of ES, benefiting different stakeholders (Rodríguez-Ortega *et al.*, 2014). However, the processes by which specific agricultural practices and management regimes affect ES (and other sustainability issues) need further investigation.

Compared to industrial agriculture, pasture-based livestock systems are associated with a wide range of ES. Nevertheless, they can also produce ecosystem disservices (EDS) or negative externalities. The positive or negative environmental outcomes will finally depend on the use of on-farm and off-farm resources, the degree of intensification, the species and the orientation of production, among others (Bernués *et al.*, 2011). The EDS most widely discussed nowadays is the emission of greenhouse gases (GHG) to the atmosphere. Many studies quantifying the carbon footprint of animal products advocate an intensification of animal production to mitigate the emission of GHG (Steinfeld and Gerber, 2010), moving away from beef and sheep/goat meat to pork and poultry; and from rustic, traditional animals to specialized, highly productive ones. The main rationale behind this proposal is the so-called 'efficiency gain'; i.e. more output with less input, and therefore less environmental impact per kg of product. Life cycle analysis (LCA) is the standard method to measure the carbon footprint of a product. However, its application to animal agriculture has a number of shortcomings.

In the first part of the paper, we present an expert-knowledge assessment of the multiple relationships between agricultural practices and a number of ES, and we introduce a Payment for Ecosystem Services (PES) system based on these relationships that can be applied at the farm level. In the second part of the paper we critically discuss the use of LCA analysis in grassland-based livestock systems using as example the carbon footprint of lamb meat. We will use the case of sheep and mixed sheep-crops systems in the Mediterranean to illustrate the results. Previous work on these systems has allowed to: (1) unravel the perceptions of farmers and nonfarmers about agricultural practices and related ES (Bernués *et al.*, 2016); (2) rank the main ES delivered by livestock agroecosystems according to socio-cultural preferences and elucidate their economic value (Bernués *et al.*, 2014); and (3) calculate the carbon footprint of lamb meat in contrasting farming systems (Ripoll-Bosch *et al.*, 2013).

Linking agricultural practices and ecosystem services for targeted policy design

In agri-environmental policy, it is crucial to recognize the delivery of ES by farmers and compensate them in economic terms, according to the demands of society, following the principle of 'public money for public goods'. Economic incentives can trigger the adaptation of farm management regimes or specific agricultural practices towards the delivery of ES. A system of Payments for Ecosystem Services (PES) can be defined as a voluntary transaction where one or more well-defined ES (or land uses that secure them) are bought by a buyer from a provider, if and only if the ES provider secures its provision (conditionality) (Wunder, 2005). According to this definition, payment mode can be oriented in two ways: by the ES outcome (target-oriented) or by the land management (practice-oriented) that secures the provision of the ES (see Reed *et al.* (2014) for advantages and disadvantages of both systems). Effectiveness of practice-oriented PES is questioned, as the link between land management and ES can be weak and might be based on assumptions that are not always backed up by scientific evidence. Therefore, designing a PES system requires elucidation of the biophysical effects of particular farming practices on the ES at the farm scale, where farmer decisions take place.

In this context, we developed a consistent and flexible PES framework for implementation of agri-environmental measures at the farm level. First, we quantified, based on expert-knowledge, the contribution of farming practices to the most relevant ES (as previously identified by Bernués *et al.,* 2014, 2016) in Mediterranean agroecosystems. Second, we applied this framework to policy scenarios targeting the actual demand of society, the conservation of biodiversity or the sequestration of carbon.

Contribution of farming practices to ecosystem services

We carried out an expert consultation with an on-line Delphi panel. The Delphi method consists of an iterative consultation process of many 'informed' individuals in different disciplines or specialties to contribute, with information or judgements, until a certain degree of judgement convergence is attained (Scolozzi *et al.*, 2012). We asked respondents to value the contribution of a number of farming practices (see below) on five ES in Mediterranean agroecosystems: the maintenance of agricultural landscapes, the conservation of biodiversity, the prevention of forest wildfires, the regulation of climate change through carbon sequestration, and the production of quality products linked to the territory (Bernués *et al.*, 2014).

From the list of 66 farming practices with potential to deliver public goods in Europe (Cooper *et al.*, 2009), we selected 36 that are currently implemented on real sheep and mixed sheep-crops farms in Mediterranean mountains and semiarid lowlands in Aragón, Spain (see Rodríguez-Ortega *et al.* (2017) for description of farming systems and monitoring). Respondents had to rate the (positive) contribution of each farming practice to the five ES separately according to a six-point Likert type scale (0: none, 1: very low, 2: low, 3: intermediate, 4: high, 5: very high contribution). We included the 'don't know' option.

The experts were chosen covering different types of knowledge and backgrounds: (1) researchers on agriculture-environment relationships (n=29) and (2) technicians/managers from the government and Non-Governmental Organizations related to agriculture and environmental conservation, as well as from agricultural associations, local agribusiness and cooperatives in the area of study (n=32). The survey implied two anonymous rounds of deliberation, with feedback information in the second round in search of the highest degree of consensus among experts.

Due to limitation of space, we graphically present the results obtained for two ES: biodiversity and prevention of forest fires (Figure 1). We performed a Kruskal-Wallis test to check for significant differences between experts in their evaluation of practices contribution to ES. Differences were very few (not presented here), so we present the two expert categories together. Figure 1 shows the ranking of importance of the top-ten farming practices for the selected ES. For example, for conservation of biodiversity the five most important farming practices were: reducing pesticides, maintaining semi-natural vegetation, applying adequate stocking rates, maintaining hedgerows, shrubs and trees, and maintaining grasslands. For prevention of forest fires, the five most important farming practices were: practicing silviculture, grazing remote or abandoned areas, grazing semi-natural areas, maintaining drove

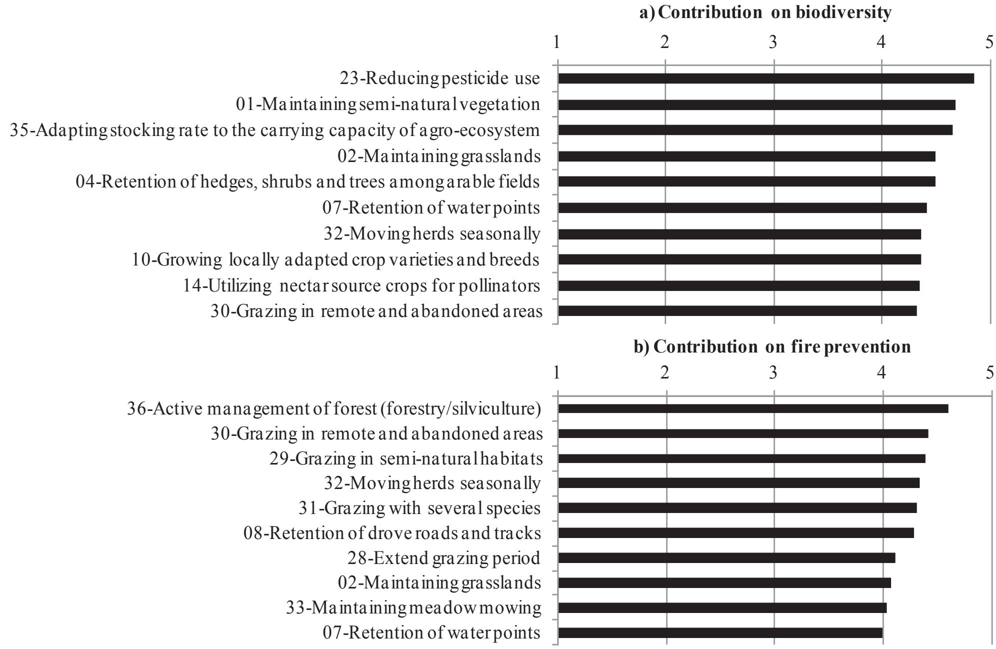

Figure 1. Contribution of top-ten farming practices to biodiversity and prevention of forest fires.

roads and paths, and extending grazing periods. Globally, the top five most important practices for the five ES considered together were: moving herds seasonally, maintaining grasslands, active management of forest (forestry/silviculture), grazing in semi-natural habitats and maintaining semi-natural vegetation. This highlights that despite single ES were highly influenced by multiple practices, synergies among these practices are common, delivering ES in bundles. In this sense, we observed that some ES such as conservation of agricultural landscape and biodiversity shared more synergies among farming practices, while other ES such as prevention of forest wildfires and production of quality products linked to the territory were influenced through more specific practices.

We grouped the 36 practices according to their nature in four categories: vegetation and other landscape elements, crops and species, use of inputs, and grazing and silviculture. We can observe the type of farming practices that are more important for the five ES under consideration (Figure 2). For preservation of the agricultural landscape, the most relevant practices referred to crops and species, followed by vegetation and other elements and grazing and silviculture. The same groups of practices also contributed the most to conservation of biodiversity. For the prevention of forest fires, the most important group of practices referred to grazing and silviculture. This group, together with crops and species contributed the most to carbon sequestration. Finally, the use of inputs had the greatest impact on the provision of quality products linked to the territory. Globally, the group with highest contribution to the delivery of ES was grazing and silviculture.

Application of a PES system to agri-environmental policy: an example

Figure 3 shows the designed framework of PES that link agricultural practices at farm level with the provision of ES as prioritized by society, the relationship among them being established according to the expert knowledge or empirical research. Policy makers can reward farmers according to the objective contribution of their practices on targeted ES, i.e. conservation priorities of a particular territory. The contribution of particular farming practices to each of the ES in Mediterranean agroecosystems is defined by the Delphi evaluation. Other variables can be customized (number of practices to include and relative importance of ES), making the framework generic and flexible. The PES system is implemented in Excel and it is fully operative.

The framework renders a different ranking of all farming practices according to their contribution to policy targets. We present the top-five (and last) agricultural practices in Table 1. For example, when policy targets correspond to the willingness-to-pay of society for ES provision in Spanish Mediterranean agroecosystems (i.e. landscape 8.2% of importance, biodiversity 18.4%, wildfires 53.2% and quality products 20.2%), as described in Bernués *et al.* (2014), the ranking of farming practices was as follows: 1st moving flocks seasonally between areas; 2nd grazing semi-natural habitats; 3rd active management of forest; 4th maintain herbaceous pastures; and 5th increase the grazing season. Globally, the groups of practices referring to grazing and silviculture (with a total contribution of 41.8%) and vegetation and

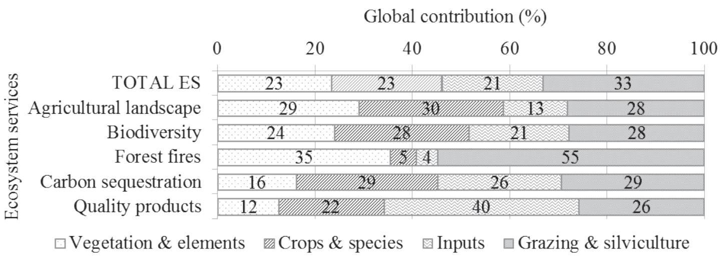

Figure 2. Contribution of groups of farming practices to ecosystem services (ES).

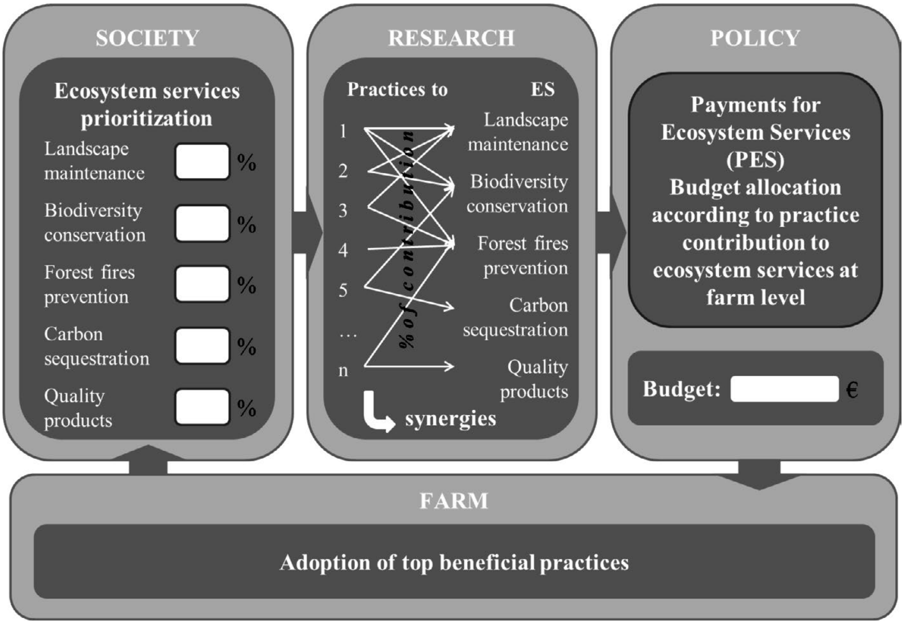

Figure 3. Framework of payments for ecosystem services.

Table 1. Ranking of top-five and last farming practices and contribution (%) to policy settings.

Rank	Willingness to pay for ES[1]		Biodiversity		Carbon sequestration	
	Practice	Cont.	Practice	Cont.	Practice	Cont.
1	Moving flocks seasonally	6.18	Reduce pesticide	3.56	Utilize manure correctly	4.92
2	Grazing semi-natural habitat	5.94	Maintaining semi-natural vegetation	3.48	Reduce ploughing	4.56
3	Management of forest	5.92	Adapt stocking rate	3.46	Maintaining semi-natural vegetation	4.44
4	Maintain grasslands	5.73	Maintain grasslands	3.33	Adapt stocking rate	4.32
5	Increase grazing season	5.49	Retention of hedges	3.33	Maintain grasslands	4.32
n	Reduce pesticide use	0.29	Reduce use concentrates	1.29	Maintain terraces	3.11

[1] Agricultural landscape 8.2% of importance, biodiversity 18.4%, wildfires 53.2% and quality products 20.2%. ES = ecosystem services.

other elements (28.2%) had the highest contribution due to their importance for wildfire prevention, which was highly prioritized by society.

If the focus of the policy was on biodiversity only, the ranking of farming practices was as follows: 1st reduce pesticide use; 2nd maintain semi-natural vegetation (trees and bushes); 3rd adapting stocking rate to the carrying capacity of agroecosystem; 4th maintaining grasslands; and 5th retention of hedges, shrubs and trees among arable fields. Globally, the groups of practices referring to grazing and silviculture (with a total contribution of 27.8%) and crops and species (27.7%) had the highest contribution.

If the focus of the policy was on carbon sequestration only, the ranking of farming practices was as follows: 1st utilizing manure correctly; 2nd reduce ploughing/tilling; 3rd maintain semi-natural vegetation (trees and bushes); 4th adapting stocking rate to the carrying capacity of agroecosystem; and 5th maintaining grasslands. Globally, the groups of practices referring to grazing and silviculture (with a total contribution of 29.3%) and crops and species (28.9%) had the highest contribution.

The framework renders a different prioritization of practices according to policy objectives. It also allows visualizing what practices are synergic, i.e. contribute to several objectives. The final user can decide how many practices to include in the agri-environmental scheme, and then allocate the available resources to farms implementing these practices. Nevertheless, the PES system would also require technical support to farmers and monitoring, in order to ensure the correct implementation of the agricultural practices. This monitoring could modulate the amount of money according to the efficacy of the management practice.

Shortcomings of LCA in grassland ecosystems: the carbon footprint of lamb meat

Life cycle assessment (LCA) has emerged as a widely accepted and standardized method to evaluate the environmental impacts during the entire life cycle of a product or service. LCA is a powerful method to provide a holistic assessment of the production processes, in terms of resource use and environmental impacts, as well as identification of hotspots (Cederberg and Mattsson, 2000; Thomassen and De Boer, 2005). However, LCA still presents significant challenges, particularly when applied to agriculture. The method presents limitations with: the comprehensive assessment of complex interconnected food chains, the production systems that have limited availability of data, and the multiple-output nature of many agricultural production systems (Gerber *et al.*, 2010). This is especially the case of grassland-based livestock farming systems (Ripoll-Bosch *et al.*, 2013), which in Europe are often located on High Value Nature (HVN) farmland and, aside from food supply, are acknowledged for providing multiple ecosystem services, such as enhancing biodiversity (Henle *et al.*, 2008), conserving cultural landscapes (Plieninger *et al.*, 2006) or preventing wildfires (Kramer *et al.*, 2003).

In an attempt to account for ecosystem services in LCA, we analysed three contrasting sheep farming systems in the Mediterranean (see Ripoll-Bosch *et al.,* 2013). Sheep farming systems in the Mediterranean are generally located in less favoured areas and considered to be pasture-based and extensive. However, a process of intensification has occurred across regions and production systems (De Rancourt *et al.*, 2006). As a result, sheep farming systems are very diverse in terms of use of inputs and land use intensity: from zero-grazing to very extensive pastoral systems. We hypothesized therefore that sheep farming systems would differ in performance (i.e. productivity), environmental impact (in this case, greenhouse gas – GHG – emissions) and in the delivery of ES or public goods (aside from meat production). We selected three farming systems: a pasture-based system (very extensive, located in the Pyrenees, Spain); a mixed sheep-cereal system (semi-intensive, the most widespread system in Spain); and a zero-grazing system (very intensive, located in the Ebro basin, Spain). The carbon footprint of lamb meat (when considered as the only output of the production system) decreased according to a degree of intensification: highest values for the pasture-based system and lowest for the zero-grazing system (Table 2). However, when allocating the carbon footprint to the different outcomes from the diverse systems (i.e. to the meat, but also the ES associated to the production system), the carbon footprint of lamb meat was reversed: lowest values for the pasture-based system and highest for zero-grazing system.

Table2. Greenhouse gas (GHG) emissions (CO_2-eq kg^{-1} of lamb meat) with or without consideration of ecosystem services (ES) and contribution (%) of CO_2, CH_4 and N_2O to total GHGs (Ripoll-Bosch *et al.,* 2013).

	Carbon footprint of lamb meat (kg CO_2-eq kg^{-1} lamb meat)		Contribution (%)		
	Without ES allocation	With ES allocation	CO_2	CH_4	N_2O
Pasture-based	51.7	27.7	7.9	61.6	30.5
Mixed	47.9	35.4	21.0	57.6	21.4
Zero-grazing	39.0	39.0	29.1	59.4	11.5

In that study we showed the tremendous effect of acknowledging multiple-output systems. System efficiency (in terms of GHG emissions) depended on the methodological choice of allocating emissions to a single output (i.e. lamb meat) or to the multiple outputs (i.e. lamb meat and ecosystem services). The approach we followed faces an important limitation, and should be understood as a proof of concept. The economic valuation to allocate GHG between lamb meat and ES was based on political decisions compensating for the loss of agricultural production rather than biophysical observations and scientific evidence. We considered the agri-environmental payments (of the Common Agricultural Policy) to farmers as a proxy of the cost for undertaking such conservation measures and, ultimately, the willingness of society to pay for the ES. There was no scientific consensus in how to incorporate ES in life cycle thinking then, and still this matter continues under development.

Despite the widespread usage and acceptance of LCA to assess the environmental impact of a product or service, the method still presents a number of challenges, especially when applied to grassland-based systems:

1. Direct comparisons between LCA studies are difficult because of potential differences in methodological choices (De Vries and de Boer, 2010). This suggests that further standardization is needed. Three main methodological issues deserve attention, as follows: (a) The functional unit. The expression of the environmental impact in relation to the product or service under study is still a matter of divergence between studies (e.g. GHG emissions per kg of product, per nutritional value, per hectare of land, or per person (Nguyen *et al.*, 2012). (b) The system boundary. The definition and delimitation of system under study will greatly influence the results. Different studies can differ, for instance, in including diverse stages of production (e.g. environmental impact up to farm gate, or to supermarket shelf), handling co-production (e.g. including, or not, the unavoidable meat production of dairy systems), or considering the carbon sequestration potential of grasslands (Soussana *et al.*, 2010). (c) The allocation method. In cases of multiple output systems, emissions can be distributed among the products in different ways (i.e. based on biophysical properties or economic valuation).
2. Land use in pasture-based systems may be difficult to measure, especially as an impact category. In many pastoral systems, animals graze in communal open areas, generally natural and semi-natural grasslands. The constant movement of animals looking for fresh forage prevents full usage or over usage (i.e. degradation) of grassland. This intermediate use of the resources allows communal-living with wild large herbivorous and hosts high biodiversity. In such cases, grassland that is not fully used may supposed to be well-managed grassland. However, the LCA framework considers land use (i.e. number of hectares used) as an impact category, implying that situations with more land used are worse. Under the LCA viewpoint the intermediate intensity usage of grasslands is not well captured.
3. Carbon (C) sequestration in grasslands has emerged as a research hotspot in recent years (Smith, 2014; Soussana *et al.*, 2010; Rodríguez-Ortega *et al.*, 2014). It is generally acknowledged that grasslands may have potential to sequester C (Lal, 2011). Hence, nowadays, many carbon footprint studies attribute a certain (default) value of C sequestered into the soils. Such methodological approach still faces important limitations: (a) There is still some controversy around the potential of grasslands to sequester C. While some authors seem enthusiastic (Soussana *et al.*, 2010), other are dubious about this potential (Smith, 2014). (b) Carbon sequestration in soils is dynamic, and C content in the soils tend to an equilibrium or saturation. This implies that grasslands cannot sequester C indefinitely in time and hence, the potential is limited (in amount and in time). The C sequestration potential will dependent on the type and maturity of the grassland, and on the management of the grassland. (c) The management of the grassland can influence C sequestration potential (sink), but more important is key to maintain the carbon sequestered into the soil (stock). A poor management of the soil cannot guarantee an actual removal of C from the atmosphere, and even less for a long period of time. (d) It is important to avoid confusion with the concepts carbon sequestration or sink and carbon stock or storage.

4. Data availability and quality is usually a constraint in low-input/pasture-based production systems (Ripoll-Bosch *et al.*, 2013). Productivity of natural and semi-natural grasslands, and the grasslands-livestock relationship is generally poorly documented. Grasslands are very diverse depending on their geographical location (e.g. latitude, altitude and/or ecosystem) and the degree of intensity of management, from natural and semi-natural rangelands (used extensively, where stocking rates and/or fire regimes are the main management variables) to intensively managed grasslands (e.g. with fertilization, irrigation, mechanization or management of species) (IPCC, 2006). In consequence, productivity of grasslands (in terms of biomass or net primary production) is very variable across locations and management, but also variable from year to year (i.e. variability in rainfall, temperature, etc.) and from season to season (i.e. seasonality within the year). Likewise, the quality of the grasslands (e.g. nutrient content, dry matter, digestibility, plant composition, etc.) varies across locations, management, years and seasons. This information, usually lacking or difficult to obtain (Lauenroth *et al.*, 2006), is crucial to properly estimate the intake and the diet composition (quality) of grazing animals, and ultimately, properly calculate the methane emissions from enteric fermentation (Moraes *et al.*, 2014).
5. The life cycle assessment or in a more generic way, the life cycle thinking, need to further recognize the multifunctionality of certain agricultural production systems, understood as services to society (Ripoll-Bosch *et al.*, 2013), and the multiple roles and functions of livestock production (Weiler *et al.*, 2014). The acknowledgement and valuation of the ecosystem services or public goods provided by grassland-based systems, especially when located in marginal areas or High Nature Value farmland, could be a key factor to increase the relevance and competitiveness of farms and farmers. Moreover, the extrinsic values of high quality products (e.g. protected designation of origin, PDO), which is highly appreciated by consumers (Bernués *et al.*, 2016) cannot be captured within the LCA methodology.

Conclusions

When analysing the relationships between pasture-based livestock and the environment, a controversial topic nowadays, conceptual and methodological challenges still remain open for discussion and further research. In this paper, we presented a novel PES system for pasture-based livestock farms that link agricultural practices and ES through expert knowledge. We showed its usability with different policy scenarios, one of which represented the societal expectations in terms of ES demand. Despite the limited success in translating the ES framework into policy design, partly due to the lack of knowledge regarding the concrete effect of practices on multiple ES at different scales, we believe that it is possible to articulate more targeted agri-environmental schemes with the existing information or with simple and quick appraisal methods to obtain it. We also argue that the LCA method to quantify the negative impact of pasture-based livestock farming systems on the environment, notably its contribution to climate change, needs to take into account the multifunctional character of these systems, and to take a more holistic approach that considers interlinked sustainability issues and broader scales.

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Combined techniques with horse-guided grazing to control gorse encroachment in highly valuable mountain habitats

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Abstract

In European high mountain ecosystems, rural depopulation, land abandonment and reduction of pastoral practices are fostering forest and shrub encroachment in grassland areas, increasing the risk of fires due to landscape homogenization and fuel accumulation. In the western Pyrenees, traditional pastoral fires and mechanical clearings are used to prevent shrub encroachment and improve grasslands. However, the effectiveness of these practices is questioned because, in many areas, the rapid resprouting of shrubs prevents the consolidation of grassland communities. The aim of this research is to evaluate the efficacy of burning and mechanical clearing combined with guided grazing to control *Ulex gallii* resprout. We performed experimental fires and mechanical clearings in three dense gorse-dominated areas located at the SCI Roncesvalles-Selva de Irati, and then established a set of fences where an extensive, rotational grazing with autochthonous breeds was planned. *U. gallii* resprout and plant community composition were surveyed during three years. Clearings produced a thick layer of ground material and a mechanical damage to shrubs, while fire appeared to stimulate a rapid gorse resprout and seed germination due to the removal of the standing biomass and the thermal shock. The effectiveness of the guided grazing measures in shrub control was detected in the mid-term, although long-term surveillance is needed to obtain reliable conclusions.

Keywords: shrub encroachment, *Ulex gallii*, pastoral fire, mechanical clearing, horse-guided grazing

Introduction

European mountain ecosystems have been created, structured and maintained by two main disturbances – fire and herbivory – which have given rise to a mosaic of open landscapes and highly valuable grasslands (Vera, 2000). Rural exodus, land-use abandonment and extensive livestock reduction during the twentieth century has accelerated shrub encroachment and forestation processes, endangering grassland ecosystems (Poschlod and Wallis De Vries, 2002). In Western Pyrenees, traditional pastoral fires and mechanical clearings are used to prevent gorse (*Ulex gallii* Planch.) encroachment. However, these practices have an economic and environmental cost, and their effectiveness in consolidating grassland communities is questioned because of the rapid resprouting of shrubs (San Emeterio *et al.*, 2016). *U. gallii* is a leguminous thorny shrub that can reach 1 m in height and it grows on acidic soils in oceanic climates. Gorse shrublands produce and accumulate a high biomass, constituting dense and persistent covers. In addition, gorse has a high inflammability and spreads flames rapidly, increasing the risk of fires (Reyes *et al.*, 2009). The aim of this study is to evaluate gorse resprout and plant community evolution, after applying prescribed burnings and mechanical clearings combined with long-term guided-grazing practices with local breeds of domestic herbivores. We hypothesize that a well-planned exploitation after the application of preliminary fuel-reduction methods allows a better control of gorse resprout, extending the effects over time and reducing the recurrence of application of starting reduction methods.

Materials and methods

The research site was located at the SCI Roncesvalles-Selva de Irati (ES0000126) in Western Pyrenees (43°1'N 1°19'W). The area has a rainy and misty climate with cold winters and cool summers (mean T = 9.2 °C; precipitation = 1,601 mm y^{-1}). Soils are organic and acidic, with clay-loamy textural classes.

The vegetation is a mosaic of beech forests, shrublands and grasslands. In shrublands, the dominant species is *U. gallii*, although heather (*Calluna vulgaris*) and heaths (*Erica vagans*, *Erica tetralix*, *Daboecia cantabrica*) are also present. Grasslands are very diverse and include perennial grasses (such as *Festuca rubra, Agrostis capillaris, Agrostis curtisii*), perennial forbs (such as *Galium saxatile, Potentilla erecta*), and legumes (*Trifolium repens*). In 2014, three dense, gorse-dominated areas (cover >90%) occurring over 179 ha, at altitudes ranging from 1,064 to 1,265 m a.s.l. were selected along a hill. The lowest area experienced a moderate grazing pressure and shrubs were younger (5-6 years) than in upper areas, which were lightly grazed and developed more mature shrubs (>10 years). Two preliminary control methods were executed at each area: experimental fires (21 March and 10 April) and mechanical clearings (27 June and 4 July). Afterwards, a set of fences was established to manage a rotational guided grazing of 76 *Burguete* horses (26 adults and 50 young mares) and 16 *Pirenaica* heifers (which grazed for a shorter period). Key facilities (watering points) and food bait delivery areas were planned to handle livestock conveniently (Sáez *et al.*, 2016). Inside and outside the fences, two permanent 1×1 m squares were established and surveyed in three years, in autumn, for species richness, species cover (adapted from Braun-Blanquet 1979) and *U. gallii* height and resprout. Overall, 72 floristic inventories (3 areas × 2 control methods × 2 outside/inside plots × 2 squares) were made.

Plant groups and *U. gallii* cover and height were compared using linear mixed models specific for repeated measures analyses. The models included control methods, grazing, date and site as fixed factors, and squares nested within the plots as random factor. Differences among the means of dates, sites and treatments were assessed by Tukey tests. Statistical analyses were performed using R software (R Development Core Team 2016).

Results and discussion

All plant communities experienced a significant evolution during the three years of study (P_{date}<0.001 for plant groups and *U. gallii*). Livestock pressure, and especially horse pressure, which included gorse consumption and mechanical damage by trampling (700 and 350 kg, mean weight of an adult horse and a heifer, respectively), affected shrub resprout in the mid-term (P_{graz}=0.070 for shrub; $P_{graz*date}$=0.048 for *U. gallii* cover; $P_{graz*date}$<0.001 for *U. gallii* height), mainly in the most intensively grazed areas (Table 1). Grazing reduced gorse height and cover at 31 months after the beginning of the experiment (at the third survey, P_{graz}=0.019 for cover; P_{graz}=0.003 for height). A temporal increase was detected in graminoid cover, with different evolution in grazed and ungrazed areas ($P_{graz*date}$<0.001), although it seemed to be influenced by initial treatments ($P_{treat*graz*date}$=0.019) and site-specific characteristics (P_{site}<0.001). Grazing favoured a gradual increase of graminoid cover in burned plots, which was not observed in non-grazed burned plots.

With regard to preliminary control methods, shrub resprout was higher in burned areas compared with cleared areas during the three years (P_{treat}=0.021 for shrub, P_{treat}=0.045 *for U. gallii*, P_{treat}<0.001 for height). Different reasons may explain these results. Clearings were performed three months later than burnings, and consequently gorse growth was delayed in cleared plots during the first season. In addition, clearings produce a thick layer of ground material (which lasts the first year) and a mechanical damage to shrubs, while fire may stimulate a rapid gorse resprout and seed germination due to the removal of the standing biomass and the effect of thermal shock (Reyes *et al.*, 2009). In addition, terrain constraints were higher in burned areas (slope$_{med}$ >35%; slope$_{max}$ >75%) compared with cleared ones (slope$_{med}$ <35%; slope$_{max}$ <75%), and persistent grazing was more difficult to achieve in the steepest, burned areas.

Conclusions

After three years, a positive effect of guided grazing on *U. gallii* resprout control was observed, regardless the preliminary method used. It seemed that previous mechanical clearings exerted a better control of

Table 1. Vegetation cover divided in groups ('Shrubs' include *Pteridium aquilinum* and 'Bare soil' includes soil mulch), and *Ulex gallii* cover and height for absence/presence of grazing, each control methods, and year of survey.

	Year	Grazed areas		Ungrazed areas		Significance[1,2]				
		Burned	Cleared	Burned	Cleared	GD×Y	UG×Y	B×Y	C×Y	
Shrubs (%)	2014	46.7±10.4	26.5±6.1	55.7±2.8	23.0±5.2	b	b	B	B	T*
	2015	56.7±14.9	38.7±8.0	84.0±11.7	42.4±8.0	a	a	A	A	G+
	2016	57.0±11.1	43.2±2.4	84.8±15.7	49.3±10.3	a	a	A	A	
Grasses (%)	2014	5.5±3.6	22.5±8.1	24.8±11.6	24.8±7.12	c	c	B	B	T+
	2015	23.4±6.8	51.2±10.0	41.0±15.3	65.9±10.8	b	b	A	A	T.G.Y *
	2016	44.0±7.4	64.2±12.1	33.4±13.6	69.4±10.1	a	a	A	A	
Bare soil (%)	2014	24.7±2.6	44.2±9.3	21.0±6.5	44.8±9.3	a	a	A	A	G**
	2015	11.8±3.2	12.5±5.7	1.5±1.3	6.7±2.5	b	b	B	B	
	2016	19.7±5.0	5.7±2.4	10.7±8.0	0.2±0.2	b	b	A	B	
U. gallii cover (%)	2014	24.7±5.5	19.9±4.8	29.2±2.8	14.7±4.9	b	c	C	B	T*
	2015	30.2±8.6	23.0±6.2	48.3±8.3	24.3±8.8	a	b	B	A	
	2016	31.0±6.5	27.0±10.8	47.7±12.0	29.5±12.3	a	a	A	A	
U. gallii height (cm)	2014	20.1±1.1	7.8±0.5	18.7±0.7	7.5±0.3	c	c	B	C	T***
	2015	19.8±1.4	14.1±1.2	32.3±2.9	13.3±1.0	b	b	B	B	G+
	2016	28.5±2.4	14.2±1.1	43.1±1.6	26.3±1.7	a	a	A	A	T.G.Y**

[1] Different capital letters for differences between dates for burned and cleared plots and lower case letters for differences between dates for grazed (GD) and ungrazed (UG) plots. T, preliminary treatment; G, grazing; Y, year

[2] Significance: + P<0.1; * P<0.05; ** P<0.01; *** P<0.001. Values are the mean ± standard errors of 6 replicates for cover and 38-42 for *U. gallii* height.

shrub than burnings, both in grazed and ungrazed areas, although further analyses of factors, such as shrub age, fire intensity, timing of treatment and grazing pressure, are needed to obtain conclusive results.

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Mil'Ouv, an innovative method of eco-pastoral diagnosis

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Abstract

The European Life+ programme 'Mil'Ouv' (standing for MILieux OUVerts/open landscapes such as limestone plateaux, grassland or scrubland) aims at optimizing the breeders' use of natural resources in Mediterranean and sub-Mediterranean regions, in order to fulfil production objectives as well as enhance the conservation status of open habitats and their biodiversity. To achieve these goals, an innovative method of eco-pastoral diagnosis has been developed. This is the result of pooling and adapting existing indicators and tools which are already used by naturalists and agricultural technicians, but which are often too specific and mutually exclusive. Thus, the Mil'Ouv eco-pastoral diagnosis is based on the conjunction of three perspectives: the naturalist, the livestock specialist ('pastoralist') and the breeder him/herself, who must combine their skills to obtain a joint definition of challenges. A second original element of this diagnosis is to provide a detailed analysis of pastoral systems by working at different scales: from farming systems to homogenous ecological plots ('topo facies'). Initially designed for French context, the Mil'Ouv eco-pastoral diagnosis has also been extended and adjusted to make it applicable to an Albanian situation (within BiodivBalkans programme).

Keywords: open habitats, pastoralism, biodiversity, eco-pastoral diagnosis

Introduction

Open habitats or landscapes demonstrate spontaneous natural growth, with little brushwood cover, and few or no trees, and they are unsuited to mechanical cultivation. They refer to a very large range of habitats showing these characteristics, such as limestone plateaux, grassland, wetland or scrubland. In the Mediterranean area, they are mostly shaped by agro-pastoral activities and host very rich biodiversity with a number of endemic species. Beyond their patrimonial and ecological value, they provide a fairly abundant and high-quality feeding resource for livestock, available all year round and suitable for every physiological stage of the animals. However, with the decline and evolution of pastoral activities in France, encroachment and landscape closure have threatened these habitats, leading to a loss of specific biodiversity and forage resource (Zarovali *et al.*, 2007). The European Life + programme 'Mil'Ouv' (standing for MILieux OUVerts/open landscapes) aims at optimizing the breeders' use of natural resources in Mediterranean and sub-Mediterranean regions, in order to ensure the conservation status of open habitats while maintaining farm production objectives. To achieve these goals an innovative method of eco-pastoral diagnosis has been developed.

Mil'Ouv: toward a shared analysis in the field

A conjunction of skills and perspectives

In the harsh areas of European countries, farmers face the challenge of finding a balance between animal production and environmental objectives. In fact, combining these two perspectives within the same area might be complex and making separate diagnoses would probably lead to contradictory recommendations. In effect, a naturalist will often rely on an inventory of species and work on a very small spatial and temporal scale (square meters and weeks), whereas the livestock specialist (pastoralist) will make observations on larger scales such as hectares and seasons (paddocks and their use during the year). The approach developed by Mil'Ouv is to merge the skills and interests of naturalists, pastoralists and breeders within the same ecosystem to obtain a joint definition of challenges. By making the diagnosis together and simultaneously, the trio will co-construct strategies and propositions to achieve the optimized use of resources.

Different scales of analysis

A second original element is the integration of several levels of analysis by gradually scaling down (working on smaller and smaller units). Three levels are investigated: (1) farm system, to consider the farmers' general feeding strategy and objectives; (2) management unit (paddock or grazing area managed in the same way), to acquire an overview of the pastureland and determine the main ecological and pastoral issues of the farm; (3) 'topo-facies', a homogenous plot in terms of vegetation and topography, which constitute a common scale of analysis in the field, between naturalist and pastoralist. By taking this approach, the diagnosis does not focus only on a targeted ecological optimum but also includes the objectives set by farmers.

A three-step process

The Mil'ouv diagnostic tools are the result of pooling and adapting existing indicators and methods (such as Aussibal *et al.*, 2010) which are already used in the field by extension services, but which are often too specific and mutually exclusive. Only the most synthetic and pertinent indicators, adapted to open landscape contexts, have been selected. Throughout the diagnosis, graphic representations help to interpret the results, in an easily readable format for the breeders. The entire diagnosis is conducted in three steps lasting 3 to 5 days:

1. Global understanding of the farming system. This first step consists of a detailed interview with the breeder concerning his production goals, the land location and use (with land mapping) and the livestock feeding strategy. Based on the functional analysis of feeding systems (Moulin *et al.*, 2001), a calendar is established in order to cross-reference animal needs with resources available on the farm. The contribution of each type of vegetation in the global livestock intake is evaluated. Indicators such as degree of autonomy, grazing rate (proportion of pastureland – meadows and rangelands – in the flock's feed) or pastoralism rate (proportion of rangelands in the flock's feed) are calculated and used to build a farm feed strategy radar plot (Figure 1). This graph can be used to evaluate the inter-annual variations of a farm or to compare farms within the same territory.
2. Eco-pastoral diagnosis. Following a description of the practices within each management unit, the farmer (grazing periods, number and type of animals grazing, animal feed supplements, cleaning or cutting operations, etc.), the pastoralist and the naturalist together subdivide the management units into several 'topo-facies'. These subdivisions can also be discussed with the breeder, according to his knowledge of the herd exploration behaviour. Each 'topo-facies' is then characterized, using a set of indicators, to evaluate the pastoral resource, ecological challenges and conservation status. This step aims at understanding the link between the operating mode and habitat dynamics. Productivity,

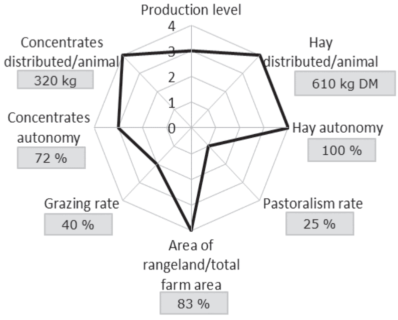

Figure 1. Example of a feed strategy radar of an ovine flock. Scored from 1 (low) to 4 (high).

herbaceous cover, attractiveness of the resource, seasonal flexibility of use, flock movement, dysfunction (over-use, under-use), plant community, composition and dynamics of woody plants, are evaluated with indicators that are selected to be used easily by naturalists as well as pastoralists and breeders themselves. A set of pastoral references is used to compare the use made by the herd (species, size of the herd and grazing duration) to the theoretical use that could be envisaged on this vegetation to fulfil animal requirements and ensure a correct conservation status. The results of this field diagnosis can also be displayed graphically by pooling and scoring the different field indicators (see example on Figure 2).

3. Management guidance and monitoring. At the end of the diagnosis, a review is carried out with the farmer to discuss the practices and, if necessary, suggest technical improvements. If wished, a monitoring phase may be put in place to evaluate the impacts of changing practices on both environment and farming system.

Adjustment to different contexts: the example of Albania

Even though it was initially designed for the French context of open-habitats, the diagnosis may easily be extended to other countries and/or contexts. After some adjustments to make it applicable, the method was transferred and tested in the Has region (Albania) in 2015 through the CIHEAM-IAMM BiodivBalkans programme.

According to Garnier *et al.*, (2016), the transferral process involved three major adaptations: (1) integration of additional levels in the scale of analysis, to understand the common management of collective pastures; (2) incorporation of wooded areas, as forests represent a large part of the pastoral area with ecological issues; (3) adaptation of diagnostic tools (questionnaire, list of natural habitats, graphic representations).

Conclusions

This eco-pastoral method provides technical advisers with a diagnostic tool, easily adaptable to other contexts, that takes into account agricultural production objectives and environmental challenges. An adaptation is currently in progress, to be used for educational purposes.

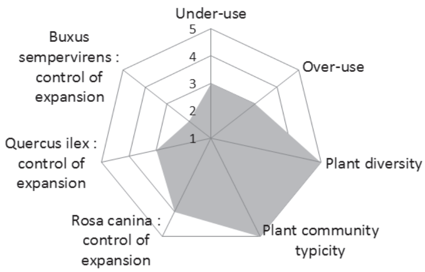

Figure 2. Conservation status of a 'topo-facies'. Scored from 1: low or dysfunctioning to 5: high or without dysfunctioning.

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Predicting the distribution of High Nature Value farmland in the Republic of Ireland

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Abstract

High Nature Value (HNV) farmland is a headline indicator for the Common Agricultural Policy. The design, implementation and assessment of policies involving HNV farmland first requires good knowledge of the distribution of HNV farmland. A significant proportion of the Republic of Ireland's territory comprises a combination of species-rich grassland, commonages and upland vegetation. The IDEAL-HNV project (www.high-nature-value-farmland.ie) developed a national map of Ireland with a distribution of the likelihood of HNV farmland. Five indicators were used to estimate the distribution of HNV farmland at 2×2 km spatial resolution: semi-natural habitat cover (CORINE); stocking density; hedgerow density, river and stream density, and soil diversity. This is the first Irish national-scale map that has used objective agri-environmental criteria to predict the likely distribution of HNV farmland, and provides a reference point for future monitoring of the distribution of HNV farmland in Ireland. It also represents an objective methodology with which to add a GIS layer of likelihood of HNV farmland to other national spatial analyses of Irish agriculture, environment and socio-economics. We also developed a typology of the types of HNV farmland in Ireland, based on farm-scale characteristics (land cover and management data). An online tool is available to assess the nature value status of individual farms.

Keywords: biodiversity, agriculture, remote sensing, GIS, sustainability

Introduction

High Nature Value (HNV) farmland has been defined as 'those areas in Europe where agriculture is a major (usually the dominant) land use and where agriculture sustains or is associated with either a high species and habitat diversity, or the presence of species of European conservation concern, or both'. Maintaining both the nature value of this farmland and the livelihoods of farmers in these areas is a key policy challenge.

Conservation of natural resources (including biodiversity) and halting the degradation of ecosystem services are key environmental objectives of the European Union. The importance of High Nature Value farming systems is now well recognised across a number of policies, not just for their biodiversity, but also for their provision of environmental public goods including clean air, clean water, stable climate, aesthetic landscapes and vibrant rural communities. The European Commission includes HNV farming and forestry systems as one of the seven headline indicators of environmental impact. Member States are required to identify areas with HNV farming practices, to support and maintain HNV farming through Rural Development Programmes, and monitor changes to HNV farmland area over time.

Due to the absence of national habitat maps, there is very limited knowledge of the spatial distribution of HNV farming systems that occur outside of designated sites in most Member States, including Ireland. Many Member States require a major effort to fill the data gaps on the distribution and character of HNV areas. With this in mind, the IDEAL-HNV project (Identification of the Distribution and Extent of Agricultural Land of High Nature Value in Ireland) was initiated in 2012, and had the following key objectives:

- Develop and apply GIS methods for identifying HNV farming systems.
- Develop a typology of HNV farming systems in Ireland.
- Examine the use of remote sensing methods to identify HNV areas at the farm scale.
- Develop bottom-up decision-support tools to assist field- and farm- scale identification of HNV farmland.
- Analyse the socio-economic status of HNV farming systems.

Here, we focus on one of the objectives, the development of Geographical Information System (GIS) methods to improve prediction of the likely distribution of Irish HNV farmland, which is dominated by grassland vegetation.

Materials and methods

We mapped the likely distribution of HNV farmland based on established European indicators adapted for Ireland using the following indicators (with weighting in brackets):

- Semi-natural land cover classes from Corine 2012 (40%)
- Stocking density from Irish Dept of Agriculture, Food and the Marine (30%)
- % hedgerow cover from Teagasc National Hedgerow cover (10%)
- Length of river and stream from Ordnance Survey Ireland (10%)
- Soil diversity calculated using the Teagasc map of soil associations (10%)

Data were modelled at the tetrad scale (2 km × 2 km), and presented here at the scale of Electoral Divisions. To maintain all the input layers in one format and range, all the input values were rescaled between 0-1. A weighted sum model (WSM) assigned weights (see above) to the input layers and combines them to create an integrated output.

Results

The resulting map (from Matin *et al.*, 2016) indicates the likely occurrence and distribution of HNV farmland in each Electoral Division, based on a scale ranging from very low (blue colour) to intermediate (yellow) to very high (green) (Figure 1). Not surprisingly, western counties such as Kerry, Clare, Mayo, Galway, Leitrim, Donegal and Cavan exhibited greatest likelihood of containing HNV farmland while Dublin, Meath and Kilkenny had lowest likelihood. Nevertheless, there is considerable variation within counties. This is the first Irish national-scale map that has used objective agri-environmental criteria to predict the likely distribution of HNV farmland. It provides a reference point for the future monitoring of the distribution of HNV farmland in Ireland. It can also assist in policy planning and development for the rural environment. For example, comparisons of the spatial distribution of HNV areas and the spatial distribution of agri-environmental and other payments can assess the degree to which payments are targeted toward HNV farming systems. In addition, these data can be used to incorporate impacts on farmland biodiversity of, for example, land use change and climate change in national-scale models or scenarios.

Discussion

As an indicator-based prediction, such maps should be interpreted within the limitations of the data used. The spatial scale of the map is restricted by the coarse scale of data at national level. Given the predictive and aggregated nature of the outputs, it is important to note that non-HNV farmland may still occur in areas with high likelihood of HNV farmland, and *vice versa*. This approach may be appropriate for other EU countries.

For this reason, this output is not suitable for strictly deciding whether farmers in certain areas should be eligible or not for agri-environmental measures aimed at HNV farming systems. Instead, there is a

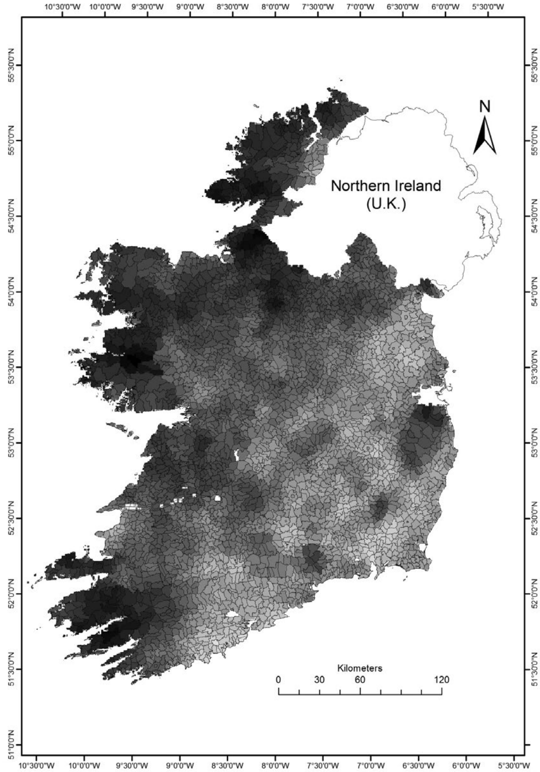

Figure 1. Likely occurrence and distribution of High Nature Value (HNV) farmland in each Electoral Division, based on a scale ranging from low (light) to high (dark). Note that non-HNV farmland may still occur in areas with high likelihood of HNV farmland, and *vice versa*.

requirement for a farm-scale assessment to confirm the high nature value of individual farms. As part of the IDEAL-HNV project, we also examine the farm-scale characteristics of HNV farmland. See the project website (www.high-nature-value-farmland.ie) for further details.

Acknowledgements

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The effect of landscape structure on biodiversity in semi-natural grasslands of high nature value

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Abstract

Semi-natural grasslands are in decline due to land use changes. To conserve grassland ecosystems, agri-environmental schemes facilitate low intensity management of semi-natural grasslands of high nature value. In addition to management of the meadows themselves, the nature value of the hay meadows also depends upon the surrounding landscape. The aim of this study was to assess the importance of the landscape on the biological value of semi-natural hay meadows implemented in the Action Plan for Hay Meadows (APHM) in Norway. The biological value was estimated by richness of all vascular plant species and of vascular plant species associated with semi-natural grassland. Richness was high in semi-natural grasslands, particularly in hay meadows implemented in the APHM. However, the landscape context also influenced the richness of the hay meadows. Species richness increased with hay meadow area and length and edge density of semi-natural habitats types in the landscapes.

Keywords: vascular plant species richness, species turnover, hay meadows, landscape, agri-environmental schemes

Introduction

Historically, semi-natural grasslands managed at low intensity were widespread and of high agricultural importance (Strijker, 2005; Emanuelsson *et al.,* 2009) but today they are in decline due to land use changes (Strijker, 2005). Semi-natural grasslands are among the most species-rich ecosystems in Europe (Veen *et al.,* 2009) and represent high cultural, biological and agricultural values (Bullock *et al.,* 2011). In Norway, semi-natural hay meadows are considered as a threatened habitat type (Norderhaug and Johansen, 2011) and in order to conserve them the Norwegian agri-environmental scheme 'The Action Plan for Hay Meadows' was initiated (Norwegian Environment Agency, 2009). The action plan encourages conservation and management of hay meadows of high biological value by developing local management plans and offering economic incentives for farmers and other managers. Landscape structure can have a strong impact on the dynamics of plant communities within remnant habitat fragments (Tscharntke *et al.*, 2012) and therefore also on biodiversity within semi-natural grasslands. In this study, we aim to assess the importance of the surrounding landscape structure on the biological value of semi-natural hay meadows included in the action plan for hay meadows.

Materials and methods

The study took place in an area (ca 50 km^2) in Norway with a relatively high density of high value semi-natural grasslands. Two data sets including recordings of all vascular plant species present were used as the basis for the analyses. Dataset 1 (DS1) was obtained July 2014 and included data from all hay meadows (area = the area of the grassland: from 1,079-6,196 m^2) within the study area that are involved in the action plan ($n_{hay\ meadows}$=13). Dataset 2 (DS2) was obtained July 2015 and included data from 87 plots (area = 16 m^2 each) distributed in all major habitat types within the study area. The ten major habitat types were semi-natural (meadows; pastures; road edges; verges of arable fields; clear-cuts; young forests that were semi-natural grasslands 30 years ago), forests (pine (*Pinus sylvestris*) forest; deciduous forest; mixed forests) as well as spruce (*Picea abies*) forest plantations.

All species were categorized as either semi-natural specialists or not, based on a list of semi-natural specialist species made available by the project 'Nature Types in Norway' (for more information, see http://www.biodiversity.no). Semi-natural specialists are defined as species distributed in semi-natural grasslands, negatively influenced by artificial fertilizers and encroachment. We calculated α- (mean ± standard deviation of the number of vascular plant species), β- (species turnover calculated as the length of detrended correspondence analysis (dca) axis 1), and γ-diversity (total number in all samples) based on the two data sets. Finally, using DS1 and area resource maps, we analysed impacts of landscape diversity features on α-diversity in the hay meadows involved in the action plan. Investigated parameters were: area of the semi-natural hay meadows involved in the action plan, total area of all semi-natural meadows and pastures, total length of field margins and road verges, and edge densities (perimeter/area) of the semi-natural meadows and pastures. The parameters were calculated using area resource maps and we delineated a landscape within a distance of 1 km from each of the hay meadows ($n_{landscapes}$=13). To estimate the influence of the landscape diversity features on the two α-diversity measures, total species richness and specialists richness, we used generalized linear models with a Poisson distribution and a log link function. Models were made sequentially and model fits were assessed with chi-square test on the log likelihood values (α=0.05) using the R 3.1.1 software (R Core Team, 2015).

Results and discussion

The total number of observed species (γ-diversity) was 199 in the 16 m^2 plots (DS2) and 127 in the hay meadows (DS1). The total number of species considered as specialists was 41 (DS1 and DS2), 33 of which were found in the hay meadows (DS1). The α-diversity in the hay meadows (DS1) was high (53.00±11.38). The semi-natural habitat plots had, on average, more species than plots in other habitat types (DS2; semi-natural: 26.17±6.60; forests: 19.46±6.01; plantations: 7.43±6.85). Even if the plantations included fewer species, this habitat had a higher number of semi-natural specialists (3.00±2.83) compared with the other forest types (2.62±1.60). The α-diversity of specialists in the semi-natural habitats (DS2) was 6.44±3.73 and in the hay meadows (DS1) was 16.31±3.90.

Species turnover (β-diversity) between the major habitat types (DS2) was also high (length of dca axis: 7.246 SD units) when only specialist species were analysed (length of dca axis: 6.772). Species composition was similar between the hay meadows (DS1) (i.e. low β-diversity; length dca axis: 1.872), but turnover was high between the different semi-natural habitat types (DS2; length of dca axis: 6.282).

The landscape analyses showed that the landscape context did influence the richness of the hay meadows (DS1; Figure 1). Species richness increased with area of the hay meadow (P<0.001), length of field margins and road verges (P=0.020), and edge density of the semi-natural habitat types in the landscapes (P=0.050).

It has been hypothesized that agri-environmental schemes are most successful when the habitat type targeted by the scheme covers 1-20% of the total landscape (Tscharntke *et al.*, 2012). In our study area,

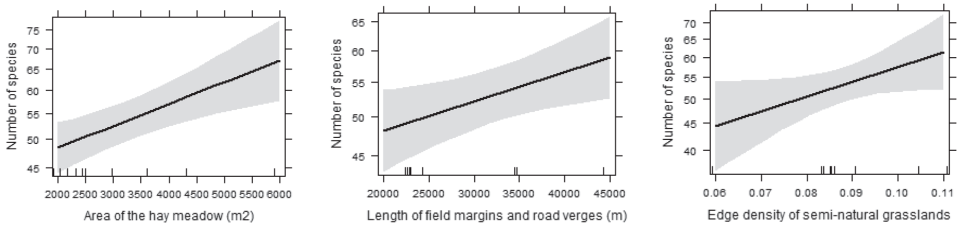

Figure 1. Effect of landscape diversity features on the number of species in 13 hay meadows.

the areas of open semi-natural habitats were 1-3% (not including field margins and road verges with semi-natural vegetation). Tscharntke *et al.* (2012) argue that, in such landscapes, management at local scale is crucial, but that the maintenance of a habitat is secure only if its distribution exceeds 20% of the total landscape. The length of the dca axis (6.112 SD) was greater than four when analysing the distribution of specialist species in the semi-natural habitat types (DS2). This means that there was a total turnover of specialists between the plots in the semi-natural habitat types. This indicates that a certain number of such habitats must be present to provide suitable 'stepping stones' for the species that give the high biological value of the hay meadows included in the action plan. A large-scale implementation of the agri-environmental schemes is to be preferred. Further, it is also important to consider proper management of field margins and road verges as well as restoration of the young forest areas that still show some characteristics of semi-natural grasslands.

Conclusions

The Action Plan for Hay Meadows in Norway targets species-rich hay meadows with high importance for conservation of biological diversity. It is vital to continue the low intensity management in order to conserve these semi-natural grasslands but conservation measures should also include actions at the landscape scale.

Acknowledgements

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Legume-rich sown permanent pastures in Iberian Dehesas: influence on biodiversity and soil carbon sequestration

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Abstract

The sowing of legume-rich mixtures in Dehesas (commercially known as Sown Biodiverse Permanent Pastures Rich in Legumes, SBPPRL) is gaining importance due to the improvements on soils, increase of biodiversity and carbon-fixation strengthening, which not only makes the Dehesa a more profitable system, but also a more resilient ecosystem to face climate change. Our research focuses on the evaluation of the persistence and the adaptation to the canopy shade microhabitat of legume-rich mixtures. The study included a chronosequence of pastures sown among 2002 and 2015, in seven Dehesa farms of western Spain. The biodiversity of sown plots increased immediately relative to unsown control plots and persisted higher during at least 12 years. A positive tendency in carbon sequestration was also observed. Positive trends were, irrespective of the habitat, beneath and outside the tree canopy projection.

Keywords: climate change, mitigation, persistence, pasture legumes, silvopastoral systems

Introduction

A Dehesa is usually defined as a two-layered silvopastoral system in which native grasslands cohabit with a layer of scattered, widely spaced trees, mostly of the *Quercus* genus. Under current climate change scenarios, improving pasture quality and strengthening the system carbon fixation capacity is a challenge for future management of pastoral systems. These goals could be achieved by increasing the proportion of legumes in extensive native pastures. However, little information exists about the persistence of legumes, both native and introduced, in low-input silvopastoral systems, such as Iberian Dehesas. On the one hand, some authors suggest a positive influence of trees over pastures in terms of nutritional status (Cubera *et al.,* 2009) or biodiversity (Texeira, 2010). On the other hand, a negative effect of water competition with trees has been observed despite the physical separation of their root systems (Moreno *et al.,* 2005, 2013). Moreover, until now, no studies have addressed the selection of pasture legume species best adapted to oak shade. This paper provides the results on the abundance of legumes, plant biodiversity and soil carbon on 30 plots of seven Dehesas sown with legume-rich mixtures, in a 15-year chronosequence.

Materials and methods

Seven Dehesa farms in Extremadura (West of Spain) were selected where a mixture of forage legume seeds (20 kg seed ha^{-1}) had been sown in different years, following a chronosequence from 2002 to 2015. In each farm, 3-5 ages (years of sown) were identified besides a control plot (parcel that has never been sown). The mixture of *Rhizobium*-inoculated seeds provided by the FERTIPRADO company was composed of *Trifolium subterraneum* (61%) (different subspecies as *brachycalycinum* and *yaninnicum*) with other forage legumes: *Trifolium michelianum* var *balansae* (7%), *Trifolium vesiculosum* (3%), *Trifolium resupinatum* (6%), *Trifolium incarnatum* (8%), *Ornithopus sativus* (12%) and *Trifolium glanduliferum* (3%). Two microhabitats were clearly defined: beneath the oak canopy and open areas. In each plot, 12 metal cages (1×1 m) were installed to exclude grazing, half of them beneath the tree canopy and the rest beyond the canopy projection (>20 m distance). Pasture production and botanical composition were estimated by cutting 50×50 cm squares inside exclusion cages. This was complemented with botanical inventory transects in which one plant per metre was recorded randomly, thus obtaining 208 plants per plot (104 beyond canopy and 104 beneath canopy) in eight 25-m transects. In each plot, eight soil samples (0-15 cm depth), one per transect, were taken. Fieldwork was conducted in spring 2016.

Total C of soil samples (equivalent in these acid soils to organic C) was analysed using the Dumas Method in a DUMATHERM® Gerhardt analyser. Species richness of different plots was estimated according to Chao (2005) with the EstimateS software (Colwell, 2016). Mean values of species richness and soil carbon were compared among ages by GLMs, including farms as a random effect. Polynomial regressions were also used.

Results and discussion

The sowing of the legume-rich mixtures had a weak but significant effect on biodiversity (species richness) of Dehesa pastures. Species richness increased gradually from the beginning, with respect to that of the control unsown pastures. The maximum species richness was reached 6-8 years after sowing, and although species richness decreased with age later on, values similar to control plots were reached only 12-13 years after sowing (Figure 1). The pattern was similar beneath and out of the oak canopy. When referring to the performance of legumes, the increase of legume cover was noticeable, especially in the first years after sowing and, on average, one decade later the cover of legumes was still higher than in the control (Figure 2). The most common species were *T. subterraneum, O. compressus, T. michelianum, T. striatum* and *T. glomeratum*, which seem to have a high self-reseeding capacity in the Iberian Dehesas, characterized by a mosaic of shade imposed by scattered trees and moderate grazing pressure (0.5 Livestock Unit ha^{-1}).

Mean values of soil carbon confirmed, as expected, a positive and significant trend with age (P<0.001) resulting in higher contents of carbon accumulated into the soils with the matureness of sown areas (Figure 2). Some farms reached noticeable levels of soil C (results not shown), probably because of differences in management practices and the nature of the soil (e.g. soil texture). There were also significant differences (P<0.001) on soil C among the two studied habitats, under and beyond canopy with higher value in the latter, but the trend with the age was similar in both habitats (data not shown).

Conclusions

Pasture biodiversity increased temporarily by sowing the legume-rich mixture, a positive effect that lasted more than 10 years. The positive effect is also evidenced by a slight long-lasting increase of legume cover in the sown pastures. Apart from the introduced species, native species also increased, which could be explained by the improved soil conditions resulting from sown species and possibly from *Rhizobium* inoculation. The content of carbon accumulated into the soil showed a positive and significant tendency with age, and under and beyond the canopy, which enhances the importance of considering this kind of management practice to increase resilience of this silvopastoral system under the current climate change scenario.

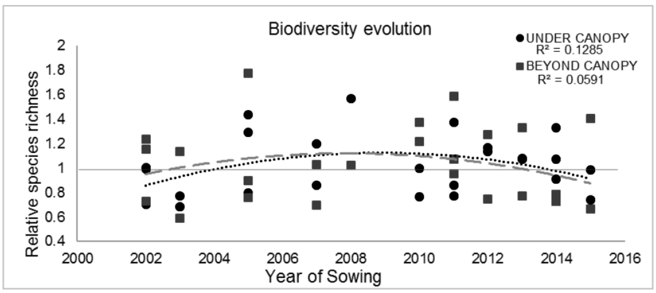

Figure 1. Evolution of relative species richness (Chao-estimated number of species) in the pastures along the chronosequence (relativized with respect to species richness of control plots). Value 1 on vertical axis indicates no change in species richness respect to control plots.

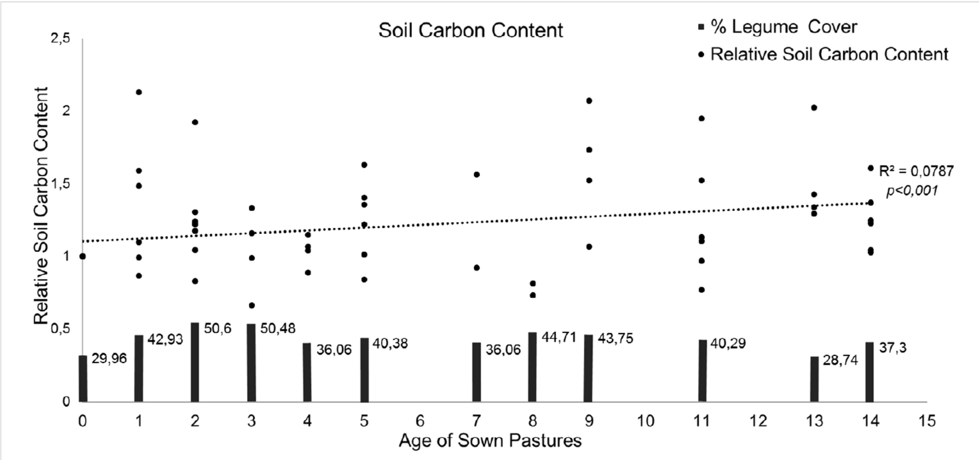

Figure 2. Evolution of soil C content (black dots and tendency line) along the chronosequence (values relativized with respect to soil C of control plots). Value 1 on vertical axis indicates no change in soil C with respect to control plots. Grey vertical bars show the cover of legumes for each of the ages analysed.

Acknowledgements

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Effects of season on nutritional value of Tedera

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Abstract

Tedera (*Bituminaria bituminosa* L.) plays an important role in the natural vegetation of coastal areas in Turkey and remains green throughout the summer period in northern areas of the country. This study was carried out with 42 genotypes in 2015. No fertilizer and water were applied during the study. Differences of some agronomic and quality properties and mineral contents in genotypes were investigated in January, April, July and November. Significant differences were observed for all aspects amongst the tested genotypes. The highest fresh yield was determined in July (267 g plant^{-1}) followed by harvests in April, January and November. The lowest ADF and NDF values were determined in the April and July harvests. Crude protein ratio reached the highest level (26.6%) in the April harvest and decreased gradually after this stage. Mineral contents of hay were significantly different among seasons and genotypes.

Keywords: yield, quality, season, minerals

Introduction

In Turkey, poor quality of feed is often observed in areas with hilly terrain and limited irrigation facilities, both during winter months when animals cannot graze, and in midsummer when pastures have largely dried up. In these conditions, planting a legume capable of maintaining its foliage during summer months on steep slopes and on stony, non-irrigated and low productive lands, would be desirable. Tedera (*Bituminaria bituminosa* L.) is considered as a very suitable forage and pasture plant for the above-described areas, because it remains green during summer when other species dry up (Gutman *et al.*, 2000), and maintains its growth during mild winters. During winter, spring, summer and autumn 2015, morphological and agronomic traits and nutrient contents of Tedera genotypes collected from the Central Black Sea Region of Turkey were studied.

Materials and methods

Seeds of different Tedera genotypes growing in the Middle Black Sea Region were collected in 2012. They were sown in a randomized block design with 70 cm inter- and intra-row spaces. Measurements and observations on 42 genotypes were performed on 15 January, April, July and November 2015 to represent winter, spring, summer and autumn productions. Morphological (plant height, leaflet length and width) and agronomic (fresh yield) traits were determined on 10 plants from each genotype. Three replicates per sample were analysed for crude protein (CP) ratio, acid detergent fibre (ADF), neutral detergent fibre (NDF) and some minerals (Ca, Mg, K, P) by using a Near Infrared Reflectance Spectroscopy (NIRS Foss 6500) device with IC-0904FE package program. All data obtained from this study were analysed with Fisher Test at 0.01 probability ($P \leq 0.01$) using SPSS 17.0 program.

Results and discussion

The mean values of all investigated traits of the genotypes showed significant differences among seasons (Table 1). Average plant height of the genotypes increased from January to July but, after harvesting in July, plant growth was limited and in November plants were very short. Although Tedera is resistant to dry and hot conditions, it is actually a cool season plant. Thus, it grows rapidly in spring and early summer periods. Plant height of Tedera increases in the growing stage and at blooming it ranges between 47 and 116 cm (Acar *et al.*, 2016).

Table1. Some traits of Tedera genotypes.[1]

Traits	Seasons			
	Winter	Spring	Summer	Autumn
Plant height (cm)	13.48 c	17.39 b	93.06 a	10.40 c
Fresh yield (g plant^{-1})	79.89 c	106.73 b	267.13 a	39.44 d
Leaflet width (mm)	26.71 b	30.95 a	20.23 d	24.45 c
Leaflet length (mm)	29.88 c	34.40 b	45.77 a	27.05 d
Crude protein ratio (%)	22.62 c	26.59 a	24.57 b	24.40 b
Acid detergent fibre (%)	19.19 a	18.02 b	18.42 b	18.41 b
Neutral detergent fibre (%)	31.40 a	28.71 c	27.74 d	29.59 b
Ca (%)	2.05 a	1.88 b	1.94 b	2.02 a
Mg (%)	0.41 c	0.38 d	0.60 a	0.44 b
K (%)	1.94 c	2.63 a	2.35 b	2.30 b
P (%)	0.31 d	0.40 b	0.41 a	0.35 c
Ca/P	6.85 a	4.72 c	4.76 c	5.88 b
K/(Ca+Mg)	0.80 c	1.17 a	0.94 b	0.95 b

[1] There are no differences among the values followed by the same letter in the same row.

The highest fresh yield (267 g plant^{-1}) was obtained in summer, at the end of the vegetative growing period; this was followed by the spring, winter and autumn values. The highest average CP ratio was 26.59% in spring and the lowest was 22.62% in winter. There were no differences between summer and autumn values. At the end of vegetative growth in spring, the fresh yield reached its highest value, but CP ratio decreased, which confirms the observations of Ventura *et al.* (2009) and Kumbasar (2015). Significant differences were observed for leaflet sizes of Tedera genotypes from winter to autumn. The highest leaflet width (30.95 mm) was determined in spring, and the highest leaflet length was measured in summer (45.77 mm). The highest ADF (19.19%) and NDF (31.40%) were found in winter for Tedera genotypes, the lowest NDF ratio (27.74%) was obtained in summer. Significant differences were found for NDF among all seasons. Conversely, there were no differences among seasons in terms of ADF ratio, except for the winter cut (Table 1). Plants seldom produce new leaves in winter. As the leaves get older, their ADF and NDF contents increase. Climatic conditions also affect the cellulose contents of plants (Kumbasar, 2015). ADF influences the digestibility rate of feeds and it should not be more than 30% for high quality feeds, while NDF ratio should not be more than 40% (Yavuz *et al.*, 2009). ADF and NDF ratios can be very different from season to season (Ventura *et al.*, 2009). Calcium content, should be at least 0.30% of DM in ruminant feed (Kidambi *et al.*, 1989); in leaves of Tedera Ca sharply decreased from winter (2.05%) to spring (1.88%) and then it started to increase again and reached values similar to winter level in autumn (2.02%) (Table 1). Ca is a structural item for plants. In the rapid growing period, an important amount of Ca is transferred to the new forming tissues from the old ones (Elinc, 2007). Gulumser and Acar (2012) and Kumbasar (2015) also found that Ca proportion of Tedera plants declines in spring and starts to increase in summer when plant growth is very slow. As regards K content, the highest value (2.63%) was found in spring, the lowest value (1.94%) in winter. There was no difference in K content in leaves between summer and autumn. K is a very mobile ion in the soil and there may be luxury uptake by plants (Elinc, 2007). Due to very high water and nutrition consumption in spring, K content of plants increased to high level. K proportion of Tedera plants was found as 2.6-3.3% by Ventura *et al.* (2009), 2.7% by Gulumser and Acar (2012). Kumbasar (2015) emphasized that K ratio of Tedera leaves was the highest (2.42%) in spring and after that it declined again. Magnesium content reached the highest level (0.60%) in summer and after this period it declined gradually until spring (0.38%). Feeds should contain 0.1-0.2% Mg for ruminants (Kidambi *et al.*, 1989), thus Mg content of the tested genotypes was enough for ruminants. Phosphorus concentration of Tedera leaves increased to the highest level in summer (0.41%) and after that it started to decline throughout autumn and winter and increased again in spring. In fact P is transported from old tissues to fresh leaves (Elinc, 2007). Similar P contents (0.36-0.41%) were found in other Tedera genotypes (Gulumser and Acar, 2012; Kumbasar, 2015). In ruminants, body Ca:P balance was 2:1, thus feeds should also have the same balance (Miller and Retz, 1995). In our experiment, Tedera Ca:P is above the desired value, so caution is required in feeding ruminants for a long time with these feeds to avoid milk fever (Acikgoz, 2001). The K/(Ca+Mg) ratio of feeds should be less than 2.2 for ruminants; if this ratio is more than 2.2 the risk for grass tetany increases (Ward, 1966).

Conclusions

If appropriate genotype/cultivars are chosen, Tedera has potential to supply fresh herbage throughout the year in non-irrigated lands of coastal area of Central Black Sea Region. Even if there has been seasonal fluctuations, leaves have high levels of protein and mineral substances and low ADF and NDF. In order to avoid milk fever, livestock should not be fed for long period only on this feed source due to its high Ca:P ratio.

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The quality of multicomponent grass swards for grazing on three soil types

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Abstract

Field trials were carried out to study the herbage quality of multi-species grass and legume-grass swards. A total of 12 mixed multicomponent swards were developed on sod-calcareous, sod-podzolic, and sod-stagnogley soils and were fertilized with P 78, K 90, and N 60 kg ha^{-1}. Dry matter (DM) was analysed for the following quality indices and the content of minerals: crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), net energy for lactation, digestibility *in vitro*, phosphorus, potassium, and calcium. The ratio of legumes and the legume species used in swards determined the herbage quality and the mineral content of DM yield. Grass-only mixtures achieved a higher average CP content (149 g kg^{-1}) on sod-stagnogley soil. Legume-grass mixtures achieved a higher average CP content (174 g kg^{-1}) on sod-calcareous soil. The results showed close positive correlation between the legume content in the sward and the Ca content in DM. A negative correlation was established between DM digestibility and the fibre fractions ADF and NDF.

Keywords: herbage quality, mineral content, grass mixture, soil types

Introduction

In Latvia's grassland farming, the use of grass-legume mixtures containing four to six species is traditional practice, because swards can secure good persistence and more stable productivity with high quality forage (Adamovics and Gutmane, 2016). Botanical composition of grassland is very important for forage quality. Legumes play an important role in high-quality herbage production from sown grasslands (Sarunaite *et al.*, 2012; Søegaard and Nielsen, 2012). Grass-mixture herbage is an important source of mineral elements for cattle feeding (Kuusela, 2006).

Materials and methods

Field trials were conducted at three experimental sites in Latvia on different soil types: sod-calcareous soil (pH_{KCl} 6.7; phosphorus (P) and potassium (K) level: 60 mg kg^{-1} and 144 mg kg^{-1}, respectively; organic matter: 24-28 g kg^{-1} of soil), sod-podzolic soils (pH_{KCl} 7.1, P and K level: 253 and 198 mg kg^{-1}, respectively; organic matter: 31 g kg^{-1}), and sod-stagnogley soil (pH_{KCl} 6.33; P and K level: 93 and 111 mg kg^{-1}, respectively; organic matter: 23 g kg^{-1}). At each of the three sites, the same 12 mixtures were sown in June 2014 without a cover crop, in three replications, with a plot size of 10 m^2. The multicomponent swards were composed of *Phleum pratensis, Dactylis glomerata, Lolium perenne, Lolium boucheanum, Festulolium, Festuca pratensis, Festuca arundinacea, Festuca rubra, Poa pratensis, Trifolium pratense, Trifolium repens,* and *Lotus corniculatus*, and grouped in four types: mixtures composed only of grasses (G); white clover (*T. repens*) and grass (Tr+G) mixtures; white clover, red clover (*T. pratense*) and grass (Tr+Tp+G) mixtures; bird's-foot trefoil (*L. corniculatus*) and grass (Lc+G) mixtures. Swards were cut four times during the vegetative season. The chemical composition of plants was determined by the following methods: dry matter (DM) – dried; crude protein (CP) by modified Kjeldahl; neutral detergent fibre (NDF) and acid detergent fibre (ADF) by Van Soest. Net energy for lactation (NEL) was calculated on the basis of the chemical composition of DM using digestibility coefficients and full-value coefficients. The mineral elements P, K, and Ca were analysed by atomic adsorption spectrometry. The experimental data were statistically analysed using analysis of variance (difference among means detected by least significant difference (LSD) at the 0.05 probability), correlation and regression analysis.

Results and discussion

The botanical composition of the sward essentially influences dry matter quality. Legume-grass mixtures achieved a higher CP and Ca content (174 g kg^{-1}) on sod-calcareous soil. Grass-only mixtures provided better forage quality on sod-stagnogley soil, a higher CP, P and K content in the DM, and a lower ADF and NDF content in the DM (Table 1).

The proportion of legumes in a sward is an important indicator for DM forage quality. The highest CP content, on average for the three soil types, was obtained for swards with the highest proportion of legumes. For the Tr+G swards (average legume content: 30.5%) and the Tr+Tp+G swards (legume content: 30.4%), the average CP content was 154 g kg^{-1} DM and 159 g kg^{-1} DM, respectively. For the Lc+G swards, which had a lower legume content (12.4%), the CP content (138 g kg^{-1} DM) was close to that of grass-only swards (132 g kg^{-1} DM). The proportion of legumes in swards showed a significant positive correlation with the content of CP and Ca in DM yield (Figure 1). Also, the proportion of legumes in swards had a significant (P<0.05) positive correlation with NEL (r=0.44) and DM digestibility (r=0.44). A significant (P<0.01) negative correlation was established between the

Table 1. Average quality of grass-only and grass-legume swards.[1]

Mixtures[2]	Soil	Content in DM, g kg^{-1}						NEL, MJ kg^{-1} DM	Digestibility, %
		CP	NDF	ADF	Ca	P	K		
G	Sod-podzolic	112.8	530.6	311.0	3.8	3.0	29.0	6.1	64.6
	Sod calcareous	132.9	594.0	345.6	3.5	2.6	34.9	5.9	62.0
	Sod stagnogley	149.4	498.5	287.1	3.8	3.7	39.6	6.3	66.5
Tr+G	Sod-podzolic	123.0	410.5	251.9	6.1	3.8	32.0	6.6	69.2
	Sod calcareous	172.6	439.5	272.6	7.3	2.0	41.6	6.4	67.6
	Sod stagnogley	166.8	397.9	249.1	5.3	4.4	41.7	6.6	69.5
Tr+Tp+G	Sod-podzolic	117.9	432.5	302.3	6.8	3.4	31.1	6.5	67.9
	Sod calcareous	190.8	432.1	261.3	8.8	2.2	41.8	6.5	68.5
	Sod stagnogley	168.7	391.4	243.2	5.7	4.4	41.9	6.7	69.9
Lc+G	Sod-podzolic	101.2	449.9	262.8	4.2	3.2	30.0	6.5	68.3
	Sod calcareous	159.9	460.9	277.7	5.9	2.4	41.8	6.4	67.3
	Sod stagnogley	154.2	392.5	231.8	4.6	4.3	41.5	6.8	70.8
Mean		149.4	440.8	270.9	5.9	3.3	37.6	6.5	68.0
$LSD_{0.05}$		15.32	42.18	29.32	1.43	0.58	2.31	0.17	

[1] DM = dry matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; NEL = net energy for lactation; LSD = least significant difference.
[2] G = grasses; Tr = white clover; Tp = red clover; Lc = bird's-foot trefoil.

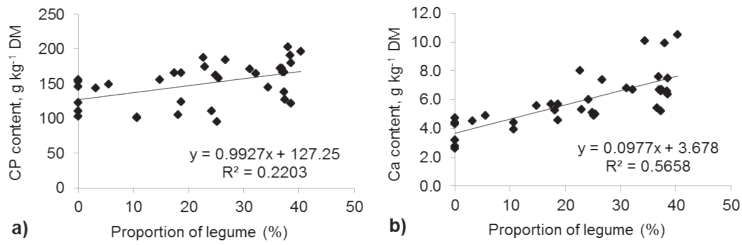

Figure 1. Relationship (P<0.01) between the proportion of legumes in the sward (average for cuts) and (A) crude protein (CP) content in herbage dry matter (DM), and (B) Ca content in herbage DM.

proportion of legumes in swards and the fibre fractions NDF (r=-0.59) and ADF (r=-0.44). The crude protein content in the DM yield had a significant (P<0.01) positive correlation with the content of K and Ca in the DM yield (Figure 2). A significant (P<0.01) negative correlation was established between the DM digestibility and the fibre fractions NDF (r=-0.96) and ADF (r=-0.80). Also, a significant (P<0.01) negative correlation was established between the NEL and the fibre fractions NDF (r=-0.96) and ADF (r=-0.80). A significant (P<0.05) negative correlation (r=-0.43) was established between the contents of Ca and NDF in herbage DM.

Conclusions

Grass-only mixtures achieved a higher average CP content on sod-stagnogley soil, but for legume-grass mixtures this was achieved on sod-calcareous soil. The use of legume-containing swards can contribute to better forage dry matter quality, higher CP content and NEL and DM digestibility, and a lower content of fibre fractions NDF and ADF. Legume-containing mixtures for forage may optimize Ca content, thus improving the quality of forages.

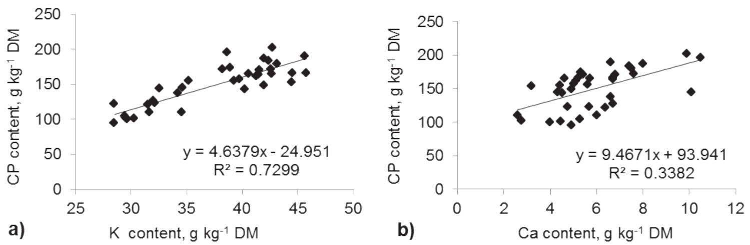

Figure 2. Relationship (P<0.01) between crude protein (CP) content in the herbage dry matter (DM) (average for cuts) and (A) K content in herbage DM, and (B), Ca content in herbage DM.

Acknowledgements

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The influence of nitrogen fertilisation on the yields and quality of multicomponent sown meadows

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Abstract

Field trials were carried out to study continuous green forage production from grass and legume-grass swards receiving three nitrogen fertilisation rates: N0, N60, and N120. A total of 12 mixed multicomponent swards were developed at three sites on sod-calcareous, sod-podzolic, and sod-stagnogley soils. The swards were cut three times during the growing season. The legumes in mixtures with grasses of various growth patterns provided continuous green forage production during the whole summer season. The botanical composition and the fertilisation affected the average productivity of the swards (11.35-25.76 Mg ha^{-1} of dry matter) and the crude protein content. The positive effect of nitrogen fertiliser on sward productivity was better expressed in legume-grass mixtures on sod-stagnogley soils and in grass-only swards on sod-podzolic soils.

Keywords: fertilisation, multispecies mixture, soil type, yield, crude protein

Introduction

The increasing role of sustainable grassland-based ruminant systems in Europe highlights the use of sown multi-species swards and stresses the need for developing comprehensive studies on the influence of different grassland management strategies in different local conditions (Peyraud *et al.*, 2014). In Latvia's farms, the use of multicomponent grass-legume mixtures is a traditional practice because of their better persistence and more stable productivity (Adamovics *et al.*, 2006). The addition of legumes in the mixtures contributes to the reduction of N fertilisation and to the improvement of forage quality (Tomić *et al.*, 2012). Legumes allow improved sustainability and stability of agroecosystems, and provide the cheapest source of nitrogen (Wilkins and Vidrih, 2000). The objective of this research was to determine the influence of nitrogen fertilisation on the performance of multicomponent swards for the production of herbage in a cutting system under the agroclimatic and edaphic conditions of Latvia.

Materials and methods

Field trials were conducted at three experimental sites in Latvia on different soil types: sod-calcareous soils (pH_{KCl} 6.7, phosphorus 60 mg kg^{-1}, potassium 144 mg kg^{-1}, and organic matter 24–28 g kg^{-1} of soil), sod-podzolic soils (pH 7.1, phosphorus 253 mg kg^{-1}, potassium 198 mg kg^{-1}, and organic matter 31 g kg^{-1}), and sod-stagnogley soil (pH 6.33, the phosphorus 93 mg kg^{-1}, potassium 111 mg kg^{-1}, and organic matter 23 g kg^{-1}).

Twelve mixtures based on *Phleum pratensis, Dactylis glomerata, Festuca pratensis, Festuca arundinacea, Festuca rubra, Festulolium, Lolium perenne, Lolium boucheanum, Trifolium pratense, Medicago sativa*, and *Galega orientalis* were used in all experimental sites. Mixtures were sown in June 2014, without a cover crop, in three replications with a plot size of 10 m^2. Mixtures were grouped in four sward types: only grasses (G); lucerne (*Medicago sativa*) and grass mixtures (Ms+G); red clover (*Trifolium pratense*) and grass mixtures (Tp+G); and galega (*Galega orientalis*) and grass mixtures (Go+G). The following fertilisation treatments were used for all mixtures: P78, K90 and N0, $N60_{(30+30)}$ and $N120_{(60+60)}$ kg ha^{-1}. Swards were cut three times during the vegetation season. The botanical composition (legumes, grasses and herbs) and the yields were determined after each cutting. Crude protein (CP) was determined by modified Kjeldahl and the neutral detergent fibre (NDF) and acid detergent fibre (ADF) by Van

Soest. The metabolisable energy was calculated on the basis of the chemical composition of DM using digestibility coefficients and full-value coefficients by Van Soest (1980).

The experimental data were statistically analysed using the three-way analysis of variance with 'mixture', 'fertiliser' and 'soil' as factors; the difference among means was detected by least significant difference (LSD) at the 0.05 probability level.

Results and discussion

The highest average DM yields were achieved on sod-podzolic soils (19.4 Mg ha^{-1}). The mixtures containing red clover (Tp+G) provided higher average DM yields (20.29 Mg ha^{-1}) on all soil types (Table 1). The influence of the levels of fertilisation on the increase in DM yields was closely connected to the botanical composition of swards. The N-fertilizer rate increase from 0 to 120 kg ha^{-1} contributed to a significant average DM yield increase of 6.11 Mg ha^{-1} for grass-only and of 4.47 Mg ha^{-1} for Go+G mixture swards on all soil types. Galega developed slowly in the first year after sowing; therefore, the increased in yield of the Go+G mixture was likely explained by the high grass proportion in the swards. For the Ms+G swards, the average increase in DM yield was less expressed – 2.27 Mg ha^{-1}. The application of N fertiliser did not provide a significant DM yield increase in the Tp+G swards.

For all legume-containing mixtures, N application negatively affected the proportion of legumes in the sward compared with that in unfertilised plots (Figure 1). CP content is the main determinant of forage quality. The N-fertilizer rate increase contributed to an average CP content increase by 18.3 g kg^{-1} DM. The CP content increase was better expressed in the only-grasses mixtures (on average by 18.9 g kg^{-1} DM). In legume-grass mixtures CP content increased, on average by 17.7 g kg^{-1} DM. Significant influences of N fertiliser on the NDF and ADF content in herbage dry matter, the DM digestibility, and net energy for lactation were not detected.

Table 1. Average dry matter yields of grass-only and grass-legume swards (Mg ha^{-1}).

Soil (F_C)	Mixtures (F_A)	N rate, kg ha^{-1} (F_B)			
		N0	N60	N120	Mean
Sod-stagnogley	G	9.04	11.34	14.59	11.66
	Ms+G	12.53	13.97	16.75	14.42
	Tp+G	15.92	16.45	17.44	16.60
	Go+G	10.15	12.79	14.96	12.63
	Mean	11.91	13.64	15.93	13.83
Sod-podzolic	G	12.60	16.40	19.46	16.15
	Ms+G	20.30	22.55	22.89	21.91
	Tp+G	24.28	23.71	24.41	24.13
	Go+G	14.72	16.12	17.71	16.18
	Mean	17.97	19.69	21.11	19.49
Sod-calcareous	G	8.48	12.69	14.41	11.86
	Ms+G	17.08	16.87	18.45	17.47
	Tp+G	19.98	19.49	20.97	20.14
	Go+G	9.50	12.22	15.09	12.27
	Mean	13.76	15.32	17.23	15.43

$LSD_{0.05}$ F_A= 0.78; F_B= 0.39; F_C=0.39; F_{AB}= 1.35; F_{AC}= 1.35; F_{BC}= 0.67

[1] G = grasses; Ms = lucerne; Tp = red clover, Go = galega; LSD = least significant difference.

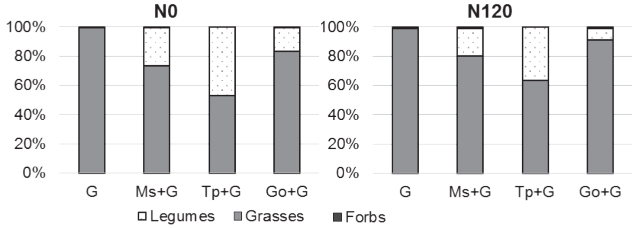

Figure 1. Proportions of the groups of herbage species (%) applying two fertilization rates (on average for cuts and three soil types). G = grasses; Ms = lucerne; Tp = red clover; Go = galega.

Conclusions

The use of legume-containing swards can contribute to the reduction in N fertilisation. The mixtures containing red clover provided higher average DM yields on all soil types. The application of N fertilizer contributed to decrease in the proportion of legumes in swards. The highest average DM yields were achieved on sod-podzolic soils.

Acknowledgements

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Breeding, design and assessment of annual and perennial legume-based forage crops

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Abstract

This study aimed to compare 25 annual or perennial forage crops grown for two years in a rainfed environment of north-west Morocco on the basis of performance data and acceptability by farmers in a participatory assessment. The crops included elite cultivars, or new germplasm developed by CREA-FLC, of pea, common vetch, Narbon vetch, oat, triticale (as annuals) and alfalfa, tall fescue and cocksfoot (as perennials) grown as pure stands, and annual legume-cereal or alfalfa-grass binary or complex mixtures. On average, annual crops outperformed perennials for dry matter yield (6.35 vs 5.14 Mg ha^{-1}) and ability to control weeds (0.03 vs 0.26 weed proportion). However, the yield of a perennial complex mixture was similar to the best annual crops, with outstanding proportion of legume biomass. On average, legume proportion in mixtures was high in both annuals (0.40) and perennials (0.53). The advantage of mixed cropping over pure stands was greater for perennials than annuals in terms of Land Equivalent Ratio (1.31 vs 1.03). Pea in mixture with oat was the only crop capable of combining high values of forage yield, legume content and acceptability by farmers. Alfalfa in pure stand exhibited greater farmers' acceptability than alfalfa-grass mixtures despite its somewhat lower forage yield.

Keywords: forage crops, ideotype, legumes, mixtures, land equivalent ratio

Introduction

Crop-livestock systems in drought-prone areas of the Mediterranean basin are challenged by over-exploitation of natural grassland and insufficiency of high-quality feedstuff (FAO, 2010). Legume-based mixtures have potential interest to increase crop forage yield and/or quality through resource complementarity of different species (e.g. Hauggaard-Nielsen and Jensen, 2001), but their actual performance and acceptability by farmers need to be assessed. More generally, breeding and design of innovative forage crops face several key questions, such as: (1) prioritizing annual or perennial crops? (2) growing legume pure stands or mixtures of legumes (with cereals or grasses)? (3) growing binary or complex mixtures? (4) which ideotype to breed for, with regard to plant morphology, e.g. tall or semi-dwarf habit in pea (Annicchiarico *et al.*, 2012), or upright or semi-erect alfalfa (Annicchiarico and Pecetti, 2010)? This study aimed to provide preliminary responses to these questions with respect to rainfed cropping in North-West Morocco, taking account of forage dry-matter yield (DMY), legume content, competitiveness to weeds, Land Equivalent Ratio (LER; Mead and Willey, 1980) and crop acceptability by farmers in a repeated participatory assessment of a large set of crops.

Materials and methods

The experiment included 16 annual and 9 perennial forage crops grown in 2013-2014 and 2014-2015 at Marchouch, Morocco (33°33' N, 6°41' W) in plots of 4×3 m in a randomized complete block design with four replications. The crop composition, inclusive of pure and mixed stands of annual legumes, cereals, alfalfa and grasses, is given in Table 1. Crop varieties were elite materials chosen following results of Annicchiarico *et al.* (2011; 2013), Annicchiarico and Iannucci (2008) and indications by breeders. A tall and a semi-dwarf pea line, and an upright (adapted to mowing) and a semi-erect (adapted also to grazing) alfalfa population, were evaluated as possible plant model alternatives. Perennials were sown in autumn 2013, whereas annuals were sown in autumn 2013 and 2014 in different nearby fields after a wheat crop. Aerial biomass was harvested in spring (at late heading/early milky stage for cereals, waxy stage for grain legumes, and flowering for perennial legumes and grasses) and, only for perennials in one year, also in autumn. Forage dry-matter yield, and legume and weed proportions, were determined on samples oven-dried at 65 °C. Farmers' assessment was performed on a plot basis by over 20 local farmers that visited the trial each spring before harvesting, who valued each crop on the basis of a 1 (very low) to 5 (very high) visual score. An analysis of variance was performed for all variables (including LER values and farmers' mean score per plot).

Results and discussion

On average, annuals exhibited higher DMY (6.35 vs 5.14 Mg ha^{-1}) and farmers' score (3.77 vs 3.49), and lower weed proportion (0.03 vs 0.26), in comparison with perennials (P<0.05). Legume proportion was rather high for both annual and perennial crop mixtures (0.40 and 0.53, respectively). Overall, binary mixtures out-yielded both legume and non-legume pure stands (6.18 vs 5.28 and 5.43 Mg ha^{-1}, respectively). The advantage of mixed cropping over pure stands was definitively greater for perennials than annuals in terms of LER (1.31 vs 1.03), partly because of the very poor yield response of grass pure stands (Table 1). LER values of perennials were always greater than unity, reaching 1.67 in the complex mixture. In annuals, the highest LER values occurred in binary or complex mixtures including Narbon vetch, whose pure stand exhibited modest yield (Table 1).

Pea in mixture with oat was the only crop capable of combining high values of forage yield, legume content and acceptability by farmers (Table 1). In addition, pea was the only annual legume that tended to combine high forage yield and farmers' acceptability also in pure stand The two pea types exhibited similar DMY, with a slight trend for the tall type (P2) towards somewhat higher legume content in mixtures than the semi-dwarf line (P1) that confirmed earlier findings (Annicchiarico *et al.*, 2012). Cereal pure stands were high-yielding, but were poorly appreciated by farmers as forage crops (Table 1) because of lower expected forage quality and protein content. On average, binary mixtures with oat, compared with those with triticale, displayed higher yield (7.14 vs 5.97 Mg ha^{-1}), lower legume content (0.33 vs 0.47), and somewhat greater farmer appreciation (4.26 vs 3.81). In perennials, the complex mixture was the only crop that achieved yield and legume content comparable with those of the best annuals (Table 1). However, alfalfa in pure stand exhibited greater farmers' acceptability than any alfalfa-grass mixture, despite its somewhat lower yield. The crops including the erect, adapted alfalfa cultivar (L1) tended towards higher yield than the semi-prostrate material (L2) both in pure stand and in mixture, and its mixtures showed markedly higher legume content, in agreement with the greater competitive ability of this plant type in Annicchiarico and Pecetti (2010). The potential advantage of L2 when adopting a mowing-grazing exploitation received just a modest appreciation by farmers, who ranked L1 and L2 likewise in pure and mixed stand (Table 1). The only difference between grass companions was some yield advantage of mixtures including tall fescue.

Conclusions

Our results can help design and breed forage crops for drought-prone Mediterranean regions. They revealed outstanding forage yielding ability and farmers' interest for pea-based crops, particularly pea-oat binary mixtures and, to a lower extent, pea monocultures. Compared with the best legume-based annual crops, legume-based perennials were lower yielding with the exception of the complex mixture (whose yield advantage over binary mixtures was manifest), and less appreciated by farmers with the exception of alfalfa monocultures. Plant ideotypes did not display large differences, but some advantage emerged for taller stature of pea grown in mixture, and erect habit of alfalfa in pure or mixed stand under the adopted mowing regime.

Table 1. Total forage dry-matter yield (DMY) of sown species, legume and weed proportion on DMY, land equivalent ratio (LER) and farmers' acceptability score, for a set of annual and perennial forage crops grown for two seasons in north-west Morocco.

Cycle	Type[1]	Crop[2]	DMY crop ($t\ ha^{-1}$)	Weed proportion	Legume proportion	LER	Farmers' score (1-5)
Annual	BM	P2-O	7.67	0.02	0.45	1.08	4.44
	Cereal PS	O	7.44	0.06	(0)	-	3.28
	BM	V-O	7.22	0.01	0.21	1.07	4.15
	Cereal PS	T	7.05	0.02	(0)	-	3.70
	BM	P1-O	6.93	0.03	0.37	0.98	4.57
	BM	N-O	6.75	0.04	0.28	1.11	3.87
	CM	P1-P2-O-T	6.69	0.02	0.48	0.95	3.39
	CM	N-V-O-T	6.64	0.04	0.33	1.15	3.91
	Legume PS	P1	6.43	0.02	(1)	-	4.63
	Legume PS	P2	6.41	0.02	(1)	-	3.96
	BM	P1-T	6.26	0.04	0.44	0.91	4.16
	BM	N-T	5.98	0.03	0.46	1.17	3.44
	BM	P2-T	5.97	0.03	0.49	0.86	4.10
	BM	V-T	5.67	0.03	0.50	1.03	3.54
	Legume PS	V	4.29	0.04	(1)	-	3.06
	Legume PS	N	4.21	0.06	(1)	-	3.92
Perennial	CM	L1-L2-F-C	7.05	0.26	0.63	1.67	3.62
	BM	L1-F	5.85	0.25	0.66	1.15	3.40
	Legume PS	L1	5.65	0.24	(1)	-	4.19
	BM	L1-C	5.56	0.26	0.69	1.08	3.51
	BM	L2-F	5.33	0.25	0.27	1.42	3.59
	BM	L2-C	4.94	0.28	0.39	1.24	3.33
	Legume PS	L2	4.70	0.30	(1)	-	4.18
	Grass PS	C	3.69	0.25	(0)	-	2.53
	Grass PS	F	3.55	0.23	(0)	-	2.89
		LSD $_{(P<0.05)}$	1.10	0.06	0.16	0.40	0.38

[1] BM = binary mixture; CM = complex mixture (four components); PS = pure stand; LER = land equivalent ratio.

[2] C = cocksfoot, cv. Kasbah; F = tall fescue, cv. Flecha; L1 = upright alfalfa, cv. Mamuntanas; L2 = semi-erect alfalfa, new population; N = Narbon vetch, cv. Bozdag; O = oat, cv. Genziana; P1 = semi-dwarf semi-leafless pea, cv. Kaspa; P2 = tall semi-leafless pea, new line; T = triticale, cv. Vivaciò; V = common vetch, cv. Barril.

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How does longer flooding affect forage value and biodiversity value in Atlantic marshlands?

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Abstract

A 5-year-long *in situ* experiment was conducted to evaluate the costs and benefits for biodiversity and for forage production of an extended flooding period during spring in wet grasslands. The results showed that biodiversity benefitted from the extended flooding period, while a detrimental effect on forage production was recorded only locally. It was shown that any detrimental effect of flooding may be alleviated by sufficient grazing intensity.

Keywords: flooding duration, agri-environmental schemes

Introduction

Flooding represents a stress for plant species (e.g. Bartelheimer *et al.*, 2010) and their response to the variations of hydrological conditions has been well studied (Blom and Voesenek, 1996; Insausti *et al.*, 2001). In contrast, only a few studies have documented plant communities' responses (Donath *et al.*, 2004; Toogood and Joyce, 2009; Violle *et al.*, 2010) and how plant community composition and diversity change according to flooding duration remain largely unexplored. Filling this knowledge gap appears a priority, as longer flooding is a widespread management tool for wet grassland with the aim of improving their environmental value. The present study was accordingly designed to investigate the response of plant communities to an increase in flooding duration in spring. Focussing on two vegetation types, we investigated the changes in their floristic composition, diversity and forage production in response to flooding changes. We tested two hypotheses expressed by local stakeholders: (1) increasing flooding period from the late winter to spring is favourable for biodiversity; (2) longer flooding is deleterious for the forage production and quality.

Materials and methods

The marshlands studied are part of the Cotentin region, Normandy, France. They constitute large areas of wet grasslands, always flooded during winter, and either grazed or mown. In the 'marais des Mottes', five sites were chosen and submitted to a longer flooding period from 1998 onwards, created by managed limitation of water discharge after the winter period. They are all grazed by cattle and horses, with a similar extensive stocking rate. The extended duration of flooding varied from few weeks to 2 months depending on the rainfall pattern of the year. Two vegetation types were present: low marsh type on organic-rich soils (sites 2 and 4) and hygrophilous vegetation type (sites 1, 3 and 5). Flooding duration was longer in the former than in the latter. Vegetation composition was surveyed in late May, June and July in all five sites every year, on 10 quadrats of 50×50 cm, distributed randomly within a homogenous area. Standing total biomass and litter were sampled from ten 25×25 cm quadrats, dried at 60 °C for 48 h, then weighed. Forage quality was measured by *in vitro* digestibility (%), which represents the proportion of the biomass that can be digestible by the herbivore by enzymatic processes. The analysis was run with three replicates per site.

Results

During the five years of the experiment, the plant species richness and its diversity increased (Figure 1), mainly because of a greater evenness in the species abundance (Figure 2). The effect of the extended

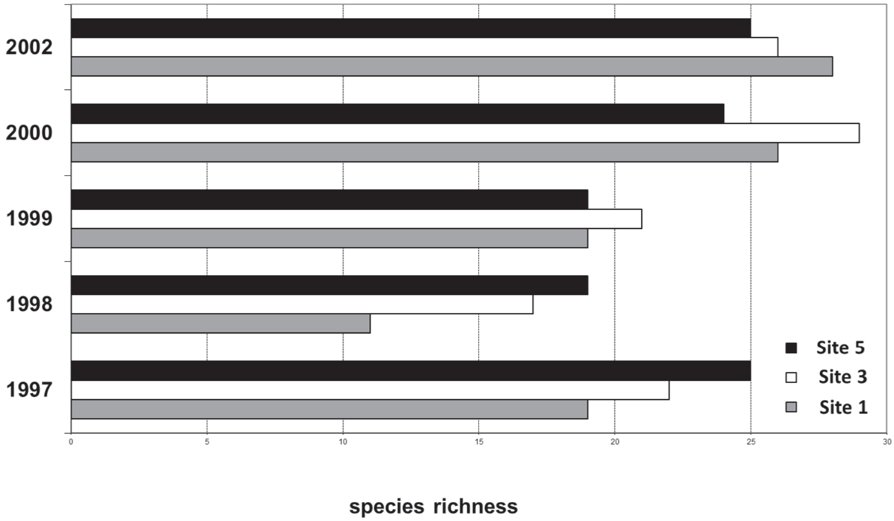

Figure 1. Species richness of the hygrophilous vegetation in three sites studied from 1997 to 2002. The first year of survey corresponds to the onset of the experimental management.

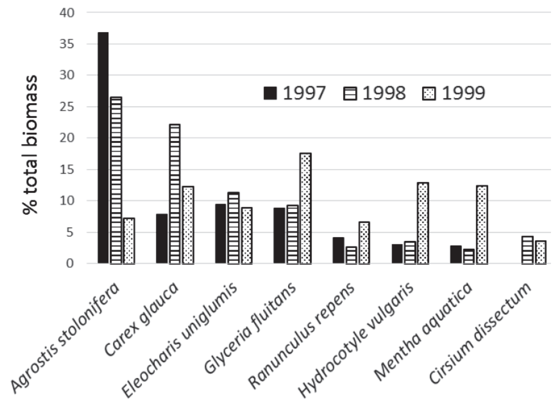

Figure 2. Variation of the species relative abundance of the most abundant species in the hygrophilous vegetation type (site 5), from 1997 to 1999, with the change of flooding pattern.

flooding management was also favourable for fauna as it corresponds to an increase in pike number (*Esox lucius*) as well as in the number of typical marshlands birds (data not shown).

For hygrophilous vegetation, the higher water level in spring and slow water discharge from the marsh resulted in an increased of forage production (Figure 3). By contrast, the forage production of low marsh vegetation type was either not modified (site 4) or reduced (site 2, Figure 3). This decrease in production was only observed under very light grazing. In such light grazing, extended flooding was found propitious for stoloniferous species (*Agrostis stolonifera* and *Glyceria fluitans)* to establish a thick 'mat' of roots and stolons at the water surface. This mat was deposited on the soil surface after water discharge in spring, and limited the recruitment of other plant species and regrowth in spring. The experimental management reduced the range of variation for forage quality: sites varying from 40 to 80% in 1998 and from 50 to 72% after 3 years of extended flooding period.

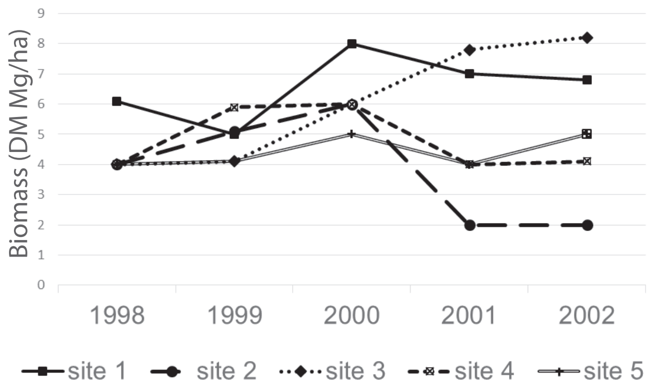

Figure 3. Forage production (Mg dry matter (DM) ha^{-1}) for the 5 studied grasslands over 5 years.

Discussion and conclusions

Longer flooding was favourable for biodiversity and vegetation evenness. By contrast, forage production and quality remained stable or increased following the extended flooding period. These good agronomic results were unexpected by local stakeholders. The absence of detrimental effects from longer flooding gives confidence and support for agri-environmental compatibility, within the range of flooding change that was tested. The fact that rainfall varied markedly from one year to another and led to variability in flooding duration has also to be taken into consideration as this may have promoted biodiversity (Bonis *et al.* 1995).

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Developing result-based agri-environmental payment schemes (RBAPS) for biodiversity conservation in Ireland and Spain

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Abstract

The Results-Based Agri-Environment Pilot Scheme (RBAPS) is a 4-year pilot, which is developing and testing result-based payment schemes for several biodiversity outputs primarily associated with grasslands in the High Nature Value farmland of Ireland and Spain. The selected biodiversity outputs are species-rich grasslands and Marsh Fritillary grassland habitat in County Leitrim, Ireland, breeding waders and flooded hay meadow in the Shannon Callows, Ireland and mosaic habitat in arable and perennial cropland typical of the Mediterranean region of Navarra, Spain. Results-based agri-environment schemes link payment levels to the quality of the environment and biodiversity produced on the farm. Higher payments go to farmers producing higher quality environmental goods such as plants, wildlife and clean water. This paper presents the identification and selection of robust result indicators for the biodiversity outputs, which are readily understood and measured by farmers, advisors and regulators. Monitoring systems aimed at evaluating achievement of RBAPS objectives. Preliminary analysis of first year monitoring data is also presented, which indicates correlations between the quality of biodiversity output and the RBAPS scoring systems.

Keywords: RBAPS, agri-environment scheme, HNV, agriculture

Introduction

Reviews of prescription-centred agri-environment schemes (AES) provide a mixed picture of efficiency, value for money and positive environmental impacts (e.g. Kleijn and Sutherland, 2003). Deliberations on CAP post-2020 reforms are underway; however, future agricultural supports under Pillar 2 (or a single integrated Pillar) may require demonstration of optimal environmental outcomes for monies invested and enhanced spatial targeting of supports (European Union, 2016). Result-based AES offer objective measures of environmental-goods quality through linkage of higher payments to farmers producing higher quality goods. This paper summarises development of the result indicators for the selected biodiversity outputs in Year 1 (2015) of the project and land-parcel scoring and monitoring results from Year 2 (2016) assessments.

Materials and methods

Results-Based Agri-Environment Pilot Scheme (RBAPS) is working in two contrasting Irish high nature value (HNV) regions: the dual SPA and SAC Natura 2000 site of the Shannon Callows and the largely-undesignated lowland areas of County Leitrim. The upland zone of Navarra's Mediterranean region supports a mosaic of cropland, and rough grazing which offer a variety of ecological niches for wildlife. The chosen biodiversity outputs (Table 1) reflect legislative requirements and conservation priorities as well as the dependence of the biodiversity output on extensive agricultural practices.

Table 1. Species and habitats trialled under the result-based agri-environment scheme (RBAPS) in Ireland and Spain.

Biodiversity output	Leitrim	Shannon Callows	Navarra, Spain
Species (habitat condition)	Marsh Fritillary	Lapwing, Curlew Snipe, Redshank; Whinchat & Curlew on flood meadows	Numerous Annex bird, reptile and mammal species
Habitat	Species rich grasslands	Species rich flood meadow	Traditional mosaic of vineyards, olive & almond groves

Potential result indicators have been developed for each output during an exploratory phase in Year 1, during which a range of ecological parameters which could act as proxies for general environmental condition and target species and habitat specific indicators were tested – largely based on the Burren Farming for Conservation system (Parr *et al.,* 2010), where quality is assessed on a score from 0 (very low) to 10 (very high). The 224 land parcels under RBAPS contract in 2016 were assessed using the relevant scoring systems. The 52 farmers in the scheme have also been afforded advice by the project team on how to achieve optimal conditions (including supports for non-productive investments in the Shannon Callows and Navarra) for the biodiversity output their land is best suited to deliver.

Monitoring of specific flora and fauna groups is being undertaken in Years 2 and 3 to examine correlation of the result indicators with quality of the target biodiversity outputs produced and scheme impact on wider biodiversity. Monitoring groups include plant richness/abundance (relevés), bird richness/abundance (counts) and insect richness/biomass (pitfall traps/counts). Output specific monitoring is undertaken for Marsh Fritillary grassland (larval web counts) and for breeding wader habitat and Whinchat/Curlew on Callow flood meadows (counts, growth stage of chicks and as well as other indicators of breeding success). As data is still being processed and analysed (using a variety of statistical methods), it is possible to give only tentative conclusions on certain monitoring aspects at this stage.

Results and discussion

Multi-functional result indicators, appropriate for the biodiversity output and reflective of quality of farm management (Table 2) have been identified. Where appropriate, indicators have been standardized across output measures and species or habitat specific result indicators added, resulting in a common framework approach to RBAPS scoring assessments.

Across all 224 land parcels assessed, the average score was 6.7 out of 10, but the range of scores varied with the biodiversity outputs. For instance, a narrow range of high scores was awarded to Marsh Fritillary grassland, reflecting the high quality habitat associated with this butterfly, and also for breeding wader

Table 2. Example of assessment indicators developed for selected habitats and species (biodiversity outputs) for results-based schemes in Ireland and Spain

Biodiversity output	Indicators assessed
Species-rich grasslands	*Common indicators*: number & cover of positive indicators; cover of negative indicators; plant litter level; scrub encroachment; damaging activities; impacts of supplementary feeding
	Species-rich grassland only: vegetation structure
	Marsh Fritillary grassland only: habitat structure suitability for Marsh Fritillary
	Flood meadows only: density of meadowsweet (indicates sub-optimal management)
Breeding waders	Sward height in breeding & late season; tussock cover; rush cover; scrub cover (predator habitat); presence of wet features
Mosaic habitat	Extent, diversity and period of herbaceous cover; evidence of traditional grazing practices and quality of traditional boundaries; rough grazing areas; structures valuable for wildlife

habitat which is supported by non-productive investments to enhance habitat condition for these species. A wider range of scores was achieved for Navarra cropland, species rich grassland and flood meadows, reflecting differing farm practices and current and past management.

Preliminary analysis of monitoring results indicates significant positive correlation between the RBAPS score and measures of biodiversity output. Some indicators are more highly correlated than others. The number and cover of positive plant species along with indicators of habitat suitability are highly weighted in the species rich grassland scoring system as they are considered to represent, and are not easily changed. Results analysed to date indicate strong correlations with total plant richness in parcels and the number of positive plant indicators in County Leitrim, Callow flood meadows and Navarra cropland. Low correlations appear to exist between some assessment indicators and the target biodiversity, such as indicators for damaging activities. This is to be expected as they are not reflecting the biodiversity output *per se*, but act as threat assessments highlighting potential concerns for the continued sustainable delivery of the biodiversity output. Data exploration and analysis will continue and will be used to inform potential revisions to scoring assessments and to the monitoring design in Year 3 of the scheme.

Conclusions

The current project is trialling at local to European level the versatility, efficacy and value of result-based approaches to conservation through agriculture in a range of other natural, agronomic and regulatory conditions. RBAPS specifically requires more engagement and understanding of the targets and greater participation of farmers than prescription based agri-environment schemes. It provides an adaptive approach to quality assessment of agriculturally delivered ecosystem services and can be a targeted support mechanism for HNV farmland and extensive farming systems.

Acknowledgements

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Is phosphorus a limiting factor for the productivity of Campos grasslands?

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Abstract

The *Campos* are natural grasslands covering 350,000 km^2 across Uruguay and southern Brazil, and mainly used for animal production in extensive grazing systems. These grasslands are characterized by soils with low phosphorus (P) availability and limited water storage capacity. Therefore, climatic variability and nutrient deficits are considered to be major limitations to their aerial net primary productivity (ANPP). To evaluate this phosphorus limitation, we (1) compiled ANPP results from 109 paired P fertilizer vs control plots, from experiments carried out throughout Uruguay between 1970 and 2015, and (2) analysed nitrogen-to-phosphorus (N:P) ratio in more than 250 herbage samples collected between 1990 and 2002 from *Campos* distributed across Uruguay. These two databases span different soil types and years with variable precipitation: ANPP of control plots ranged from 400 to 6,000 kg dry matter ha^{-1} y^{-1}. P fertilization generally increased ANPP, but the magnitude was low and stable across the gradient of ANPP (mean: 450 kg DM $ha^{-1}y^{-1}$). No differences were detected either between soil types or fertilizer sources. Almost all herbage samples had N:P ratio lower than 16 (92% had N:P lower than 14). Together, these results suggest that P deficiency is a minor determinant of annual ANPP of *Campos* grasslands in Uruguay.

Keywords: natural grasslands, campo natural, phosphorus limitation, ANPP, N:P ratio

Introduction

Campos grassland is an extensive ecosystem spread over a wide variety of soils in Uruguay and southern Brazil (Allen *et al.*, 2011), characterized by low fertility and limited water storage capacity. These natural grasslands, a hotspot of biodiversity with more than 3,000 species of temperate and subtropical plants, are mainly used for animal production in extensive grazing systems, and provide several ecosystem services (Modernel *et al.*, 2016). Climatic variability is considered to be the main limitations for productivity, and nutrient deficiencies are also widespread (Berretta *et al.*, 2000). Average available soil P (Bray-Kurtz), for instance, is typically lower than 5 ppm (Hernández *et al.*, 1995), which would indicate that aerial net primary productivity (ANPP) may be limited by P deficiency. The N:P ratio of plant biomass could be used as an indicator of the relative limitation of N, P or both. A N:P ratio less than 10-15 identifies a N-relative limitation, while a ratio more than 20 to 25 indicates a clear P relative limitation in grassland (Güsewell, 2004). The aim of this study is to evaluate the hypothesis that available soil P is one of the major limitations of ANPP of *Campos* grasslands.

Materials and methods

We compiled ANPP from 109 short- and long-term experiments with paired P-fertilized vs control treatments, carried out in Uruguay between 1970 and 2015. This dataset included 15 soils of different origin from 3 pedologic regions (Basaltic, East and Sedimentary), P rates from 6 to 140 kg P ha^{-1} y^{-1}, different fertilizer sources and interannual variable precipitation. Further, we compiled a separate dataset of nitrogen-to-phosphorus (N:P) ratio in more than 250 herbage samples collected between 1990 and 2002 from well managed to overgrazed (degraded) *Campos*. Statistical analyses were performed with Infostat (Di Rienzo *et al.*, 2015).

Results and discussion

ANPP of control plots varied greatly, from 400 to 6,000 kg dry matter (DM) $ha^{-1}y^{-1}$, mainly due to interannual variability of precipitation. Increases in ANPP on P-fertilized plots were relatively significant (P<0.05), but little and stable across the gradient of ANPP and soil types: on average 443 kg DM ha^{-1} y^{-1} (Figure 1). Annual or cumulative P rate used as fertilizer had no effect on forage yield production. No significant difference was also detected between more soluble vs more insoluble P fertilizer sources (482 and 422 kg DM ha^{-1} y^{-1}, respectively).

The N:P ratio data further support the idea of little P deficiency in these grasslands: more than 90% of herbage samples had N:P ratios lower than 14, and less than 5% of the samples had N:P ratios higher than 16 (Figure 2). This suggests a more important N limitation than P limitation in these grasslands (Güsewell, 2004).

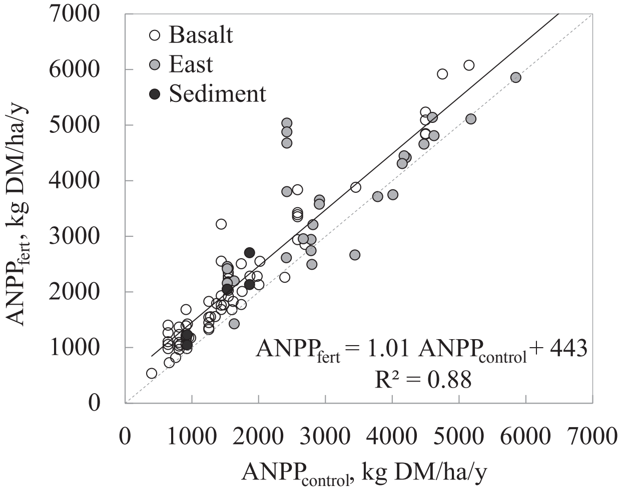

Figure 1. Relationship between ANPP of control and P-fertilized plots. White, grey and black symbols indicate sites from Basaltic, East, and Sedimentary pedologic regions.

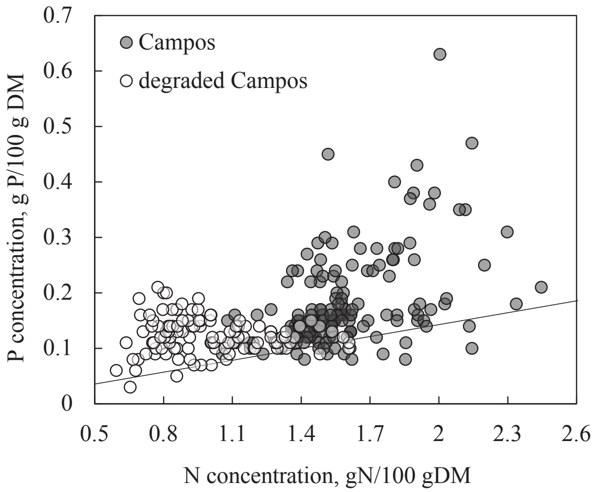

Figure 2. Relationship between N and P concentration (on a % of dry matter basis), for *Campos* grasslands. The line indicates the N:P ratio = 14.

Conclusions

In spite of low available soil P levels, as indicated by available P, primary productivity of Uruguayan *Campos* grassland appears not to be largely limited by P availability.

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Comparison of different doses and forms of selenium on its content in aboveground mass of cocksfoot (*Dactylis glomerata* L.)

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Abstract

Selenium (Se) is an essential element that significantly influences the health status of animals and humans. Its adequate intake may affect the health, production and reproduction of livestock. The aim of this study was to determine the effect of foliar applications of selenium in different forms and doses on the content of selenium in the forage of cocksfoot (*Dactylis glomerata* L., cv. Dana). The experiment took place in a climate chamber. For foliar application comparisons, solutions of selenium at doses of 2 and 4 mg m^{-2} of Se (as selenate sodium or selenite sodium) were used. After the applications, samples of aboveground mass of each group were sampled at regular 14-day intervals. Samples were analysed by atomic absorption spectrometry. After successful foliar application of selenium, a significant (P<0.05) increase of selenium content in aboveground mass of cocksfoot was found. No difference was found between selenite and selenate after application of the lower doses (2 mg m^{-2}). In comparison with selenite, selenate showed significantly (P<0.05) higher efficiency of accumulation of selenium (dose 4 mg m^{-2}) in the aboveground mass. The lower doses of selenium were also effective and pose a lower risk than higher doses of selenium.

Keywords: cocksfoot, foliar application, selenium, aboveground mass

Introduction

Selenium (Se) is an essential element significantly influencing health status of animals and humans. The insufficient supply of this element to organisms leads to many disorders. Conversely, higher intake can be toxic (Rahman *et al.*, 2015). Selenium as a part of selenoproteins regulates the antioxidant system and thus prevents the oxidative destruction of biological membranes and prevents the damage to the body by heavy metals. As a result of the involvement of selenium compounds in numerous biological functions, its deficit impairs overall health status of animals: it increases the susceptibility of juveniles to infectious diseases, causes reproductive disorders or may be the direct cause of an illness (Horky, 2014). The selenium concentration in plant biomass is derived from its content in the soil and may vary considerably depending on the region (Guerrero *et al.*, 2014). The amount of selenium deposited in animal products depends on the content this element in the feed (Meyer *et al.*, 2014). Cocksfoot (*Dactylis glomerata* L.) is a perennial grass which provides high yield of the good quality aboveground mass. It has wide ecological amplitude and it is an early species. The forage is grazed on pastures or used to produce hay or silage (Skladanka *et al.*, 2014). The aim of this study was to determine the effect of foliar application of selenium in different forms and doses on the content of selenium in the forage.

Materials and methods

The experiment used cocksfoot, cv. Dana, and 5 g of seed was planted into soil in each prepared pot. Subsequently, the pots were stored in a climate chamber and remained there throughout the experiment. In the climate chamber, daily temperature was set at 24 °C and 20 °C overnight, with 65% of humidity, and the length of day light lasted 12 h (light intensity of 380 $\mu mol \cdot m^{-1} \cdot s^{-1}$). The selenium was applied in two forms, as sodium selenate or sodium selenite, on the leaves in the concentrations as 2 or 4 mg m^{-2}. The control samples were not affected by selenium during the whole experiment. The leaves from selenium-affected and non-affected plants were sampled at the 14th, 28th and 42nd day after application of the target substances. The experimental groups were divided into (A) selenate and (B) selenite. Selenium was analysed by atomic absorption spectroscopy with a continuous source of radiation with high resolution ContrAA 700 (ANALYTIK JENA, Germany, 2012). The effect of selenium on plant health was evaluated based on the yield of above ground mass. Statistical significances of the differences between content of selenium, were determined using STATISTICA.CZ. Differences with $P<0.05$ were considered significant and were determined by using of multifactor way ANOVA test, which was applied for means comparison.

Results and discussion

After treatment, the selenium content was significantly (P<0.05) increased in all periods of sampling in the experimental groups. In the comparison with the control group, both forms (selenate and selenite) and doses (2 and 4 mg m^{-2}) of selenium increased Se content in the aboveground mass. After application of selenium (dose 2 mg m^{-2}) no statistically significant difference between forms of selenium was observed. On the other hand, after using the dose of 4 mg m^{-2}, a higher (P<0.05) content of selenium was found after application of selenate then selenite (Figure 1). Uptake of selenium in the form of selenate was significantly higher, which corresponds to the findings of other authors (Shand *et al.*, 1992). Hu *et al.* (2010) also confirmed that foliar application increases the selenium content in forage of alfalfa. Higher doses of selenium than this may have a negative effect on health status of plants. In this case, none of these signs was observed even when using the higher doses of selenium.

Conclusions

The foliar application of selenium proved to be a very effective method to increase selenium content in the aboveground mass of cocksfoot. After successful foliar application of selenium, there was significant (P<0.05) increase in the selenium content in aboveground mass of cocksfoot. Despite the use of higher selenium doses, no toxic effects on plants were observed. Adequate content of selenium in forage can contribute to health of plants, livestock and subsequently people.

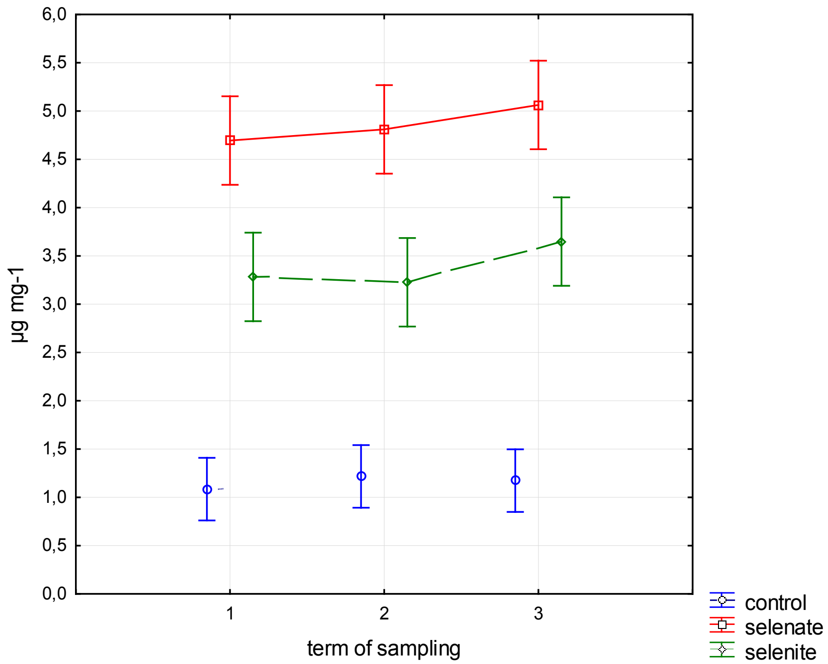

Figure 1. Content of selenium in aboveground mass after application dose 4 mg m^{-2} of selenium.

Acknowledgements

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Factors affecting grazing in protected wetlands of North-East Poland

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Abstract

Extensive grazing is one of the tools for protecting nature valuable grasslands. With this perspective, the Polish Primitive Horses Breeding Centre, in the Biebrza National Park in northeastern Poland, has promoted, since 2004, Koniks grazing on wetlands. In a previous study, *Carex panicea* L., *Molinia caerulea* (L.) Moench, *Carex flava* L. and *Agrostis canina* L. were the species most often grazed by Koniks. The aim of this study was to evaluate Koniks' preferences on the basis of chemical composition of the plant species most often grazed by horses. In 2009 and 2010 chemical analyses were conducted on the above-mentioned species collected from the most frequented areas grazed by Konik horses. Among them sedges (*Carex* species) are characterized by having the highest digestibility and fibre fractions. Preferences of the animals towards grass species in June may result from significantly higher content of minerals in *Agrostis canina* and of NDF in *Molinia caerulea*. As the vegetative season progresses, with reduced quality of plant species, digestibility of fodder increases in importance for the Konik horses and sedges become grazed more often.

Keywords: Konik horse, grazing selectivity, *Carex panicea*

Introduction

Extensive grazing is one of the tools that may be used effectively in the protection of nature valuable grasslands, particularly when located in marginal areas. Among domestic animals, cattle and horses are widely used in Europe, especially in wetlands (e.g. Menard *et al.,* 2002; Vulink, 2001). The primitive animal breeds seem to be especially suited to this because they can adapt to very difficult environmental conditions. For this reason, Konik horses – the native polish breed of horses and the descendants of Tarpan – are kept in different protected areas, not only in Poland but also in other European countries like Holland, France or Belgium (Cosyns *et al.,* 2001).

Grazing animals are characterized by showing variable selectivity towards the species available at pasture (e.g. Cosyns *et al.*, 2001). Their dietary choices depend on a number of factors including floristic composition and structure of the sward, and chemical composition (contents of nutrients or toxic compounds) of species. Horses are regarded as grazers, not browsers, and prefer mainly monocotyledonous species. In the diet of Koniks grazed in the Biebrza National Park, among the available species, *Carex panicea* L., *Molinia caerulea* (L.) Moench, *Carex flava* L. and *Agrostis canina* L. (Chodkiewicz and Stypiński, 2011) had the highest share. Even though sedges (*Carex* species) dominated among the plants that were bitten and they were abundant in the sward, they were generally avoided by horses in summer months, although both grasses and sedges were selected but only in June. The aim of this study was to evaluate how the selectivity of Konik horses towards the four above-mentioned species depends on their chemical composition.

Materials and methods

The study was conducted in the Middle Basin of Biebrza National Park located in north-eastern Poland. In 2010, samples (about 300 g of fresh mass) of the above ground parts of *Carex panicea*, *Molinia caerulea*, *Carex flava,* and *Agrostis canina* (species eaten by horses) were collected in two different periods (late June and late August) from two vegetation communities. One community was dominated by *Carex panicea* and one had *Molinia caerulea* and *Potentilla erecta* (L.) Raeusch). Samples were collected in the areas more intensively grazed by horses (Chodkiewicz and Stypiński, 2010). In total four samples of each species were gathered each month. After oven drying (24 h at 60 °C) and grinding, nitrogen analyses were performed using Kjeldahl method. The content of crude protein (CP), ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), organic and dry matter digestibility (OMD and DMD), water soluble carbohydrates (WSC), and cellulose were determined by near infrared spectroscopy (NIRS) using available calibration for dried grasses and sedges (INGLOT). The content of Ca, Cu, K, Mg, Mn, Na, P and Zn was analysed by atomic absorption spectrometry (ASS). The data were analysed by using mean and least significant differences (LSD) computed with restricted maximum likelihood function (REML) in SAS 9.3.

Results and discussion

Among the four analysed species, sedges (*Carex* sp.) are characterized by better digestibility of organic matter as well as dry matter, whereas grasses had the highest percentage of fibre fraction, about 2-4% on average (Table 1).

The palatability of plant species for horses is positively correlated with their organic matter digestibility and the content of simple carbohydrates, but negatively with cellulose and lignin (e.g. Vulink, 2001), which can also explain why sedges are grazed in this study. Although their chemical composition is quite similar, *Carex flava* had lower OMD and DMD, which most probably explains their lower preference by Konik horses. *Molinia caerulea* may be harmful for grazing animals; however, Vulink (2001) reported its exploitation by Konik horses. This species was characterized by the worst feeding value relative to other species in the study. As compared to both sedges, *M. caerulea* differs because of a higher NDF content, which is important for the proper functioning of horse digestive system. Probably, as suggested by Vulink (2001), Konik horses preferred *M. caerulea* in June in order to fulfil their requirements of that compound in diet.

The nutritional value of all species decreases over time, and in this experiment there was a lowering of digestibilityWSC content and increasing NDF and CP. In August, the reduction of feeding value of *M. caerulea*, in connection with the poor content of minerals (especially its Ca and Mg content was significantly lower than in sedges and *A. canina*; Table 2) may be the reason for decreased exploitation of this species by animals. *Agrostis canina* is a small species, whose grazing requires a high energetic expenditure for Konik horses. The high share of this grass in the horse diet probably results from larger

Table 1. Chemical composition of the species grazed by Koniks [% of DM].[1,2]

Species	Period	Ash	CP	NDF	ADF	ADL	Cellulose	WSC	OMD	DMD
Agrostis canina	June	7.33cd	33.21c	56.99c	35.33cd	4.56ab	30.77bcd	10.93cd	43.45bc	42.98b
	August	10.25e	27.91a	58.51c	37.48de	5.47c	32.01cd	5.19a	37.38a	35.47a
Molinia caerulea	June	5.18ab	33.06c	57.49c	34.93bc	4.58ab	30.35bcd	10.94bcd	43.93bc	43.90bc
	August	4.76a	35.85d	62.04d	38.53e	5.42c	33.11d	7.31ab	34.39a	33.79a
Carex panicea	June	6.89cd	28.04a	48.35a	30.74a	4.03a	26.71a	13.55d	54.33e	55.06e
	August	6.29bc	31.16bc	53.79b	34.70bc	5.03bc	29.67abc	8.56bc	46.92cd	47.53cd
Carex flava	June	7.66d	30.04ab	50.43a	32.90ab	4.41ab	28.49ab	12.13d	48.85d	49.96d
	August	6.55bcd	31.75bc	54.04b	36.25cde	5.37c	30.88bcd	8.76bcd	42.30b	43.91bc
LSD		0.88	1.21	1.43	1.19	0.33	1.53	1.67	2.30	2.06

[1] Letters identify statistical differences of a given variable within a column. LSD = least significant difference.

[2] DM = dry matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; OMD = organic matter digestibility; DMD = dry matter digestibility; WSC = water soluble carbohydrates.

Table 2. Content of the minerals in the four species grazed by Konik horses.[1]

Spiecies	Period	Na ($g\ kg^{-1}$)	P ($g\ kg^{-1}$)	K ($g\ kg^{-1}$)	Mg ($g\ kg^{-1}$)	Ca ($g\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)	N ($g\ kg^{-1}$)
Agrostis canina	June	0.32bc	1.52a	15.30c	1.52b	3.06b	99.82a	23.22a	5.31c
	August	0.42c	1.91a	11.23b	2.95c	5.51e	345.94b	50.49c	4.46a
Molinia caerulea	June	0.09a	1.72a	10.18ab	0.98a	1.89a	64.38a	23.97a	5.29c
	August	0.06a	1.04a	7.43a	1.81b	2.95abc	99.35a	39.06b	5.74d
Carex panicea	June	0.16ab	1.26a	12.35b	1.77b	4.06bcd	87.17a	19.84a	4.49a
	August	0.19ab	1.34a	11.47b	2.87c	4.65de	165.17a	33.31b	4.98bc
Carex flava	June	0.16ab	1.42a	10.91ab	1.63b	3.33bc	78.23a	16.08a	4.81ab
	August	0.16ab	1.26a	7.92a	2.93c	4.22cd	138.05a	23.69a	5.08bc
LSD		0,12	0.51	1.58	0.24	0.59	48.89	4.35	0.19

[1] Letters identify statistical differences of a given variable within a column. LSD = least significant difference.

content of some minerals, especially Na, K in June and Zn, Mn in August, in comparison with the other species.

Conclusions

The grasses preferred by Konik horses are characterized by having worse digestibility, compared with the sedges. However, these grasses may play a role in complementing the intake of mineral components. As the vegetative season progresses, and with reduced quality of plant species, digestibility of fodder increases in importance for the Konik horses and sedges become grazed more often. In the light of these results, farmers should consider complementing the diet of Konik horses with appropriate mineral salts.

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Soil properties, yield and quality of permanent grasslands in Bačka region, Vojvodina (Serbia)

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Abstract

The northern part of Serbia (Vojvodina Province) is very diversified in terms of soil types and agricultural use of the land, including areas with arable land, natural grasslands, forests, and sandy terrains. In this research, nine permanent grassland sites were selected in this province in the region of Bačka. The analyses included soil analysis, assessment of floristic composition, dry matter yield and grassland forage quality. Regarding the soil properties, pH values varied from neutral to highly alkaline. The soil in all locations had a high content of nitrogen, low to very high content of phosphorus and optimum to very high content of potassium. The selected grasslands mainly consist of grasses, legumes and other herbaceous species. Dry matter yield of the studied permanent grasslands varied from 1.95 Mg ha^{-1} (Bečej 1) to 3.60 Mg ha^{-1} (Bečej 2), while the percent of crude protein was around 10%. Although most of these soils are not appropriate for crop production, with adequate agricultural measures (timely cutting and basic fertilization) improving the yield and quality of these permanent grasslands becomes possible.

Keywords: floristic composition, permanent grassland, quality, soil properties, yield

Introduction

According to their characteristics, natural grasslands in Vojvodina are typical lowland grasslands that have developed on different types of soil unfavourable for crop production (Eric *et al.*, 2007). The grasslands cover approximately 200,000 ha, of which two-thirds are pastures. As a result of the local pedo-climatic conditions, extensive production and the absence of adequate agro-technology, the productivity of these natural grasslands is low. While the yield of temporary meadows averages 2.0-2.5 t ha^{-1}, that of pastures is only 0.5-1.0 t ha^{-1} of hay of poor quality. The climate in Vojvodina Province, in terms of annual rainfall and its distribution, is generally not suitable for high forage production. The beginning of the growing season is characterized by a sufficient amount of precipitation, with a peak in May and June. However, a semi-arid period from mid-July to late September negatively affects sward growth (Ćupina *et al.*, 2011). The objective of this paper is to describe the natural grasslands in Bačka region by assessing the soil properties, sward floristic composition, yield and forage quality.

Materials and methods

During the research carried out in 2009, nine permanent grassland sites were selected in the region of Bačka, Vojvodina Province (Titel, Novi Sad, Kula, Crvenka, Sivac, Bačko Gradište, Bečej 1, Bečej 2 and Srbobran). The analyses included soils, assessment of floristic composition, dry matter yield (t ha^{-1}) and quality of the selected sites. The soil properties were analysed on the basis of an average value of the collected samples. Yields were measured in 5 m^2 plots (one replicate). Dry matter yield was obtained by drying samples (1 kg each) to a constant mass at 70°C. Samples for dry matter yield and chemical analyses were taken from the first cut at the end of May. The chemical analyses included assessment of crude protein (CP), crude fibre (CF), crude fat (CFat), ash, nitrogen, phosphorus and potassium, while the content of nitrogen-free extract matter (NFEM) was calculated. Floristic composition was determined according to Šoštarić-Pisačić and Kovačević (1974), but only for groups of species (grasses, legumes, other herbaceous species).

Results and discussion

Soil properties

The reaction of the soil solution ranged from 6.70 (Bečej 1) to 8.20 (Kula); i.e. at the majority of the studied sites pH ranged from slightly acid to alkaline (Table 1). Bearing in mind the requirements of grass in relation to the reaction of the soil solution, pH value in some locations is a limiting factor for growth and development of certain species. The majority of the studied soils were slightly carbonic, with high humus content, which is typical for natural grasslands. All locations showed low to very high content of phosphorus and optimum to very high content of potassium. A high content of P_2O_5 and K_2O in the locations of Titel and Srbobran can be accounted for by permanent presence of cattle on the grasslands, which affects soil quality.

Floristic composition

Grasses accounted for more than 50% of the grasslands at the majority of sites, with a significant presence of legumes and a smaller presence of other species (10-27%) (Table 2). The grassland nearby Novi Sad consisted of 44% grasses, with a significant share of legumes (34%), which makes this grassland favourable for forage production.

Dry matter yield and quality

An average yield of dry matter at the studied sites was 2.01 t ha^{-1}. The lowest yield of dry matter was registered in the locality of Titel (1.91 t ha^{-1}), while the largest was in the locality of Bečej 2 (3.60 t ha^{-1}). The forage quality of dry matter depends primarily on the floristic composition of grasslands, so it varied depending on the studied location (Table 3). The grasslands were characterized by low contents of crude protein (average 9.33%) and a high proportion of crude fibre (average 26.24%), as a result of the extensive use of the land or lack of agronomic management practices, especially fertilization. In

Table 1. Soil properties of permanent grasslands in Bačka region, Vojvodina Province.

Locality	pH-value		$CaCO_3$ (%)	Humus (%)	Total N (%)	Al-P_2O_5 mg 100 g^{-1}	Al-K_2O mg 100 g^{-1}
	KCl	H_2O					
Titel	7.31	8.17	5.14	4.22	0.28	61.0	25.50
Novi Sad	7.02	7.58	8.00	4.21	0.29	8.50	18.50
Kula	8.20	9.10	15.57	7.15	0.47	7.88	39.47
Crvenka	8.20	9.10	18.57	5.83	0.38	12.05	23.44
Sivac	7.90	8.40	20.80	5.69	0.37	21.87	25.68
B. Gradište	7.60	8.10	2.27	6.02	0.40	5.86	24.04
Bečej 1	6.70	7.70	0.71	5.01	0.33	5.81	16.00
Bečej 2	7.10	7.80	0.85	4.62	0.30	17.53	26.06
Srbobran	8.12	7.39	10.98	4.30	0.22	38.87	30.12

Table 2. Floristic composition (relative abundance) of permanent grasslands in Bačka region, Vojvodina Province.

Plant species	Locality								
	Novi Sad	Titel	Kula	Crvenka	Sivac	B. Gradište	Bečej 1	Bečej 2	Srbobran
Grasses	44	60	54	49	59	53	60	58	58
Legumes	34	24	24	24	23	22	21	32	19
Other herbaceous species	22	16	22	27	18	25	19	10	23
Total	100	100	100	100	100	100	100	100	100

Table 3. Dry matter (DM) yield and quality of permanent grasslands in Bačka region, Vojvodina Province.[1]

Locality	DM yield (t ha^{-1})	Quality (% of DM)							
		CP	CF	Cfat	Ash	NFEM	N	P_2O_5	K_2O
Titel	1.91	9.39	20.05	1.49	11.06	43.20	1.79	0.29	1.23
Novi Sad	2.40	10.86	26.21	1.48	12.65	47.56	1.89	0.30	1.53
Kula	2.65	10.35	27.01	1.45	12.98	47.00	1.59	0.36	1.58
Crvenka	2.31	10.23	28.23	1.56	12.97	46.23	1.61	0.40	1.70
Sivac	2.46	10.01	27.65	1.65	12.84	45.98	1.71	0.42	1.89
B. Gradište	1.98	9.89	28.00	1.59	13.00	47.20	1.65	0.49	1.97
Bečej 1	1.95	9.90	27.63	1.61	13.12	46.95	1.56	0.60	1.69
Bečej 2	3.60	10.65	26.32	1.46	12.99	47.23	1.69	0.74	1.71
Srbobran	3.50	12.9	25.82	1.47	12.72	46.92	1.98	0.73	1.75
Average	2.53	10.46	26.32	1.53	12.70	46.47	1.72	0.48	1.67

[1] CP = crude protein; CF = crude fibre; Cfat = crude fat; NFEM = nitrogen-free extract matter.

addition, since summer months are predominantly dry with high temperatures and unfavourable for the plant development (May-September), the grasslands of Bačka could provide an additional source of feed for livestock.

Conclusions

According to the floristic composition, yield and quality of the analysed permanent grasslands it is notable that they have good potential for forage production. Permanent grassland properties have shown differences between localities, giving the advantage to the area of Bečej 2 with high yield and quality. Even though this paper is not based on scientific research, its results are of significance in that they represent the state of grasslands on marginal lands which are important feed source in dominant extensive farming systems in the West Balkan Countries.

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Social valuation of ecosystem services provided by livestock farming in the Italian Alps

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Abstract

This study shows the preliminary results of work using the framework of ecosystem services to value the main market and nonmarket functions of dairy cattle farming systems in the eastern Italian Alps (Autonomous Province of Trento). We used qualitative methods (face-to-face and on-line interviews of stakeholders in the study area) to identify the social perception of the positive and negative externalities of the dairy livestock sector in the study area. In general, stakeholders perceived more positive aspects associated to the livestock sector rather than negative ones. Producing high quality food and maintaining a beautiful landscape attractive to tourists were considered the most important farming functions. These results stress the attention to the need for holistic policies that could address the development of rural areas from a more sustainable point of view, considering stakeholders' perceptions and farmers' objectives. This study is the basis for a socio-economic analysis that will apply a choice model to evaluate the ecosystem services related to the mountain agroecosystem.

Keywords: dairy cattle, landscape, quality products, grasslands, mountain

Introduction

Traditional alpine dairy livestock farms are grassland-based systems, which rely on valley meadows for forage production and they practise transhumance in summer to highland pastures to integrate the forage supply (Sturaro *et al.*, 2013). Due to the abandonment that mountain areas are experiencing the use of these farming practices, which are fundamental for shaping the mountain landscape, is decreasing (MacDonald *et al.*, 2000; Otero-Rozas *et al.*, 2014) and this influences the supply of ecosystem services (ES). This paper shows the preliminary results of a study that aims to evaluate, from a socio-economic point of view, the ES of a mountain agroecosystem. Hereafter we show the perception that different stakeholders have on positive and negative impacts of the mountain dairy cattle systems.

Materials and methods

The study area was in Trento Province (north-eastern Italian Alps). This mountainous territory extends for 6,200 km^2 with an elevation ranging from 66 to 3,769 m a.s.l. Almost 80% of the Utilized Agricultural Area (UAA) is represented by meadows and pastures (ISTAT, 2010). Pastures are usually located above 1,500 m a.s.l. Agriculture in the province is devoted to dairy cattle livestock farming, with more than 1000 active dairy farms (ISTAT, 2010) strongly connected with cooperative dairy factories and the production of specific quality cheeses. Almost all the permanent dairy farms still practise transhumance, moving their animals in summer farms to highland pastures for forage supply (Sturaro *et al.*, 2013). The study used a non-random sampling with a questionnaire directed to four categories of stakeholders in the Trento province. Face-to-face interviews were carried out with 47 farmers and 15 stakeholders working in the tourism industry, whereas on-line interviews were designed for 15 technicians of the local farmer federation and 15 public servants of governmental agencies. We referred to 47 farmers as 'farming stakeholders', whereas the other 45 stakeholders were grouped as 'nonfarming stakeholders'. They expressed their level of agreement on a list of positive and negative impacts of mountain dairy cattle systems using a 5-point Likert scale. We analysed the data with a Kruskal-Wallis test (PROC

UNIVARIATE, SAS 9.3; SAS Institute, 2011) comparing two stakeholder categories: farmers and other stakeholders. The positive aspects were assigned to the different categories of ES described by TEEB (2010).

Results and discussion

Results of the study are shown in Table 1. According to all stakeholders, dairy cattle systems generally have only positive functions and no negative aspects. However, regarding water pollution, a significant difference between the two stakeholder categories was found. Non-farming stakeholders gave a neutral response, suggesting that they recognized the problem, but they did not consider the livestock system to be the only cause. Despite a significant difference, all stakeholders were not worried about animal welfare.

Regarding the positive aspects, farmers assigned the highest scores. They appreciated all the functions related to the maintenance of a beautiful natural landscape, without grassland encroachment, that can easily attract tourists. A well-managed mountain territory presents a high level of heterogeneity, with patches of pastures and meadows that interrupt the continuity of the forest. A heterogeneous landscape, besides being more attractive to society (Daugstad *et al.*, 2006), provides numerous ES and better sustains biodiversity (MacDonald *et al.*, 2000). Therefore, the confidence expressed by stakeholders agreeing on other positive aspects (the maintenance of soil fertility, of a suitable habitat for wild animals, of high biodiversity and of traditional cultural landscapes) was well justified. Moreover, farmers assigned a

Table 1. Median of agreement and disagreement on negative and positive aspects of the mountain dairy cattle system as expressed by farmers and non-farming stakeholders.[1]

	Farmers			Other stakeholders			*P*-value[2]
	Median	Min	Max	Median	Min	Max	
Positive aspects							
Control grassland encroachment	5	2	5	4	2	5	0.2961
Maintain beautiful natural landscapes	5	2	5	4	2	5	0.0420
Maintain traditional cultural landscapes	4	1	5	4	2	5	0.9166
Maintain a high biodiversity	4	2	5	4	1	5	0.1579
Maintain a suitable habitat for wild animals	4	1	5	3	2	5	0.0732
Maintain soil fertility	4	1	5	4	1	5	0.1979
Prevent soil erosion	4	2	5	3	1	5	0.0052
Prevent avalanches risk	4	1	5	3	1	5	0.0046
Maintain cultural heritage	4	1	5	4	1	5	0.0554
Maintain tourism attractiveness	5	3	5	5	2	5	0.1372
Produce high quality food	5	4	5	4	2	5	0.0495
Negative aspects							
Pollution of water	2	1	4	3	1	5	0.0057
Emission of greenhouse gas	2	1	4	2	1	4	0.3225
No respect for animal welfare	1	1	3	2	1	5	0.0608
Contamination of soil	2	1	4	2	1	3	0.5764
Compaction/erosion of the soil	2	1	4	2	1	3	0.9299
Contaminate the air (bad smell)	2	1	5	2	1	5	0.2434
Produce low quality food	1	1	3	1	1	5	0.6348
Cause loss of natural vegetation	1	1	4	1	1	4	0.5398
Cause loss of natural landscapes	1	1	5	1	1	4	0.9488

[1] Scores: 1: strongly disagree; 2: disagree; 3: neutral; 4: agree; 5: strongly agree.

[2] Probability levels of significance at Kruskal-Wallis test.

higher score to the production of high quality food. Characteristics of the production systems, such as preservation of the environment or cultural heritage, are often associated with the local or traditional food production (Bernués *et al.*, 2003). Therefore, these goods can play a key role in the maintenance of specific practices and processing favourable to the grassland maintenance, such as the use of forage and transhumance to highland pastures. On the other hand, aspects as the maintenance of a cultural heritage and the prevention of soil erosion found non-farming stakeholders with a neutral opinion. Moreover, they did not think that farming can be involved with the prevention of avalanche risk. Differences in the perceptions of functions related to the environment depend on the local ecological knowledge of the stakeholders' category (Oteros-Rozas *et al.*, 2014). The definition of the local perception of the ES delivered by the mountain dairy cattle systems will allow us to perform a survey-based stated preference method to rank and economically value the most important ES identified. The selected ES were 'Landscape' (Cultural ES: maintain traditional, cultural landscape attractiveness for tourists), 'Biodiversity' (Supporting ES: preserve biodiversity, control forest growth), 'Water quality' (Regulating ES: not compromise water quality), and 'Quality products linked to the territory' (Provisioning ES: produce high quality food, maintain tourism attraction).

Conclusions

The first part of the study showed that people living in the study area do not consider the dairy cattle farming system as negative for the environment and instead they highly appreciate the positive aspects of the system. Once completed, the results of the choice model will help us to discuss some implications for agri-environmental policy design in this province.

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Foraging behaviour of cattle grazing alone or mixed with goats on partially improved heathlands

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Abstract

This study aimed to evaluate diet selection and grazing behaviour of beef suckler cows (Asturiana de los Valles breed) managed alone (twenty cows) or mixed (ten cows with seventy-five Cashmere goats) on heathlands associated with improved grassland areas (25% of total area). Grazing behaviour was determined by recording the grazing activity during daylight on each vegetation type in May and July, whereas diet selection was estimated using alkane markers in May and October. Cattle managed alone spent less daily time grazing than under mixed grazing (503 vs 614 min day^{-1}). Goats grazed for longer time (734 min day^{-1}). Cows spent most of their grazing time on grasslands in both seasons (92% on average). By contrast, goats concentrated their grazing time on heathland (75%) in May, whereas an opposite trend was found in July (25%). Results on diet composition were consistent with direct observation, showing that cattle diet was almost exclusively composed of herbaceous plants in both seasons (96% on average for both grazing managements). Goats incorporated significant quantities of shrubs on their diets (about 29%), with greater preference for heather species over gorse.

Keywords: grazing behaviour, diet selection, mixed grazing, cattle, goats

Introduction

Heathlands constitute the dominant plant community in the north-west of the Iberian Peninsula and their very low nutritive value does not allow the establishment of viable animal production systems. As a consequence, rural abandonment, woody plant invasions and occurrence of wildfires have been observed in these areas. Previous studies (Celaya *et al.*, 2007; Osoro *et al.*, 2017) showed that the incorporation of adjacent areas of improved pasture can help animals to meet their nutritional requirements and, consequently, to achieve the sustainability of the systems. Due to distinct morpho-physiological adaptations (feed prehension, muzzle anatomy, digestive strategy) cattle and goats exploit differently the vegetation communities, with cattle showing an almost total preference for herbaceous species, whereas goats tend to incorporate large amounts of woody species in their diets (Ferreira *et al.*, 2013). This distinct grazing behaviour of cattle and goats led us to study different management strategies that could be later implemented in the territory. In this study, data on the grazing behaviour of cattle grazing alone or mixed with goats in heathland vegetation with improved pasture areas are presented.

Materials and methods

This study was carried out in two plots of 22.3 and 18.9 ha, established on heathland vegetation, with an adjacent area of improved pasture (*Lolium perenne* and *Trifolium repens*) representing 25% (IP25) and 69% (IP69) of the total area. Heathland vegetation consisted mainly of heather species (*Erica* spp. and *Calluna vulgaris*), gorse (*Ulex gallii*) and grasses (*Pseudarrhenatherum longifolium* and *Agrostis curtisii*). Twenty Asturiana de los Valles beef cows (550±79.1 kg live weight (LW)) were managed alone in IP69, whereas ten Asturiana de los Valles beef cows (532±98.7 kg LW) were managed in a mixed herd with seventy-five Cashmere goats (41±6.6 kg LW) in IP25. Animals stayed grazing from late April to late October. Grazing behaviour was evaluated by recording the time spent grazing by each animal species on each vegetation type (heathland, natural pasture or improved pasture area) every 15 min from dawn to dusk during two consecutive days in two occasions (May and July). Diet selected by each animal was estimated using n-alkane markers in May and October. For that estimation, samples of faeces from individual animals and of the main plant components were collected. Alkanes were determined by gas chromatography and their faecal concentration was corrected for their incomplete faecal recovery using data obtained in validation studies (Ferreira *et al.*, 2005, 2007). Diet composition was calculated using 'EatWhat?' software (Dove and Moore, 1995). The effects of grazing type (monospecific vs mixed grazing), season (May or October) on the composition of the diet selected by cattle were analysed by analysis of variance (ANOVA) using JMP version 9 (SAS Institute, Inc., 2015).

Results and discussion

Grazing times observed in this study (Table 1) for both cattle and goats are consistent with those found elsewhere for ruminant species (Vallentine, 2000) ranging between 7 and 12 h day^{-1}. Over both periods, cattle tended to spend more time in grazing activities when managed together with goats compared with in a single flock (averaging 614 vs 503 min day^{-1}, respectively). These results are consistent with those observed by Benavides *et al.* (2009), who found greater daily grazing times of both cattle and sheep managed together with goats compared with single flocks. Goats presented the highest grazing times, averaging 734 min day^{-1}, possibly because their higher selective behaviour obligates them to spend more time in selection/prehension activities. Differences between both measurement periods in the grazing time of cattle were also observed, with animals showing, unexpectedly, lower values in July when the improved pasture availability was lower. In fact, grazing time is the primary compensatory variable that ruminants use when the bite size decreases as a result of a lower availability of feed in the pasture (Vallentine, 2000). The hot conditions found in July could explain the reason why less time was spent by both cattle and goats in grazing activities.

Cattle spent the majority of their grazing time on the improved pasture area in single and mixed herds (87 and 77%, respectively) in both May and July (78 and 85%, respectively). These results were expected, as cattle are intensive users of herbaceous vegetation (Celaya *et al.*, 2007; Ferreira *et al.*, 2013) tending to reject woody species that have lower nutritive value. Data on diet composition (Table 2) were consistent with these observations, with the herbaceous species representing in average 0.957 of cows' diet. Reluctance of cattle to incorporate woody species in their diet is well known and could be related to their morpho-physiological specialization (tongue prehension form, large and flat muzzle) for herbaceous-feeding (Putman *et al.*, 1987). In contrast, goats combined the more nutritive herbaceous vegetation (on average 71%) with large amounts of heath species (29%) even when the improved pasture availability was high. This browsing behaviour of goats was also observed in different plant communities (Vallentine, 2000).

Table 1. Grazing behaviour (min day^{-1} animal^{-1}) of cattle managed alone or mixed with goats on heathlands associated with improved grassland areas in two grazing periods.

	Cattle alone		Cattle mixed		Goats mixed	
	May	July	May	July	May	July
Improved pasture	408.8	454.5	546.0	400.8	197.0	479.4
Natural herbaceous	67.5	0.0	54.0	96.7	0.0	49.8
Heathlands	75.8	0.0	61.5	69.2	565.4	176.3
Negative[1]	303.0	340.0	253.5	258.3	152.6	119.5

[1] Animals not showing any grazing activity.

Table 2. Diet composition of cattle managed alone or in mixed grazing with goats on heathlands associated with improved grassland areas in two grazing seasons.[1]

Season (S)	Spring		Autumn		SEM	Effects		
Herd (H)	Alone	Mixed	Alone	Mixed		H	S	H × S
Herbs	0.954	0.994	0.916	0.963	0.0239	0.0384	0.0938	0.8738
Gorse	0.000	0.000	0.054	0.000	0.0187	0.1106	0.1159	0.1159
Heaths	0.046	0.006	0.030	0.037	0.0053	0.0145	0.2315	<0.001

[1] SEM = standard error of the mean.

Conclusions

As expected, results showed that cattle and goats have different and complementary grazing behaviour on these poor and marginal areas. Although slight differences in the grazing behaviour of cattle were observed when they were mixed with goats, utilization of mixed flocks will allow a more efficient and sustainable use of these vegetation communities.

Acknowledgements

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Short- and long-term effects on yield of grassland monocultures and mixtures exposed to simulated drought

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Abstract

At two sites (Zürich, Switzerland and Wexford, Ireland) we evaluated the effects of experimentally imposed summer drought on intensively managed grassland communities (3×5 m plots) of varying richness (1 and 4 species), and comprising four species (*Lolium perenne* L., *Cichorium intybus* L., *Trifolium repens* L., *Trifolium pratense* L.). The short-term drought effects in the individual growth period resulted in strong yield reductions of -39 and -85% in the average across monocultures at Zürich and Wexford, respectively. However, during the same period, the yield advantage from mixing species could compensate for this drought impairment at Zürich. In the three combined harvests across the drought and post-drought period and under control and drought conditions, mean yields were higher (P<0.05), and yield variance was lower (P<0.05) in four-species communities than in monocultures, indicating that yield stability was enhanced in four-species mixtures. The total annual yield (across all harvests) of mixtures under drought exceeded the average annual yield of the rain-fed monocultures. The benefit due to mixtures (~33% increase in annual yield, P<0.05, across sites) was substantially greater than the weak impact of drought (-5% reduction in annual yield, across sites). These results illustrate the high potential for multi-species mixtures to compensate for drought-induced yield losses from the scale of individual harvests to the whole year.

Keywords: diversity, ecosystem function, drought, resilience, forage yield, sustainable intensification

Introduction

In experiments with four-species mixtures in intensively managed conditions, grass-legume combinations consistently yielded more than the best-performing monoculture (Finn *et al.*, 2013). However, there have been relatively few tests of whether such an advantage of multi-species mixtures remains evident under environmental stress, e.g. drought conditions. Both increased variability in precipitation and the amount of precipitation per event (prolonged periods of drought or waterlogging) can act in combination to result in reduced yields in grassland systems (Swemmer *et al.*, 2007). In light of the predicted increase in climate variability, understanding the effects of climate change and severe weather events on grasslands is imperative, given the importance of grasslands as a global resource.

Here, we collate some of the main results from our recent research on the effects of experimental drought on the yields of intensively managed temperate grasslands. We specifically consider the relative effects of a drought event on single harvests, a period including the harvests during the drought and in the post-drought harvest, and the total annual yield.

Materials and methods

A common field experiment to manipulate precipitation was established at two sites, and conducted on two consecutive years. Sites were in Switzerland near Zurich, and in Ireland at Wexford. We selected the following four forage species based on the factorial combination of their specific functional traits related to rooting depth and nitrogen (N) acquisition: a shallow-rooted grass (*Lolium perenne* L.), a deep-rooted herb (*Cichorium intybus* L.), a shallow-rooted legume (*Trifolium repens* L.), and a deep-rooted legume (*Trifolium pratense* L.). Using these four species, plots of 5×3 m were established in monocultures and mixtures of four species in different relative species abundances, including a four-species equi-proportional mixture. Monocultures and mixtures were established as control treatment under ambient rainfed conditions and as drought treatment, in which a summer drought event of nine weeks was simulated at both sites using rainout shelters. The annual yields comprised six harvests at Zurich and five at Wexford; there were two regrowth periods during the drought treatment, and one post-drought harvest. All plots of a site received the same amount of mineral N fertiliser: 200 kg N ha^{-1} year^{-1} at Zurich and 130 kg N ha^{-1} year^{-1} at Wexford (see Hofer *et al.*, 2016a for details). The effects of drought on yield and yield differences between monocultures and mixtures were analysed by linear mixed regression.

Results and discussion

At the scale of individual harvests under drought (Hofer *et al.*, 2016a), on average across monocultures and mixtures there were extreme effects of drought on yield at Wexford (-86%) and severe effects at Zurich (-33%). Compared to the average of the monoculture yields, the mixtures provided a yield advantage of 27 and 67% at Wexford and Zurich, respectively (Table 1). Regarding three combined harvests across the drought and post-drought harvests, (Haughey *et al.*, in review) the average effects of drought on yield were -27 and -23% at Wexford and Zurich, respectively. Considering the total annual yield at each of the sites (Hofer *et al.*, 2016b), the average effects of drought on yield were substantially lower, and were only -5 and -8%, respectively. Taking the average of total yield across the drought and control treatments at both sites, the mixture benefit expressed as a proportion of the average monoculture yield was ~33%.

Overall, there was a consistent reduction in the effect of drought as the number of harvests increased. This might be expected as the drought effect is averaged out over a number of harvests, but will only occur if there is a relatively quick recovery after the cessation of drought within the same growing season. This seemed to be the case in our experiment (Hofer *et al.*, 2016a). At the scale of individual harvests, there can be severe or extreme effects of drought on yield; however, yields recovered as soon as soil moisture levels were restored after the drought event (see Hofer *et al.*, 2016a,b). Recovery in the plots exposed to drought was likely aided by the availability of mineral N (applied during the drought) when soil water supply increased after the drought shelters were removed (Hofer *et al.*, 2017).

Table 1. Comparison of (A) change in aboveground biomass in the average of the four monocultures (av. mono) and four-species mixtures in the drought treatment compared to the rainfed control, and across three temporal scales. (B) Yields of the drought harvests (kg dry matter ha^{-1} year^{-1}), and the % yield advantage (% yield adv.) from mixtures in comparison to the average monoculture yield.[1,2]

		Single harvest during the second regrowth of the drought period		Three harvests during drought and recovery period		Total annual yield	
		Drought Wexford	Drought Zurich	Drought Wexford	Drought Zurich	Drought Wexford	Drought Zurich
A	av. mono	-85%	-39%	-23%	-23%	-5%	-6%
	mixtures	-88%	-27%	-30%	-22%	-5%	-9%
		t/ha	t/ha	t/ha	t/ha	t/ha	t/ha
B	av. mono	0.15	1.13	3.24	3.78	9.7	10.9
	mixtures	0.19	1.89	3.90	5.70	12.5	14.8
	% yield adv.	27%^{ns}	67%*	20%*	51%**	28%***	36%***

[1] *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns: not significant.

[2] The single harvest occurred during the second regrowth of the drought period (see Hofer *et al.*, 2016a); the three harvests occurred across the drought (n=2) and post-drought period (n=1), and the total annual yield across all harvests (n=5 at Wexford, n=6 at Zurich). Note that mixtures are represented by the four-species equi-proportional mixture, except in the three-harvest comparison that used all of the four-species mixtures and averaged over two years of sampling in the same experiment.

Conclusions

At the scale of individual harvests, there can be severe or extreme effects of drought on grassland yield. However, once drought ceases and soil moisture is restored, yields can recover quite rapidly. At the scale of total annual yield, the effects of drought were considerably smaller, at least with the application of a single drought, and in systems with a relatively long growth season. With the exception of the extreme drought effect on the single harvest during drought at Wexford, the yield benefit from the use of mixtures was maintained under drought. Yield benefits of mixtures (~33% increase in annual yield) were much greater than the effect of drought (5% reduction in annual yield). These results illustrate the high potential for multi-species mixtures to compensate for drought-induced yield losses.

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Effectiveness of heifers grazing on extensive grassland situated in the B. Papi Reserve

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Abstract

In 2007-2010 an experiment was carried out to investigate the effectiveness of beef production from heifers under extensive grazing conditions on grassland situated in the B. Papi Reserve. Experimental animals (112-118 heifers) were purchased in spring, grazed 24 h day^{-1} during grass-growing season lasting 182-202 days and were sold in November. The cycle was repeated four years in succession. The botanical composition of the sward and dry matter yield of pastures were estimated. Daily gains of heifers were measured by weighing and the effectiveness of beef production was evaluated. During the grazing season daily liveweight gains of animals ranged on average between years from 495 to 554 g depending on weather conditions and age of animals. Effectiveness of heifers grazing on this type of grassland can be improved by adjusting the stocking rate to the yield potential of the pasture. Extensive grazing of heifers appeared to be an economically justified and ecologically sustainable method of agricultural utilization of nature conservation areas with positive effects on grassland biodiversity.

Keywords: beef production, grazing, heifers, nature conservation area, extensive grasslands

Introduction

In low-input grassland management, grazing with beef cattle is considered an appropriate method of sward utilization (Isselstein *et al.*, 2005). For both organizational and economic reasons, it is recommended to prolong the grazing season and keep grazing animals at pasture for as long as possible during the day. In search for balanced, environmentally and landscape-friendly forms of management of permanent grasslands, experiments on the possibilities of grass-fed beef production in areas under legal protection were carried out. The objective of this work was to evaluate the effectiveness of extensive grazing of heifers on grassland situated in the B. Papi Reserve in Poland.

Material and methods

During 2007-2010, studies to evaluate the effectiveness of beef production from heifers under extensive grazing conditions on pastures situated in a nature conservation area (B. Papi Reserve established in 1974) near Brody (52°26'N, 16°18'E) Experimental Station of Poznan University of Life Sciences were carried out. Since the area is under legal protection because of feeding and nesting habitats for many bird species, it requires special extensive agricultural management. For this reason, the pasture were established in 1988 on a muck soil formed on the drained fen (pH_{KCl} 6.5, N_t – 0.68%, P_2O_5 – 53.2 mg 100 g^{-1}, K_2O – 37.0 mg 100 g^{-1}, Mg – 7.8 mg 100 g^{-1}). Since the beginning the area was dedicated for extensive grazing of beef cattle. The pasture area (42.4 ha) was divided into six paddocks. Mineral fertilization of pasture was restricted to 50 kg ha^{-1} N, 50 kg ha^{-1} P_2O_5 and 90 kg ha^{-1} K_2O applied in spring. After 20 years of extensive grassland management the *Poo-Festucetum rubrae* association developed. Shannon-Wiener diversity index of the vegetation reached the level of 1.85. In the study period experimental animals (112-118 Polish Holstein-Friesian heifers), were purchased in spring from among animals that were selected as unsuitable for milk production, and they grazed during the growing season lasting 182-202 days. In the rotation the herd grazed each paddock 6 days in the first cycle and 8 days in the next cycles. One employee managed the herd for 4 h day^{-1}. Forage from the pasture was the only feed the heifers received. Fresh water was brought to the animals twice a day and they also received straw and mineral supplements.

At the end of the grazing season in November all the animals from the herd were sold. The cycle was repeated four years in succession. The botanical composition of the sward using point method and dry matter yield of pasture using a rising plate meter were estimated. The daily liveweight gains of heifers were measured by weighing. The evaluation of the economic effectiveness of beef production using the method of differential computation and calculation of total costs (Ströbel, 1987) was performed. All calculations were made on the basis of 2016 prices, assuming the purchase and sale price on the same level of 6.2 PLN kg^{-1} (1 PLN = 0.22 €).

Results and discussion

Changes in sward botanical composition during successive years of pasture utilization were caused by weather conditions, which affected the intake and turf compaction by grazing animals (Figure 1). The dominant sward species was *Poa pratensis*, which well developed in the muck soil site and under grazing conditions, reaching 34.8% in 2010. The remaining grasses sown in the mixture comprised 10.5-12.2% depending on the year. *Festuca rubra* appeared in the sward spontaneously and its share reached 16.1% in 2010. The share of *Trifolium repens* in the sward ranged from 8.2% in 2008 to 10.8% in 2007. The remaining species of the pasture were various species of herbs and forbs. The differences in botanical composition between paddocks were not significant and did not affect the animal production. An increase in the number of species in the sward was observed, from 37.3 (on average) in 2007 to 48.7 in 2010.

The dry matter yield varied slightly over the years and ranged from 4,930 to 5,580 kg ha^{-1}. The highest mean liveweight gains (554 g day^{-1}) were recorded in 2010, the lowest (495 g day^{-1}) in 2007 (Table 1). Both yearly averages and individual data show that higher gains were associated with the older age of grazing animals. The lowest liveweight gain in 2007 can be explained by the rainy weather, which influenced the forage intake and resulted in damage to the sward. Pavlu *et al.* (2001) found that heifers can maintain a good individual performance through selective grazing when the herbage offered exceeds the demand. The productivity of beef ranged from 261.5 to 283.9 kg ha^{-1} (Table 2). The highest beef yield on a surface area basis was reached in 2010 by the best individual animal performance. In 2007, despite this year having the lowest gains per heifer, the beef productivity per hectare was satisfactory because the stocking rate and sward yield were better matched. The highest return of beef production inputs for pasture and labour were recorded in 2010, and for capital in 2008. From 2004 when Poland accessed the EU, the economy of beef production on extensive grasslands increased. Additionally, since 2004 the farmers have had the opportunity to receive financial compensation when they integrate nature conservation objectives into their grassland systems. In the case of the B. Papi Reserve extensive grasslands, the key for effective heifer grazing is to balance individual performance, beef yield per unit area, and conservation goals.

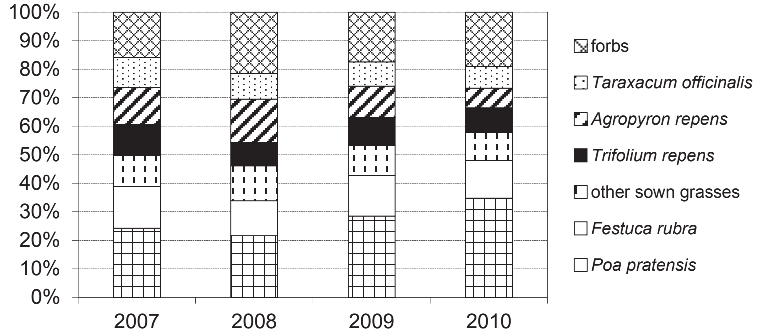

Figure 1. Changes in sward botanical composition over successive years.

Table 1. Characteristics of heifers beef production on extensive pasture in particular years.

Item	Unit	2007	2008	2009	2010
Number of heifers in the herd	heifers	116	112	115	118
Buying weight of heifers[1]	kg	338±47	324±72	346±58	360±43
Selling weight of heifers[1]	kg	438±53	423±78	447±62	462±54
Grazing period	days	202	182	194	184
Daily liveweight gain[1]	g	495±134	544±194	521±147	554±96
Stocking rate	LU ha^{-1}	2.12	1.97	2.15	2.29
Labour engagement per grazing period	lh	808	728	776	736
Average capital engaged in production	PLN	243,090	224,986	246,698	263,376

[1] Values are mean ± standard error.

Table 2. Productivity and return of production inputs on extensive pasture in particular years.

Production input	Unit	2007	2008	2009	2010
Natural productivity – beef					
Pasture	kg ha^{-1}	273.6	261.5	273.9	283.9
Labour	kg lh^{-1}	14.4	15.2	15.0	16.4
Capital	kg 100 PLN^{-1}	4.8	4.9	4.7	4.6
Financial productivity – money					
Pasture	PLN ha^{-1}	7,429.5	6,927.6	7,516.8	7,971.7
Labour	PLN lh^{-1}	389.9	403.5	410.7	459.2
Capital	PLN 100 PLN^{-1}	129.6	130.6	129.2	128.3
Return of beef production inputs					
Pasture	PLN ha^{-1}	1,408.9	1,358.0	1,420.1	1,488.0
Labour	PLN lh^{-1}	73.9	79.1	77.6	85.7
Capital	PLN 100 PLN^{-1}	24.6	25.6	24.4	24.0

Conclusions

The values of return of beef production inputs showed that extensive grazing of heifers is an economically justified method of agricultural utilization of nature conservation areas in the B. Papi Reserve with positive effects on biodiversity. During the grazing season daily liveweight gains of animals ranged from 495 to 554 g depending on the age of the heifers and weather conditions in particular years. Effectiveness of heifer grazing on extensive grassland can be improved by adjusting the stocking rate to the yield potential of the pasture.

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The Dehesa silvopastoral systems as high nature value areas: interaction between agricultural uses and natural values

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Abstract

The High Nature Value (HNV) concept has been increasingly used in both research and policy to support agroecosystems with high species richness and habitat diversity. Most of the HNV areas are exposed to severe threats, usually associated with management changes and market pressures, as in the case of Dehesa silvopastoral systems. Setting indicators and assessment methods are necessary to preserve Dehesas as HNV areas against the drivers of change. However, this objective is difficult to achieve due to the multifunctionality of such systems and the diversity of management options. Because of this, new approaches are required to better understand the relationships between environmental conditions, management practices and the values defining dehesas as HNV systems. In this paper, a methodology based on the analysis of different information layers (sets of indicators on different relevant perspectives) is presented and tested. Thirty-four pastureland estates of the Demonstrative Dehesas Network of Life BioDehesa (Andalusia) were analysed using a multivariate approach. The results show the importance of management options to determine the landscape structure and the natural values associated. The method enables a comprehensive assessment of farms and discussion of the effects of management options (livestock, commodities, etc) on these HNV areas.

Keywords: HNV, dehesa, montado, multifunctionality, silvopastoral systems

Introduction

High Nature Value (HNV) farmlands are defined as areas where agriculture is a major land use and it is related to high biodiversity rates and conservation values (Andersen *et al.*, 2004). Dehesas (called Montados in Portugal) are silvopastoral systems considered as HNV due to their ecosystem services provision. These agroecosystems are characterised by a variable density evergreen oak tree cover, under which there is a mosaic of dispersed shrubs and pastures varying in specific composition, cover and phenology. The management changes during the last decades have promoted both intensification and abandonment processes, which are threatening such agroecosystems (Gómez Sal, 1997). In this context, it is important to achieve better understanding of the relationships between management and the landscape structure of these systems to improve their resilience (Ferraz-de-Oliveira *et al.*, 2016). Setting indicators and assessment methods is necessary to preserve Dehesas as HNV areas. Nevertheless, the diversity of current management options and the multifunctionality of these systems, make this a complex endeavour.

We propose a hierarchical conceptual evaluative model based on different information layers (Figure 1); in this way, each layer can be evaluated by a set of specific indicators. The data for thirty-four representative estates allowed us to compare the current situation with the model and to assess the conflicts that may affect the natural value (HNV). At the basis of the model, the Ecological conditions layer (C in Figure 1) provides support and resources, which are used by the technical-productive system (B, 'Agricultural uses'). The market conditions (A) act over them, according to a vertical hierarchical scheme. The natural and cultural values of the dehesas (D) as well as the effects on the surrounding territory (E) would be the main results of the adequacy of uses to the ecological conditions (coherence and sustainability of human uses) (see Gómez Sal *et al.*, 2003).

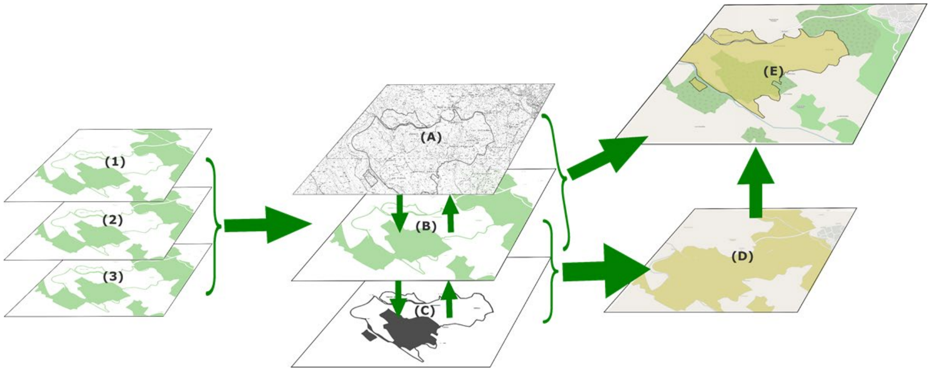

Figure 1. Hierarchical scheme of information layers acting in high nature value farms. (1) Production system target, (2) Type of crops, (3) Animal husbandry, (A) Market facilities, (B) Agricultural uses, (C) Ecological conditions, (D) Natural and cultural values and (E) Effects on the territory/social context.

Materials and methods

A multivariate approach was used to analyse thirty-four estates of the Demonstrative Dehesas Network of Life BioDehesa (Andalusia). Different indicators were set for each information layer. The 'Market' (A) layer has not yet been analysed due to the lack of information. The technical-productive (B) system 'Agriculural uses' was divided for the analysis, due to its complexity into three components: (1) 'Production system target', (2) 'Types of crops' and (3) 'Animal husbandry'. The estates have been analysed using Detrended Correspondence Analysis (DCA) for each set of indicators (information layer). The first two axes of each DCA were compared by Pearson correlation index as a measure of similarity. For E layer, only the first axis was used because of the low information (variance percentage) provided by axis 2. Also, a Mantel Test was performed with the dissimilarity matrix of the different layers. The resulting model of correlations and Mantel Test were compared with the proposed conceptual model. The used statistical software were Past3 and R (Vegan and Corrgram package).

Results and discussion

The analysis of the Pearson correlation average for each axis (Table 1 and Figure 2) indicates that the ecological conditions layer is the layer most correlated to the rest of the layers, which explains its basic general influence on the functioning and structure of the analysed estates. The Mantel Test (Figure 3) supports these results.

A second nucleus of high correlations is formed by the components of the agricultural uses ('Production target' and 'Animal husbandry'). The low relation with the ecological conditions (C) indicates the importance of the management in the structure of the dehesas. The capacity of the management models to obtain specific commodities beyond physical boundaries modulates the silvopastoral structure and heterogeneity. That could put at risk the suitability of the production system. The 'Effects on the territory' (E) layer shows a low relation with the rest of the layers, showing significant correlation with 'Values' (D, axis 2) only. Estates with lower 'natural value', estimated on protected areas cover and threatened species, are associated with lower population density territories. This allows to hypothesize that, in the set of

Table 1. Pearson Correlation Average of first two axes of the different layers.

a1_(C)	a1_(2)	a2_(1)	a2_(D)	a2_(C)	a1_(E)	a1_(D)	a1_(3)	a1_(1)	a2_(3)	a2_(2)
0.2528	0.2133	0.1958	0.1924	0.1829	0.1713	0.1704	0.1638	0.1486	0.1388	0.1169

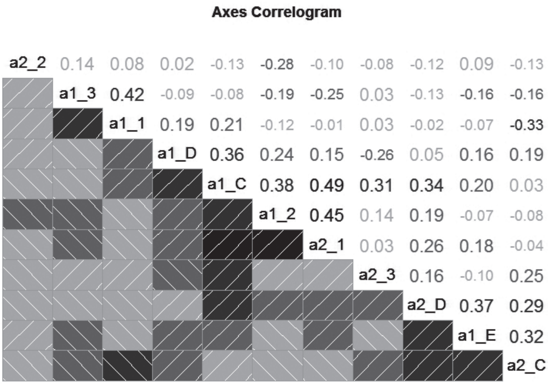

Figure 2. Inter-axes correlogram. The upper panel shows correlation values, the lower panel, right oriented lines indicate positive relations, the contrary negative ones, to mayor intensity, greater values.

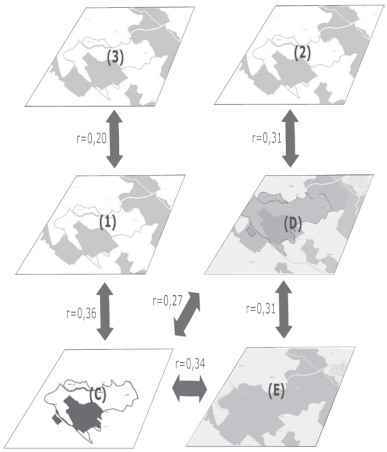

Figure 3. Mantel test significant results.

studied estates, conservation based in both species and spaces does not covariate with multifunctional agricultural landscapes, but with areas where the land uses are more polarized (cities vs protected areas). However, the 'Value' axis 1, referred with Biosphere Reserve (BR) and EU priority habitats, occupies an intermediate position in the correlation ranking, having numerous but low correlations with the rest of the axes. That indicates a greater fit for these conservation figures (specially BR) to the diversity of natural values throughout the land (not only coming from biodiversity, but also from traditional uses).

Significant results from the Mantel Test (Figure 3) endorse the 'Ecological conditions' (C) layer as the basis of the system, related to 'Productive target' (1), 'Values' (D) and 'Effects on the territory' (E). Despite the above, it is relevant that management practices (current agricultural uses) seem not to be so related to the physical-ecological conditions in this set of representative studied dehesas.

Conclusions

The application of the proposed model, considering the interaction between different information layers (set of indicators), allows a comprehensive assessment of different types of dehesa estates, depending on their capacities to provide multifunctionality and ecosystem services. However, the market layer (A) should be integrated in future analysis (in this case it was considered indirectly). Consequently the model is a useful tool for a comprehensive evaluation of pasturelands, which would also be applicable to other types of agroecosystems. We can conclude that the proposed model is worthwhile for the study of HNV farms and agricultural systems.

Acknowledgements

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Renovating grazing lands on the Greek islands through the introduction of forage legumes

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Abstract

Sheep and goat farming is an important agricultural activity in many Greek islands that are limited by the shortage of forage, which leads to high production costs. A renovation project was established with the aim of finding low cost and environmentally friendly practices to ensure high quality forages. For this purpose seed mixtures of four forage legumes [*Medicago littoralis* (cv. Angel), *Medicago polymorpha* (cv. Scimitar), *Trifolium subterraneum* (cv. Seaton Park) and *Trifolium michelianum* (cv. Paradana)] were sown on medium quality land on five Aegean islands (Lesvos, Chios, Ikaria, Kea and Rhodes). Renovated fields were monitored during two years for forage productivity, forage quality and introduced species persistence. These parameters were compared with those of neighbouring unimproved fields. Results showed that forage dry matter production was significantly different between islands and was higher in renovated fields. Crude protein and crude fibre contents of forage samples and crude protein productivity were significantly higher in renovated fields. It was concluded that the introduction of forage legumes might be an advisable practice for the renovation of degraded grazing lands.

Keywords: rangeland renovation, forage legumes, Mediterranean islands

Introduction

Isolation and scarcity of resources, observed in the past centuries, forced islanders in the Mediterranean to develop specific systems of land management of which agriculture and animal husbandry were vital parts (Margaris, 1992). Hilly landscape and limited water supply forced the development of terrace agriculture for the cultivation of either annual (cereals, legumes, etc.) or perennial (olives, almonds, figs, etc.) crops. At the same time, sheep and goat farming was employed to utilize more marginal lands (rangelands) and the by-products of crops (Hadjigeorgiou et al, 2008). The pastoral system was organized usually under a dizonic form (each year 50% of the rangeland was under grazing and the other 50% was rested). Developed through centuries of experience, this form of management permitted a continuous but sustainable exploitation of environmental resources (Margaris, 1992). This sustainable system collapsed dramatically during recent decades mainly because of trade globalization and agriculture intensification in the lowlands of the Greek mainland, which forced the islanders to abandon their usual agricultural activities. The abandonment of traditional land management practices together with the EU policy of *per capita* subsidies for small ruminants resulted in animal husbandry specialization (mainly dairy), which dominates in all the islands. Moreover, the increase in feeding costs, due to recent increases in global feed prices and transportation costs, has increased grazing pressure on the land (Hadjigeorgiou *et al.*, 2008). The result was the acceleration of desertification processes, a phenomenon that requires immediate action to be mitigated. To reverse this situation, physical and economically sustainable approaches should be employed. Land renovation through the introduction of forage legumes appears a promising option (Aronson *et al.*, 1993; Ewing, 1999; Henkin, 2016; Ovalle *et al.*, 2008). Therefore, a project was established, funded by the Hellenic Ministry of Rural Development and Food, involving five Aegean Sea islands with the aim of increasing forage production, provide quality forage appropriate for dairy animals and act against desertification.

Materials and methods

Seeds of the following legume species were used: *Medicago littoralis* (cv. Angel), *Medicago polymorpha* (cv. Scimitar), *Trifolium subterraneum* (cv. Seaton Park), *Trifolium michelianum* (cv. Paradana). Two rhizobia inoculant commercial products (ALOSCA C and AM) were also used. The above legume seeds were mixed at a rate of 25, 25, 20 and 30%, while inoculants mixed at a ratio of 60 and 40%, respectively. Finally, legume mixtures and the inoculants were used at a ratio of 70 to 30% by weight and the final mixture was applied at a rate of 50 kg ha^{-1}. The project involved the voluntary participation by 50 farmers from five Aegean Sea islands, i.e. Chios, Ikaria, Kea, Lesvos and Rhodes, representing different climatological and soil conditions. Farmers allocated a plot on their land ranging in size between 0.2 and 2.0 ha. The seedbed was prepared by disk or tooth harrowing, and a superphosphate fertilizer (0-46-0) was added at a rate of 50 kg/ha before sowing (mainly in second half of November). At the end of growing season (second half of May, when indigenous herbaceous vegetation was fully ripe) representative forage samples were harvested both from the renovated field and non-treated nearby land. Forage samples were oven dried at 55 °C for 48 h for dry matter (DM) determination, and analysed for ash, crude protein (CP) and crude fibre contents according to AOAC procedures (AOAC, 1990).

The fields were then either grazed intensively or cut for hay and left intact to regrow next season before applying the same recordings as above. Data were explored through a GLM procedure for the effects of 'island', 'year' and 'treatment'.

Results and discussion

Meteorological data obtained from the nearest recording station of the National Weather Network showed substantial differences in annual precipitation, annual average temperature and mean minimum temperature between islands and between years. The introduced species represented on average about 50% of the DM yield on the sown plots. An average of 399 g DM m^{-2} of forage was produced with differences between the five islands and between 'sown' and 'indigenous' fields (514.3 and 284.2 g DM m^{-2} respectively), while the year effect was not significant (Table 1). Differences in the total amount of precipitation between the islands in the entire period of this two-year study (Chios 1,430 mm, Ikaria 3,019 mm, Kea 2,015 mm, Lesvos 1,952 mm and Rhodes 1,890 mm) did not correlate with herbage yield.

Table 1. Forage production and forage quality characteristics from five Aegean islands averaged over two years on either renovated with forage legumes or indigenous fields.

		Observations	Forage production (g DM m^{-2})	Ash (% DM)	CP (%DM)	CF (%DM)	CP production (g m^{-2})
Island	Chios	20	417.5	9.21	10.22	35.00	46.43
	Ikaria	16	345.1	9.17	10.52	34.58	39.37
	Kea	24	380.8	9.29	10.11	34.71	41.13
	Lesvos	26	458.1	9.51	9.54	35.75	49.86
	Rhodes	12	394.7	9.15	10.35	34.45	43.70
Year	A'	50	392.7	9.39	10.09	35.24	43.17
	B'	48	405.7	9.13	10.20	34.55	45.03
Treatment	Indigenous	49	284.2	8.72	6.81	39.28	19.43
	Sown	49	514.2	9.80	13.48	30.51	68.76
			***P*-value**	***P*-value**	***P*-value**	***P*-value**	***P*-value**
Factor effects	Island		0.0005	0.5691	0.0713	0.1923	0.0050
	Year		0.4484	0.1132	0.6680	0.0877	0.3644
	Treatment		0.0000	0.0000	0.0000	0.0000	0.0000

[1] CP = crude protein; CF = crude fibre; DM = dry matter.

Chemical traits of forage differed significantly only among treatments. Percentage of CP doubled in the sown fields, and CP yield was 3-fold higher than in unsown fields.

Forage productivity was substantially improved by the introduction of forage legumes in the indigenous vegetation. Among the sown species, *Medicago* species (*M. polymorpha* and *M. litoralis*) produced the most abundant biomass followed closely by *T. michelianum*, which did not survive well in the second year. Finally, subterranean clover did not succeed in any year on most of the sites. It appears that the ability of these species to set seeds very early in the season is critical in these arid and semi-arid environments.

Conclusions

Rangeland restoration and rehabilitation in arid and semi-arid islands appears feasible through the introduction of forage legumes, both for the benefit of local agriculture and the environment (to mitigate desertification). Proper choice of plant species for this purpose appears vital in the success of this action.

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Influence of first cut timing on the performance of forage grass varieties in variety testing

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Abstract

In different species of temperate forage grasses, a range of different maturity types with different development rates are available. The question is how to select the optimal harvesting time in official variety testing to evaluate this. This project evaluated two different times (normal, adjusted) for the first cut of forage grasses in official variety testing in Sweden. The study was conducted in two consecutive years at three sites in central and southern Sweden, using tall fescue (*Festuca arundinacea* Schreb.), festucoid festulolium hybrids (*F. arundinacea* × *Lolium multiflorum*) and meadow fescue (*Festuca pratensis* Hudson). The normal time of first cut in Swedish variety testing is at the heading of meadow fescue. The adjusted first cut time tested was five days before heading of the control (*F. pratensis* cv. SW Minto) for meadow fescue and tall fescue varieties and five days after heading of the control for festucoid festulolium hybrids. The results showed that the normal first cut time still provided a good basis for comparison for total yield and that time of first cut did not affect the relative performance of varieties.

Keywords: *Festuca pratensis*, *Festuca arundinacea* × *Festulolium,* yield, variety testing

Introduction

In different species of temperate forage grasses, a range of different maturity types with different development rates are now available. The question is how to select the optimal harvesting time in official variety testing to evaluate and compare these different types. The aim of this project was to evaluate two different times (normal, adjusted) of first cut of different forage grasses in official variety testing in Sweden.

Materials and methods

The study was conducted in two consecutive years at three sites in central (Uppsala) and southern Sweden (Rådde, Lilla Böslid), using tall fescue (*Festuca arundinacea* Schreb., cvs. Swaj, Rahela, Stockman and Karolina), festucoid festulolium hybrids (*F. arundinacea* × *Lolium multiflorum*, cv. Hykor and Fojtan) and meadow fescue (*Festuca pratensis* Hudson, cvs. SW Minto, Norild, Cosmolit, Lipoche, Tored, Liherold, Pardus). SW Minto was taken as the control variety. Uppsala (59°50'18.2'N; 17°42'11.8'E) and Lilla Böslid (56°35'54.4'N; 12°57'07.3'E) are close to sea level, while Rådde (57°36'26.9'N; 13°15'33.9'E), in the southern Swedish highlands, is at about 185 m above sea level. The trial had a two-factor split-plot design, where the main factor was time of first cut, with two options: (1) Normal, at heading of SW Minto, and (2) adjusted, at five days prior to heading of SW Minto, when heading begins in early varieties of tall fescue (e.g. cv. Rahela) and festucoid festulolium hybrids. The adjusted harvest time was included to see if ranking of varieties was affected by their rate of development. The stage of heading is defined when the inflorescence is visible to 50% at 50% of the shoots. All species were established in pure stands. Non-sown species were removed before determination of total yield. In year 1 (ley 1), the normal cut (at control heading) was taken on 11 June, 3 June and 4 June at Uppsala, Little Böslid and Rådde, respectively, and the adjusted (earlier) cut of meadow fescue and tall fescue (at heading) was taken on 4 June, 29 May and 30 May, respectively. There was an average of 6 days between first cut times. In year 2 (ley 2), there was an average of 5 days between first cut times and plant development was 6 days earlier than in 2013. Statistical analysis was performed using the Mixed model in the SAS software package (SAS, 2016), with first cut time, variety and location as fixed variables and block (three) as random variable. The results reported are least square means.

Results and discussion

There was significantly lower total yield in ley 2 in Uppsala at the adjusted first cut time (Table 1). At the other sites, there was no significant effect of either first cut time on total yield. The results demonstrate that there is no difference in ranking of varieties between normal and adjusted harvest dates. This is believed to be valid in other situations for the species investigated. In ley 2, there was a significant interaction (P<0.003) between first cut time and location, while in ley 1 there was no significant interaction (P<0.798) (Table 1). However, there was a large difference between Uppsala and the other two sites in terms of total yield, which was caused by drought in Uppsala in ley 1.

The interaction between variety and site was strong for all cuts and total yield in both ley years. Table 2 shows the results of total yield for ley 2. The tall fescue and festucoid festulolium varieties gave considerably greater total yield, particularly in Uppsala. Among the meadow fescue varieties, Tored and Pardus gave high total yield at most sites. In the statistical analysis, there was no significant interaction between time of first cut and variety, which can be taken to mean that relationships between varieties were maintained regardless of time of first cut.

Table 1. Effect of time of first cut (normal, adjusted) on total yield (kg dry matter ha^{-1}) of all test varieties in ley 1 and 2 at the three test sites (Uppsala, Lilla Böslid, Rådde).

Time of first cut	Site[1]	Ley year		Significance[2]
		1	2	
Adjusted	Uppsala	5,270	13,550	b
	Lilla Böslid	13,640	14,260	ab
	Rådde	12,170	13,830	ab
Normal	Uppsala	5,640	14,630	a
	Lilla Böslid	14,490	14,500	ab
	Rådde	12,300	13,970	ab

[1] Interaction between time of first cut and site not significant.

[2] Values with different letters are significantly different (P<0.05).

Table 2. Total yield (kg dry matter ha^{-1}) in ley 2 of the different varieties grown at the three test sites (Uppsala, Lilla Böslid, Rådde).[1]

Variety	Ley 2					
	Uppsala		Lilla Böslid		Rådde	
SW Minto	11,930	mop	12,780	jklmno	12,490	lmno
Hykor	18,360	ab	17,660	bc	16,190	efg
Norild	10,500	r	12,450	klmno	10,180	r
Swaj	16,650	cdefg	17,070	bcde	14,390	hi
Cosmolit	11,960	mop	13,230	ijklm	13,840	ijk
Fojtan	16,910	cdef	16,390	defg	15,420	gh
Lipoche	11,770	opq	13,150	ijklmo	13,780	ijk
Tored	13,240	ijkln	13,290	ijklm	13,810	ijk
Liherold	12,730	jklmno	12,990	ijklmno	12,680	klmno
Pardus	10,630	qr	13,470	ijkl	13,940	ij
Rahela	17,160	cde	16,170	efg	16,420	cdefg
Stockman	19,450	a	17,040	bcde	15,560	fg
Karolina	17,600	bcd	16,500	cdefg	14,190	i

[1] Values with different letters are significantly different (P<0.05).

Conclusions

It was shown that carrying out the first cut at heading of the control (meadow fescue), as is normal practice in Swedish variety testing, provided a good basis for comparison of total yield of varieties of meadow fescue, tall fescue and festucoid festulolium hybrids and that choice of first cut time did not affect the relative performance of varieties.

Acknowledgements

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Grass-legume mixtures sustain yield advantage under cool maritime growing conditions over a period of five years

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Abstract

Grassland-based livestock systems play an important role as a provider of food in the more marginal areas in Nordic regions. Such production systems are commonly dominated by grass monocultures receiving relatively high levels of fertilizer. Here, we investigated whether grass-legume mixtures can improve the productivity and robustness of cultivated grassland under extreme growing conditions over a period of five years. Monocultures and mixtures of *Phleum pratense* L., *Festuca pratensis* Huds., *Trifolium pratense* L., and *Trifolium repens* L., differing in species' relative abundances, were established in Iceland in spring 2008 and maintained under three N levels (20, 70 and 220 kg N ha^{-1} $year^{-1}$) for five consecutive harvest years. We observed significant positive diversity effects in all individual years and averaged across the five years. Across years, the four-species equi-proportional mixture was 71% (N20) and 51% (N70) more productive than the average of monocultures, and the highest yielding mixture was 36% (N20) and 39% (N70) more productive than the highest yielding monoculture. Importantly, diversity effects were evident also at low relative abundances of either species group grasses or legumes in the mixture. Our results demonstrate that northern grassland production can benefit from grass-legume systems, that these benefits can sustain five years and that they were robust over years, N fertilizer treatments and a wide range of relative abundances of legumes in the mixture.

Keywords: grass-legume mixtures, sustainable grassland production, species diversity, overyielding

Introduction

Grassland-based livestock systems play an important role as a provider of food in the more marginal areas in Nordic regions (Helgadóttir *et al.*, 2014). It has been argued that basing these systems on grass-legume mixtures would improve both their productivity and sustainability (Lüscher *et al.*, 2014). The obtained benefits have primarily been attributed to the ability of the legume component to make use of atmospheric N_2 through symbiotic N_2 fixation, thus reducing their requirements for soil and fertilizer N. However, under marginal growth conditions in northern areas, symbiotic N_2 fixation is limited by low summer temperatures (Rasmussen *et al.*, 2013). Furthermore, winter survival of the legume component is challenged by various stress factors such as low temperature, ice encasement and desiccation due to ground freezing (Bélanger *et al.*, 2006; Helgadóttir *et al.*, 2008). Farmers in these regions have been reluctant to use grass-legume mixtures, probably because of poor persistence of the legumes, thus making mixture performance unpredictable, both within and between years. When it comes to inter-annual yield stability, agronomically relevant studies with grassland mixtures rarely, if ever, extend beyond three harvest years. The latter would be particularly relevant in the more marginal areas of northern Europe, where grass fields commonly are only renovated every six to ten years. In the current study, we investigated whether (1) grass-legume mixtures can improve the productivity of cultivated grassland under extreme growing conditions, (2) such positive effects can persist over five years and (3) how they depend on the legume proportion in the mixture.

Materials and methods

The experiment was carried out at Korpa Experimental Station, Iceland (64°09'N, 21°45'W, 29 m above sea level). The growing conditions at Korpa are characterised by relatively cool summers with long photoperiods. Monocultures and mixtures of *Phleum pratense* L. (cv. Snorri), *Festuca pratensis* Huds. (cv. Norild), *Trifolium pratense* L. (cv. Bjursele), and *Trifolium repens* L. (cv. Norstar), differing in species' relative abundances following a simplex design (Cornell, 2002), were established in spring 2008 and were maintained under three N levels (20, 70 and 220 kg N ha^{-1} $year^{-1}$). All plots received 40 kg P and 60 kg K ha^{-1} yr^{-1} in early spring. The plots were harvested twice a year for five years (2009-2013) and the four sown and pooled unsown species proportions were determined by manually separating plant samples from permanent sub-plots. Total biomass yield for each harvest year, and averaged across the five years, was analysed by multiple linear regression following principles established by Kirwan *et al.* (2009). Pairwise interactions between species were grouped together to specifically reveal diversity effects (D) between grass and legume species (BGL) and within grass and legume species (WGL), and were only included in the model if significant at $P<0.05$. An initial analysis revealed that the legume proportion in mixed stands at N220 strongly decreased from the first year onward and all mixtures at N220 were therefore excluded from the regression analysis.

Results and discussion

Mixing grass and legume species (BGL effects) resulted in significant positive diversity effects in all individual years and averaged across the five years, as demonstrated for the four-species equi-proportional mixture in Figure 1. In years 3 and 4, positive diversity effects appeared also through the grass-grass and legume-legume interactions (WGL); these were approximately half the size of the BGL effects. The relative magnitude of overyielding (mixture yield higher than the average of monocultures) was largest in year 3 (119% at N20, 79% at N70), yet overyielding was generally maintained over the five years. Averaged across the five years overyielding was 71 and 51% at N20 and N70, respectively. Further, transgressive overyielding (mixture yield higher than the best monoculture yield) of the highest yielding mixture was 36% and 39% across the five years at N20 and N70, respectively. As a consequence of these strong BGL effects (Figure 1), transgressive overyielding of the mixture was significant over a large range of legume proportions in mixtures, ranging from 43 to 90% at N20, and from 15 to 93% at N70.

The diversity effects obtained for the equi-proportional mixtures under these marginal growing conditions in Iceland are fully comparable to results obtained in more favourable climates where diversity effects were maintained for two to three years (Finn *et al.*, 2013). As found by Nyfeler *et al.* (2009) the diversity effect could mainly be attributed to the interactions between grasses and legumes (BGL), while the effect of mixing two grasses or two legumes (WGL) was much smaller, even though the two legumes (and the two grass species) differed strongly in their growth form and/or temporal development

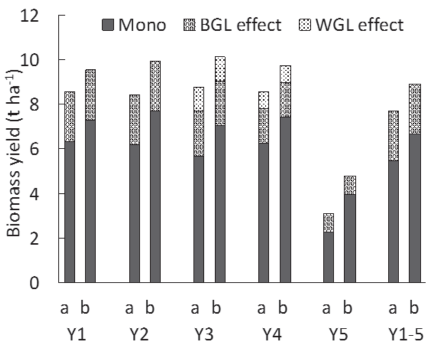

Figure 1. Predicted total yield (including unsown species) of mean monoculture yield (Mono), and significant ($P<0.05$) diversity effects between grass and legume species (BGL effect) and within grass and legume species (WGL effect) in the four-species equi-proportional mixture, grown at N20 (a) and N70 (b) over five harvest years (Y1-Y5).

of competitive ability over the years. This strong BGL effect suggests that additional N input into the system, through the legumes' access to atmospheric N_2 and a stimulation of legumes' symbiotic N_2 fixation due to the presence of the grass partners in the mixture (Nyfeler *et al.*, 2011), also occurred under the Icelandic growth conditions. Importantly, in our study, the diversity effects sustained over a period of five years and high levels of transgressive overyielding were reached over a large range of legume proportions in mixtures. The proportion of *T. pratense* sustained over time, even when sown in sub-dominant proportions. Contrary to expectations, *T. repens* did not increase in relative abundance, probably due to shading by the grasses.

Conclusions

Our results indicate a robust benefit of mixing grass with legume species. This was evident from sustained transgressive overyielding at both N fertiliser treatments and over a wide range of legume proportions in the mixtures. Thus, northern grassland production can benefit from grass-legume systems on the short- and the medium-term scale.

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New land classification system in Hungary: grassland production estimation in practice

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Abstract

Grassland management should adjust to environment-friendly and sustainable progress expectations in the near future. The Hungarian grassland evaluation criteria are now outdated because the utilization of parameters were determined in the past. In particular, this evaluation system cannot be used nowadays for assessing the true value of temporary grasslands. These inconsistencies can be bridged by the evaluation method called D-e-Meter. The focus of our analysis was to create and test a new grassland rating system, which will became a module of D-e-Meter. The measured parameters were: dry matter yield, soil water management, agro-ecological districts, slope gradient category, date of the grassland establishment, annual weather features, farming intensity and forage quality. This method can be used for extensive agricultural production areas, as well as in vulnerable or high nature value areas. According to our previous investigations, this method can be accurate to characterize all types of grasslands in Hungary based on the results of experiments undertaken to date.

Keywords: grassland, evaluation parameters, qualification, vulnerable grasslands

Introduction

The important role of grassland management and production in Hungary has brought up the need to develop an informative evaluation system. Grasslands occur over a significant area in Hungary, representing 14.2% of the total agricultural area (Dér *et al.*, 2008). The issue of an appropriate evaluation of grasslands has not been solved, so predicting grassland yields is difficult. Both mowing and grazing, as means of land use, are regulated and have close connection to environment-friendly farming. A new grassland evaluation method should be introduced, which can be used for both the low or extensive agricultural production areas and for vulnerable or high nature value areas. An important part of the choices in farm management relies on the actual quality and value of the land, instead of the old qualification (established more than 100 years ago) based mainly on geographical position (Várallyay, 2003). In the past, several scientists set up methods for estimating grassland quality and value, from Braun-Blanquet (1954), to the new methodology published by Ren *et al.* (2008). In Hungary, Máté (2003) and Tóth *et al.* (2007) should be cited, but their methods approach the quality of arable land and grasslands only by means of productivity, without evaluation of sustainability and conservation values. During the last five years, a new method called D-e-Meter was developed in Hungary, which hopefully will be suitable for the estimation of characteristics of both the arable lands (crops) and grasslands. This method has been only partially developed up to now. The aim of our study was to introduce inside D-e-Meter and clarify a new land estimation method called 'Grassland module', which can meet the requirements of recent sustainable guidelines of agricultural land use. The main aim was to focus on the 'land use' and 'land quality' points of view.

Materials and methods

Evaluation of the 'Grassland module' of D-e-Meter began in 2005. Soil analyses (nutrient and water content) were performed from autumn 2005, till autumn 2008 in five plots at Kaposvár University Farming Centre. Results on soils, yields and carrying capacity were published in a previous work (Hoffmann *et al.*, 2014). Based on such results, we focused on the theoretical background of the mentioned estimation method discussed in present work. Due to limited space only one example of the estimation and calculation is given in the results. We investigated the following variables: botanical composition (Braun-Blanquet method), dry matter yield (t/ha), soil water features (rainfall, water table depth), agro-ecological districts, slope gradient category, date of establishment, annual weather features (temperature, rainfall), farming intensity, nature conservation status, and forage quality (nutritive values measured with Weendei analysis). These factors can have positive or negative effects on grassland dry matter yield and carrying capacity, as supported by relevant literature (Vinceffy, 1995) and earlier observations of the authors (Dér *et al.* 2008). The first results of D-e-meter application are reported in Dér *et al.* (2008).

Results and discussion

Table 1 presents the classification of Hungarian grasslands resulting from potential dry matter yields (t ha^{-1}). Classification was refined afterwards using the 'tuning' factors of Table 2 as specified hereafter. Specifically:

1. Quality factor: grasslands are complex phytocoenoses with different nutritive values. Their yields were modified by multiplying by a factor based on annual species composition and ranging between 0.8 and 1.
2. Proportion of plants valuable from nutritional point of view based on percent cover (factor ranging between 0.8 and 1.2).
3. Soil water management: grass production is frequently limited by low levels of rainfall and their temporal distribution. We introduced a water-table depth factor varying between 0.5-1.
4. Agro-ecological districts: based on seven main agro-ecological districts and its 35 sub-districts of Hungary, we set a factor varying between 0.6 and 2.
5. Slope: since the possibility increases on steep slopes, we introduced a factor varying between 0.6 and 1.2.
6. Date of establishment: soil management, fertilization and grassland composition affect productivity, so that for the year of establishment we set the factor to 0.8 because of reduced cover, while from the second year the factor varied between 1.2 and 1.6.
7. Year effect: based on the effect of annual weather, the value was set to 0.8-1.2.
8. Intensification: intensive grasslands are well fertilized and frequently used (grazing or cutting), whereas extensive grasslands are unfertilized and seldom used. The values ranged between 0.7 and 1.5.
9. Quality: grassland nutritive value changes according to botanical composition. We introduced a quality factor varying between 0.7 and 1.5 to better assess grassland real value.
10. Use (grazing or cutting): this factor is affecting mainly species composition and potential dry matter yield consequently. It ranges between 0.8 and 1.2.

One example of the estimation and calculation results is compiled in Table 3 for one location and three years. The mentioned classification will be adapted and will be used to improve agricultural practice in the near future. This method can make land use planning easier in particular cases.

Table 1. Distribution and utilisation of Hungarian grasslands according to Dér *et al.* (2008), modified.

Designation of the grassland	Categories	Size (1.060 ha)	Productivity (t ha^{-1} DM)
Productive grassland	Unfertilized, or barely fertilized grasslands with medium productivity	54%	3-7
	Frequently fertilized grasslands with high productivity	3%	8-14
Protected and/or vulnerable grassland	Strictly protected grasslands	3%	No data
	Vulnerable, and other natural and seminatural grasslands	15%	2-4
	Soil protecting grasslands	25%	1-2

Table 2. Modifying factors and multiplying factor based on Fábián *et al.* (2010).

Dimension	Factor value
Starting point: potential dry matter yield (t ha^{-1})	0.9-4.0 t ha^{-1} (most common 36 types)
Quality factor	0.8-1.0
Valuable plant proportion (cover factor)	0.8-1.2
Soil water management	0.5-1.0
Agro-ecological districts	0.6-2.0
Gradient (slope) category	0.6-1.2
Grassland establishment date	In the first year 0.8; after 1.2-1.6
Year effect	0.8-1.2
Farming intensification	0.7-1.5
Purpose of use (grazing or cutting)	0.8-1.2

Table 3. Example of estimation and calculation of dry matter in one location for three years.

Plot name, Leader grass species	Egyenestető, *Dactylis glomerata*					
Year	2006		2007		2008	
	factor	DM t ha^{-1}	factor	DM t ha^{-1}	factor	DM t ha^{-1}
Dry matter (DM) based on the old qualification system	-	3.00	-	3.00	-	3.00
Modified with quality factor	0.90	2.70	0.90	2.70	0.90	2.70
Modified with cover factor	1.10	2.97	1.10	2.97	1.00	2.70
Modified with soil water factor	0.90	2.67	080	2.38	0.90	2.43
Modified with agro-ecological districts factor	1.60	4.28	1.60	3.80	1.60	3.89
Modified with slope factor	1.20	5.13	1.20	4.56	1.20	4.67
Modified with establishment date factor	0.80	4.11	1.60	7.30	1.60	7.46
Modified with year effect	1.20	4.93	0.90	6.57	1.00	7.46
Modified with farming intensification factor	1.00	4.93	0.70	4.60	1.00	7.46
Modified with grazing or cutting factor	1.20	5.91	1.00	4.60	1.00	7.46
Estimated DM yield t ha^{-1}		5.91		4.60		7.46
Measured DM yield t ha^{-1}	-	5.80	-	4.80	-	7.70
Difference between estimated and measured DM yield		-0.11		0.20		0.24

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Improving short grass prairies by contour furrowing in Northern Sonora, Mexico

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Abstract

The study was conducted at Cananea, Sonora, Mexico to monitor changes in vegetation in a *Bouteloua-Aristida* grassland with and without contour furrowing. Changes were monitored in triplicate in three permanent plots 400 m^2, each. Plant density, height, basal coverage and forage production were monitored during the summers of 2013-2014 on five permanent quadrats of 1 m^2 per plot. Forage production was estimated by clipping in ten quadrants of 1 m^2 per plot. A randomized block design was used and the information was analysed by ANOVA ($P \leq 0.05$). The precipitation was near average during the study period. All variables were higher ($P \leq 0.05$) in the contour furrowed areas than in the control. Plant density averaged 9.6 plants m^{-2} in the control plots and had increased by 65% two summers later on the contour furrowing areas. Plant cover and height increased by 37.5 and 46.8% after two summers in the contour furrowing treated plots. Total production averaged 724 kg forage dry matter (DM) ha^{-1} in the control and 1,243 kg DM ha^{-1} in treated plots, and it increased by 71.6% in the contour furrowed areas. We conclude that water harvesting by contour furrowing is an effective way to promote the establishment, development and productivity of grasses in short grass prairies.

Keywords: deterioration, short grass prairies, forage production, water harvesting, contour furrowing

Introduction

Grasslands are of ecological and economic importance and constitute strategic areas for the cattle industry of North America. They are plant communities where short and medium-sized grasses predominate with few trees and shrubs. The main genera are: *Bouteloua*, *Buchloe*, *Andropogon*, *Eragrostis*, *Leptochloa*, and *Hilaria*, and among the shrubs and trees the genera *Prosopis*, *Acacia*, *Mimosa*, *Fouqueria*, *Juniper* and *Quercus*. They are found in semi-arid regions with a cold temperate climate, with average annual temperatures of 12 to 20 °C and mean annual precipitation of 300-600 mm. Most of the grasslands are used for breeding cattle, horses, and for wildlife, and are considered sources of feed, fibre and fuel. They contribute to climate regulation, pollination, purification, aquifer recharge, control of invasive species and carbon sequestration. Grasslands reduce the negative impact of raindrops on bare soil, maximizing water infiltration and reducing their loss through runoff, which prevents nutrient loss due to erosion. They play a major role in the recharge of aquifers and supporting habitats of various species of reptiles, migratory and resident birds and other wildlife (Milchunas and Lauenroth, 1993), and are key factors in mitigating the effects of climate change by preventing soil degradation. Intensive cattle breeding on grasslands characterized by intense and frequent overgrazing has caused strong changes in most of the palatable and forage grass species. The goal of this research is to evaluate if water harvesting by contour furrowing will enhance plant establishment and forage production in short grass prairies.

Material and methods

The study was conducted during the summers of 2013 and 2014 at the University of Sonora Experimental Ranch, located 15 km east of Cananea, Sonora, (30° 58'00 'N and 110° 08'30' W), in a short grass prairie of regular condition, at an elevation of 1,417 m a.s.l. The site has relatively uniform topography with low planes and hills, and slopes varying from 3 to 7%. The climate is temperate semi-dry BS1 kw (x') (e')

(Garcia, 1973). Mean annual precipitation and temperature is 520 mm and 16.3°C, respectively. The vegetation consists of native grasses. An area of 1 ha was selected, where 6 experimental plots (20×20 m) each were located, three in areas with contour furrows and three in flat areas (control). The furrows were constructed on level curves at intervals of 30 to 50 cm of unevenness, with an 8-disc plough of 90 cm diameter each. The experimental area was excluded from cattle grazing.

Plant density, basal cover and height of the plants were monitored during the summer of 2013 and 2014, in 5 permanent quadrats 1 m^2 per plot. Plant density and basal cover were estimated for each species by counting the total number of plants and the sum of the crown of all the plant species present, respectively. Forage production was estimated by clipping in 10 quadrats of 1 m^2 each, randomly distributed per plot. A completely randomized block design with two treatments and three replicates was used. All variables were analysed using ANOVA ($P \leq 0.05$). Statistical analyses were run using the SAS package (SAS, 1988).

Results and discussion

Precipitation at the study site was below the regional average mean (512 mm) 395.0 and 429.0 mm in 2013 and 2014 respectively. The density of plants per species and the total density was significantly higher ($P \leq 0.05$) in plots treated with contour furrows than in the control (Table 1). All grass species increased their density in plots where rainwater was retained. Total plant density was 64.6% higher in the contour furrowed areas than in the control. The height of plants was also different ($P \leq 0.05$) between treatments. All pasture species reached greater height in the plots where furrows were drawn and the water was retained, relative to the control plots. Plants were 46.8% higher in the contour furrowed areas. The basal cover of plants was also different between treatments ($P \leq 0.05$). All species reached greater basal coverage in plots where contour furrows were drawn and rainwater was retained, relative to the control. The total basal cover of plants was 37.0% higher in furrowed areas than in the untreated control.

Forage production of plants by species and the total forage production of all species was higher ($P \leq 0.05$) in the plots treated with contour furrows than in control. Forage production was increased by 71.6% in plots where furrows were constructed to retain rainwater, representing an additional 519 kg of dry matter per ha. The already established grasses were favoured by the additional moisture in the soil as a result of the retention of rainwater with the construction of contour furrows. All species showed higher crown growth, height, as well as increased forage production, as compared to the control. These results agree with Ali *et al.* (2010), who indicates that water harvesting in micro-structures in the soil captures excessive runoff, available for subsequent use by plants, helping the development of roots and foliage and reducing the mortality rate of seedlings. Forage production increases of 380 to 1,151 kg ha^{-1} have been reported with the use of water retention practices (Abu-Zanat *et al.*, 2004). For instance, in Sudan, increases of 2.25 to 3.65 Mg ha^{-1} of forage are reported in ditches with retention of rainwater, compared with the control 0.65 Mg ha^{-1} (Ezzat *et al.*, 2013).

Table 1. Total plant density, plant height, basal cover and forage production of grass species in two summer growing seasons after the construction of contour furrows on a short grass prairie at Cananea, Sonora, México.

Variables	**Treatments**[1]	
	Contour furrows	**Untreated control**
Plant density (number of plants m^{-2})	15.8 a	9.6 b
Plant height (cm)	106.4 a	72.5 b
Basal cover (%)	12.2 a	8.9 b
Forage production (kg dry matter ha^{-1})	1,243.5 a	724.5 b

[1] Means among treatments with the same letter are not significantly different ($P \geq 0.05$) according to Duncan's test.

Conclusions

The construction of contour furrows in the soils of Short Grass Prairie favoured the basal growth and height of the pasture grasses, which together increased individual and total pasture forage production. The practice allowed the capture and conservation of additional water for the plants, compared with the control areas, increasing the germination and ensuring the establishment of new seedlings. The rainwater harvested resulted in an increase in grass density, height, basal cover and biomass production of the plants in soils with potential runoff, which increases the potential of production for the feeding of the livestock and wildlife.

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The effect of mowing time on flower resources for pollinators in semi-natural hay meadows of high nature value

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Abstract

Semi-natural grasslands are important habitats for pollinators because of high abundance of flowering plants. The aim of this study was to assess effects of mowing time on flower resources for pollinators in semi-natural hay meadows. Flowering species in semi-natural hay meadows throughout a landscape in Maramures, Romania were used as indicators of flower resources for pollinators. Botanical surveys were performed in 31 hay meadows and all herbs and shrubs were recorded in hay meadows cut early, intermediate and late in the growing season. All hay meadows included a high number of flower species but the mowing time influenced the available floral resources for pollinators. If mowing time varies between hay meadows within a landscape, flower resources for pollinators will be available throughout the growing season, which is essential in pollination conservation in agricultural landscapes.

Keywords: pollination, hay meadows, semi-natural grasslands, flower resources, mowing time

Introduction

Pollination is an ecosystem service of vital importance for human well-being because of its importance for global food production. Populations of insects are declining because of multiple anthropogenic pressures and this decline can have strong economic and environmental consequences. Highly intensive agricultural practices have negative effects on bee abundance and richness (Kremen *et al.,* 2002; Steffan-Dewenter and Tscharntke, 1999). Semi-natural hay meadows, however, represent low-intensity land use that several pollinators are benefitting from because such meadows provide both nesting sites and floral resources (Kovács-Hostyánszki *et al.,* 2016). Increase in flower diversity and density has been associated with higher bee abundance and diversity (Holzschuh *et al.,* 2007; Potts *et al.,* 2003; Steffan-Dewenter and Tscharntke, 2001) and floral diversity leads to wider range of foraging niches for various pollinators (Fenster *et al.,* 2004; Holzschuh *et al.,* 2007). High floral diversity throughout the growing season helps to ensure nectar resources for pollinators during the whole season (Ebeling *et al.,* 2008; Nicholls and Altieri, 2013). We therefore hypothesise that varying heterogeneous cutting times in an agricultural landscape will benefit pollinators. The aim of this study was therefore to assess the effect of hay mowing times on flower resources for pollinators in an agricultural landscape, which includes several semi-natural grasslands.

Materials and methods

The study was performed in an agricultural landscape with a high density of semi-natural hay meadows in the village of Botiza in Maramures, Romania (47°40'05.30'N, 24°09'04.27'E). A mosaic of small parcels of low-intensity hay meadows characterizes the landscape in the study area. The study area represents a typical traditional land use system. Richness of flowering herbs and shrubs, and proportion of such species flowering were used as indicators of flower resources for pollinators. Botanical surveys were performed on 3 August 2016 in 31 semi-natural hay meadows (1×1 m plots) and all flowering herbs and shrubs and the number of their inflorescences were recorded in hay meadows cut early (beginning of July; 10 meadows), intermediate (late July; 10 meadows) and late (after mid-August; 11 meadows).

General linear models were used to estimate the influence of mowing time on the number of flowering species (Poisson distribution) and proportion of species flowering (binomial distribution) using the R 3.1.1 software (R Core Team, 2015).

Results and discussion

All the studied hay meadows had a high flower species richness (mean = 20.032; standard deviation = 4.231) that did not vary among mowing time categories (P=0.41). The proportion of species flowering varied among the mowing time categories (P<0.001) and was highest in hay meadows cut early and lowest in the intermediate mowing time category (Figure 1).

These results indicate that varying the mowing times within a landscape benefits pollinators. First, a high plant species richness in all the hay meadows studied represents flower resources for the pollinators throughout the landscape. Secondly, by mowing at different times floral resources are available for pollinators during the whole season. The semi-natural grasslands in the study area were located in a continuous agricultural landscape with short distances between hay meadows mowed at different times. Hence, floral resources were available for pollinators at a local scale throughout the season. A spread of mowing times may have consequences for hay digestibility and yield (Flaten *et al.,* 2015). Even so, the farmers in the study area used this practice because there is insufficient labour for cutting all the meadows at the same time.

The study area represents a farmland with low-intensity agriculture and high biodiversity that is uncommon in Europe today. Land use change from low-intensity to highly intensive farming is one of the strongest drivers for biodiversity loss in Europe (Stoate *et al.,* 2009). Landscapes with low-intensity farming, as in Botiza, therefore have strong conservation policy relevance in situ but also for generating knowledge about management methods that can be used for conservation of hay meadows in other parts of Europe.

Conclusions

Pollinators depend upon floral resources during the whole growing season. Regardless of mowing time, all hay meadows are species rich, but the available flower resources for pollinators depend upon the mowing time. Our study demonstrated that having different mowing times in a landscape yields floral resources for pollinators throughout the season, and that mowing times need to be considered in the conservation of pollinators in agro-ecosystems. Pollinators are threatened due to land use change and agricultural intensification. Hence, the few landscapes that remain in Europe with low-intensity farmland, as in Romania, give a unique opportunity to generate knowledge about management methods used in conservation of pollinators.

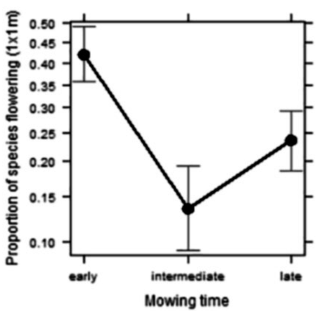

Figure 1. Estimated effects and standard errors for proportion of species flowering in semi-natural hay meadows cut early, intermediate or late in the growing season.

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Effects of irrigation on phosphorus in soil, soil microbes and plants of semi-natural grasslands

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Abstract

Water and phosphorus (P) are two critical resources for high nature value farming in semi-dry mountains. They are major drivers of grassland productivity but can negatively affect plant diversity. Soil moisture is known to affect soil microbial activity and plant P nutrition but the effects of irrigation on soil P status, soil microbial P and plant P nutrition in semi-natural grasslands remain largely unexplored, which impedes the optimization of fertilization. The aim of this study was to assess the mid-term effects of irrigation on P availability in the soil, the size of the soil microbial P pool and plant P nutrition in semi-dry mountain grasslands. At the end of a five-year irrigation-fertilization factorial experiment on eleven semi-natural grasslands of an inner-alpine valley, soil P availability and herbage P content did not significantly differ between the irrigated and the not irrigated treatments. Fertilization increased herbage P content from 2.2 to 3.1 mg P kg^{-1} dry mass (P<0.001). The size of the microbial P pool remained unaffected by irrigation and fertilization. We conclude that for semi-natural mountain grasslands, no correction factor for irrigated compared to not irrigated meadows is necessary to estimate the P fertilization requirement per unit of herbage yield.

Keywords: irrigation, fertilization, P availability, soil microbial P, nutrition index

Introduction

Irrigation and fertilization of mountain grasslands must be finely tuned in order to save resources and because mountain ecosystems are sensitive to agricultural inputs. Grassland-based ruminant production strategies require herbage that has adequate P content. In the short-term, water shortage may negatively affect grassland P uptake (Meisser *et al.*, 2013). In the mid-term, irrigation might nevertheless decrease the size of the plant available P pool because of greater P offtake and losses (Condron *et al.*, 2006). Moreover, water availability might alter the competition for P between plants and soil microorganisms (Dijkstra *et al.*, 2015). The impact of irrigation on grassland P nutrition thus remains difficult to quantify. The objective of this study was to assess the mid-term effects of irrigation on P availability in the soil, the size of the microbial P pool, and plant P nutrition in semi-dry mountain grasslands.

Materials and methods

The study was carried out on 11 semi-natural montane to subalpine hay meadows on loam soils from an inner-alpine valley with continental climate and annual average precipitation of 570 mm. Four treatments, arranged in a RCB design with the 11 meadows used as blocks (no replicates within meadow), were applied from 2010 to 2015. These treatments were I_0F_0: neither fertilized nor irrigated; I_1F_0: only irrigated; I_0F_1: only fertilized; and I_1F_1: both irrigated and fertilized. Irrigation was 20 mm water per week from May to mid-September, except when over 20 mm of rain fell during the previous week. Fertilization was applied as manure pellets dissolved in water with an N:P:K ratio of 1.0:0.36:2.7. The fertilization level was adjusted to the productivity potential of the meadows, which correlates with altitude (880 to 1,770 m), by categorizing them into three altitudinal groups with fertilization levels of 1.0, 0.75 and 0.5 from the lowest to the highest altitudinal group. For P, this resulted in yearly inputs of 19.4, 14.5 and 9.7 kg P ha^{-1} respectively. In spring 2016, plant samples were harvested when the dominant grass species were at late boot to mid-heading stage. Plants were sorted into grasses, legumes and forbs, and analysed for P and N content. The P nutrition index (PNI) of the grass fraction was calculated following Duru and Ducroq (1997). For the soil, we show the results for the 0-5 cm soil layer sampled in spring 2016. The pool of P extractable with anion exchange resin (Resin P) was used as an indicator of plant available soil P (Myers *et al.*, 2005). For resin extraction, field-moist soil samples were shaken with distilled water and a resin-strip for 16 hours at a soil:water ratio of 1:15. Soil microbial P was assessed by the difference between the amount of P extracted by hexanol-fumigation combined with resin extraction and the amount of P extracted by resin extraction without hexanol (Kouno *et al.*, 1995). Hexanol-labile P was corrected for incomplete P recovery due to sorption by adding a P-spike of 50 mg P kg^{-1} soil to a third subsample. Soil organic carbon (SOC) was extracted by the Walkley-Black method. The effects of irrigation and fertilization were analysed by ANOVA with the irrigation-effect as irrigated (I_1F_0 and I_1F_1) vs not irrigated (I_0F_0 and I_0F_1), and the fertilization-effect as fertilized (I_0F_1 and I_1F_1) vs unfertilized (I_0F_0 and I_1F_0). The meadows were included as random factors in the model. No significant irrigation × fertilization interaction was observed.

Results and discussion

Irrigation and fertilization increased P offtake by 28% (P<0.05) and 131% (P<0.001) respectively at the last harvest of the treatment application period (summer 2015). Fertilization had a carry-over effect on biomass in spring 2016, but irrigation did not (Table 1). Soil P availability (Resin P) was low in the unfertilized treatments and, correspondingly, herbage P content was substantially improved by fertilization (Table 1).

The five-year period with summer irrigation did not affect herbage P content in spring. This contrasts with the short-term results of Meisser *et al.* (2013) who observed a substantially larger herbage P content after a growing season without drought stress than following a severe summer drought. Resin P was not yet affected by irrigation, which is in contrast to the long-term (52-years) results of Condron *et al.* (2006). Resin P explained 51% of the variability in PNI of the grass fraction, with a similar relationship for the irrigated and the non-irrigated plots (Figure 1). Microbial P remained unaffected by the treatments (Table 1). Converted to kg ha^{-1}, the size of the microbial P pool was approximately 4.5 times larger than the average annual P fertilization, which shows the importance of this P pool in permanent grassland soils. Soil microbial activity is known to influence plant P availability (Dijkstra *et al.*, 2015). In our experiment, soil P availability and herbage P content were not correlated to the size of the microbial P pool (data not shown). SOC content was, on average, higher than 5% (Table 1) and it explained only 29% of the variability in soil microbial P (Figure 2). Further studies are thus required to assess the main factors of microbial P variability in grassland soils rich in organic matter.

Table 1. Standing biomass, herbage P content and P nutrition index (PNI) in the spring succeeding five years of contrasting irrigation and fertilization, as well as the content of resin-extractable P, microbial P and organic carbon (SOC) in the 0-5 cm soil layer. The irrigation-effect is the contrast of (I_1F_0, I_1F_1) vs (I_0F_0, I_0F_1) and the fertilization-effect the contrast of (I_0F_1, I_1F_1) vs (I_0F_0, I_1F_0).[1]

Treatment	Biomass (t DM ha^{-1})	Herbage P (g kg^{-1} DM)	PNI	Resin P (mg kg^{-1} soil)	Microbial P (mg kg^{-1} soil)	SOC (%)
I_0F_0	1.36	2.2	51	1.5	88	5.5
I_1F_0	1.40	2.3	51	1.4	95	5.0
I_0F_1	2.03	3.1	78	7.4	88	5.8
I_1F_1	1.74	3.0	79	5.2	94	5.3
Mean SD	0.48	0.5	13	2.3	18	1.6
Irrigation-Effect	ns	ns	ns	ns	ns	ns
Fertilization-Effect	***	***	***	***	ns	ns

[1] DM = dry matter; SD = standard deviation; *** P<0.001; ns = not significant.

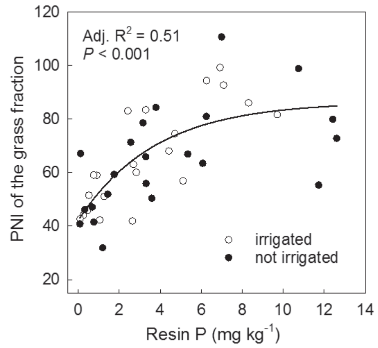

Figure 1. Relationship between the P nutrition index (PNI) of the grass fraction and the resin P in the topsoil layer (0-5 cm).

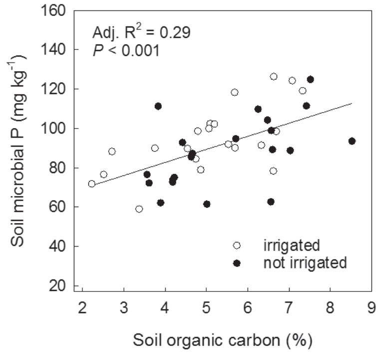

Figure 2. Relationship between the soil microbial P content and the soil organic carbon content in the topsoil layer (0-5 cm).

Conclusions

After five years of experimentation in semi-dry mountain grasslands, a weekly irrigation of 20 mm during the dry season did not affect soil P availability, the size of the microbial P pool, or plant P nutrition. We thus conclude that for semi-natural mountain grasslands, no correction factor for irrigated compared with non-irrigated meadows is necessary to estimate the P fertilization requirement per unit of herbage yield.

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Interspecific hybridisation of white clover and Caucasian clover confers grazing tolerance

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Abstract

The rhizomatous growth habit of Caucasian clover (*Trifolium ambiguum* M. Bieb.) has been introgressed into a white clover (*T. repens* L.) background by interspecific hybridisation and a programme of backcrossing and selection. Previous studies have demonstrated the utility of such hybrids in improving persistence in marginal areas through enhanced tolerance of water stress. In the present study the response of these hybrids to grazing was investigated. Dry matter yield and persistence of hybrids was compared with the performance of conventional white clover varieties. The results of the study indicate that *T. ambiguum* × *T. repens* hybrids show enhanced persistence under grazing. It is postulated that this enhanced persistence is conferred by the presence of rhizomes in the hybrids.

Keywords: interspecific hybrids, marginal areas, rhizomatous growth, stress tolerance

Introduction

Caucasian clover is a close relative of white clover and is indigenous to the alpine Caucasus region (Taylor and Smith, 1997). It is noted for its cold tolerance, water-stress tolerance, pest tolerance and disease resistance, as well as its ability to thrive on steep slopes (Duke, 1981; Marshall *et al.*, 2015). These qualities are attractive, particularly for upland areas, but it has not been widely adopted as a forage legume due to its slow establishment and rate of growth (Abberton, 2007; Speer and Allinson, 1985). Of particular note is its rhizomatous growth habit, which is thought to play a role in stress tolerance (Speer and Allinson, 1985).

One strategy to combine the desirable traits of Caucasian clover with the higher yield of white clover is to produce interspecific hybrids. While pollen of white clover readily fertilises Caucasian clover ovules, the resulting embryos abort at an early stage and do not naturally develop into mature seed. Thus the development of hybrid varieties is not an easy process. Through embryo rescue techniques and a programme of backcrossing, the first hybrid variety, AberLasting, has been developed. The response of this variety to grazing has been investigated in this study.

Materials and methods

AberLasting, a hybrid *T. repens* × *ambiguum* variety, was bred as described by Marshall *et al.* (2001). F1 interspecific hybrids were established by hand crossing and ovule culture. Progeny selected for rhizomatous growth habit were backcrossed over two generations using white clover (cv. Menna) as the recurrent parent. The synthetic cultivar was stabilised at BC2 Syn3. Field plots (5×5 m) of AberLasting and three comparable small-medium leafed white clover varieties/breeding lines (AberHerald, Crusader and Ac 4835) were sown in replicate plots (4) in a randomised complete block design at a lowland site in 2013 at Gogerddan, Aberystwyth, Wales (52°44N, 04°27W; 20 m a.s.l.). The soil at the site was freely drained acid brown earth of the Rheidol series, pH 6.26, P 25.47 ppm; K, Mg and Ca concentrations were 0.29, 1.20 and 7.40 meq 100 g^{-1} respectively. Plots were sown by broadcasting the seed of AberLasting and the three white clover varieties at a rate of 3 kg ha^{-1} with the perennial ryegrass (*Lolium perenne* L.) variety AberMagic at 33 kg ha^{-1}. On establishment, fencing was erected, bisecting the plots such that there was a 3 m width plot in the contained grazing area and a 2 m width plot on the external area to be managed by cutting.

The external cut plot areas were managed as per UK National List protocols and harvested with a Haldrup forage harvester at a cutting height of 5 cm. In the internal grazed plot areas, sheep (various breeds) were introduced at the time of the first harvest cut and plots were grazed to a target sward height of 5 cm. Circular exclusion cages (1 m diameter) were used to assess sward growth on the grazed areas of the plots. These were moved after each cut to a new location in the plot and the excluded section harvested with a reciprocating blade mower. Fresh weights of the harvested forage and the separated grass and clover fraction was analysed on a 200 g subsample from each plot. Dry matter yields were calculated after drying the subsample in a forced draught oven at 80 °C for 24 h. Stolon and rhizome characteristics were examined in the third harvest year by collecting soil cores (5 from each plot) and manual separation and measurement of stolon and rhizome. Ground cover percentages were visually estimated at the end of the third harvest season. Data were analysed in AGROBASE Generation II version 38.8.1 using a completely balanced analysis of variance.

Results and discussion

Ratios of total clover dry matter yield over three years under the grazing management to total clover DM yield over three years under the cutting management are shown in Figure 1. There was no significant difference between the three conventional white clover varieties with grazed yield being approximately 1.6-1.7 times higher than under the cutting management. The higher yields measured in the grazed area of the plots is most likely to be due to nutrient returns to the sward from livestock. AberLasting, on the other hand, had an increased grazed/cut clover yield ratio of 2.5±0.4. No preferential grazing was observed between varieties.

Morphological characteristics of the varieties under the cutting and grazing managements at the end of the third harvest year are shown in Table 1. The conventional varieties show a higher stolon density and a higher ground cover percentage than AberLasting under the cutting management. This pattern is reversed under the grazing management where the conventional varieties had a lower ground cover relative to the cutting management, while AberLasting had a greater stolon density and a higher ground cover percentage under grazing.

The difference in performance of AberLasting in comparison with the conventional white clover varieties is probably due to an enhanced ability of its stolon network to regenerate following grazing. While the stolons of conventional varieties are prone to being uprooted as sheep graze, the rhizomes of AberLasting are retained in the soil and provide a refuge from where the plants can regrow. This suggests that the variety AberLasting, and material developed from this, will have great potential in improving the ability of white clover to tolerate grazing particularly in environments where white clover is challenged by moisture stress.

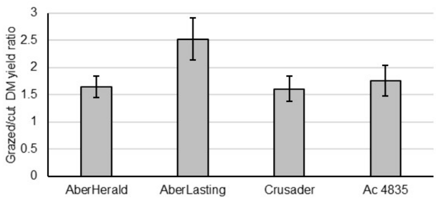

Figure 1. Mean ratio of 3-year total dry matter (DM) yield under the grazing management to total annual dry matter yield under the cutting management ± standard error of the mean (P<0.05).

Table 1. Morphological characteristics of varieties at end of the third harvest year. Figures are quoted in length (m) of stolons or rhizomes per $m^2 \pm$ standard error of the mean (SEM) and percentage ground cover ± SEM.[1]

	Stolons ($m\ m^{-2}$)		Rhizomes ($m\ m^{-2}$)		Ground cover (%)	
	Cut **	**Grazed** ***	**Cut** ***	**Grazed** ***	**Cut** **	**Grazed** **
AberHerald	110.3±13.0	7.6±4.7	0	0	74±6	13±3
AberLasting	43.3±9.2	62.2±8.5	7.2±1.5	5.6±1.3	28±3	50±8
Crusader	61.8±3.7	21.4±4.4	0	0	60±7	28±8
Ac 4835	66.6±5.7	14.8±3.4	0	0	73±11	16±3

[1] Significance: ** = $P<0.01$; *** = $P<0.001$.

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Feasibility study of directly georeferenced images from low-cost unmanned aerial vehicles for monitoring sward height in a long-term experiment on grassland

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Abstract

Monitoring sward height is an important step in the assessment of overall grassland quality and quantity. A common approach involves measuring compressed sward height with a rising plate meter (RPM), which has a limited spatial coverage. The concept of multi-temporal Crop Surface Models (CSM) based on unmanned aerial vehicles (UAV)-imaging offers non-invasive near real-time analysis of vegetation with high spatial and temporal resolution. CSMs provide similar information as RPM measurements, but require precisely measured ground control. The rapidly evolving technology of UAVs, however, provides low-cost systems with direct georeferencing of images during flight with a moderate accuracy. Therefore in this study we evaluated the accuracy of sward height derived from directly georeferenced images of a low-cost UAV in an experimental grassland field in Germany. The good correlation of RPM based sward height and UAV-based sward height (R^2 = 0.86) indicate that an automated monitoring approach for sward height is possible.

Keywords: sward height estimation, rising plate meter, UAV, direct georeferencing

Introduction

The non-destructive estimation of forage or herbage mass is of key interest for the management of grasslands. The usage of rising plate meters (RPM) is well established. It is a robust method for non-destructive forage mass estimation, which produces the compressed sward height representing a height/density value (O´Donovan *et al.,* 2002). The disadvantage of RPMs is in their limitation of spatial coverage. Using RGB images captured by unmanned aerial vehicles (UAVs) is a means to facilitate non-invasive near-real-time analysis of vegetation with high spatial and temporal resolution. The concept of multi-temporal Crop Surface Models based on UAV images is well suited to estimate plant height, as shown for barley and rice by Bendig *et al.* (2013) and is also transferable to grasslands (Bareth *et al.,* 2015). This concept requires precisely measured ground control, which cannot always be guaranteed. However, the rapidly evolving technology of UAVs provides low-cost systems with direct georeferencing technique, thus omitting the need for referencing the images to a coordinate system manually. Direct georeferencing of image data from UAVs has several advantages: It allows flights in remote areas and areas with difficult access and reduces flight preparation time, since no ground control points (GCPs) need to be installed in advance. Finally, direct georeferencing reduces human interaction in the processing chain to the final data product, thus allowing an automated approach (Turner *et al.,* 2014). Therefore, the main objective of this contribution was to evaluate the capabilities of a low-cost UAV with direct georeferencing to predict sward height from digital surface models (DSM).

Materials and methods

The study was conducted in 2016 on the Rengen Long-term Grassland Experiment (RGE), established in 1941 in the Eifel region in Germany. The experimental setup comprises five fertilizer applications (Ca, CaN, CaNP, CaNPK_2O, CaNPK_2SO_4) with ten replicates in two separate blocks, resulting in 55 plots (3×5 m) (Schellberg *et al.,* 1999). For this study 25 plots were selected for investigation.

Image data were collected with a DJI© Phantom 3 Professional (P3Pro) on 4 April and 18 May 2016 (flight altitude 20 m). The P3Pro is a low-cost, low-weight consumer grade UAV. The manufacturer reports a horizontal accuracy of ±1.5 m and ±0.1 m vertical accuracy of the UAV´s positioning system. The vertical accuracy refers to the ability of the P3Pro to hold a constant position above ground level, not the GPS-recorded altitude information.

18 GCPs were installed on the RGE and measured with a highly accurate real time kinematic (RTK) positioning system. To derive sward height information from UAV images, 134 multiple overlapping images per sampling date were processed in the Structure from Motion software AgiSoft Photoscan® v1.2.6. The computed DSM for 18 May 2016 were georeferenced to the DSM of 4 April 2016 to match their relative positions to one another for further analysis. Differences in elevation information of the DSMs were normalized using a simple approach. The elevation-value of the UAV take-off point – which was the same in both campaigns – was subtracted pixelwise from the respecting DSM. The sward height was then computed by subtracting the normalized base-DSM (4 April) from the normalized DSM of the second campaign (18 May). Mean sward height per plot was extracted by the zonal statistics tool of ArcGIS®. For the evaluation of UAV-based sward growth, sward height was measured manually with a RPM. Four measurements per plot were randomly collected and averaged to obtain mean sward height per plot.

Results and discussion

Compared to the RTK measurements of the GCPs, the DSM of 4 April had a horizontal root mean square error (RMSE) (x-y direction) of 0.85 m and a vertical RMSE (z direction) of 8.3 m. Horizontal RMSE for 18 May was 0.86 m while the vertical RMSE was 34.4 m. Thus horizontal accuracy lies within the range stated by the manufacturer. As expected, the absolute altitude information measured by the positioning module of the UAV was not reliable enough to be used without accurate control points. The approaches described in the methods section were an easy and reliable way of referencing both sample dates relative to one another to extract meaningful information. This is shown by the results of the sward height prediction.

Compressed sward height data from RPM and UAV measurements were compared using the mean sward height per plot. The coefficient of determination shows a strong relationship between UAV and RPM derived sward height (R^2=0.86; Figure 1A).

It turned out from UAV-based sward height, that two plot-means were negative (plots fertilized with Ca/CaN, see Figure 1B), due to the height-normalization approach and low sward height. In general, all other values showed good correlation with the manual measurements. This indicates that, although the absolute altitude information recorded by the positioning system of the P3Pro had a high error, it was reliable relative to the ground level (within the range of ±0.1 m). Thus, variations of sward height in sub-decimetre range could be detected.

This study indicates the potential of directly georeferenced images from low-cost consumer grade UAVs to derive plant height information for monitoring purposes. These findings also indicate that an automated approach for monitoring grasslands is possible. Such remote sensing-based sward height estimates can also be applied in precision farming, where sward height and biomass are key parameters to account for in-field heterogeneity (Schellberg and Verbruggen, 2014). Further, the products derived from UAV-based off-the-shelf cameras can also be used to support grazing management, fertilizer application and yield estimation.

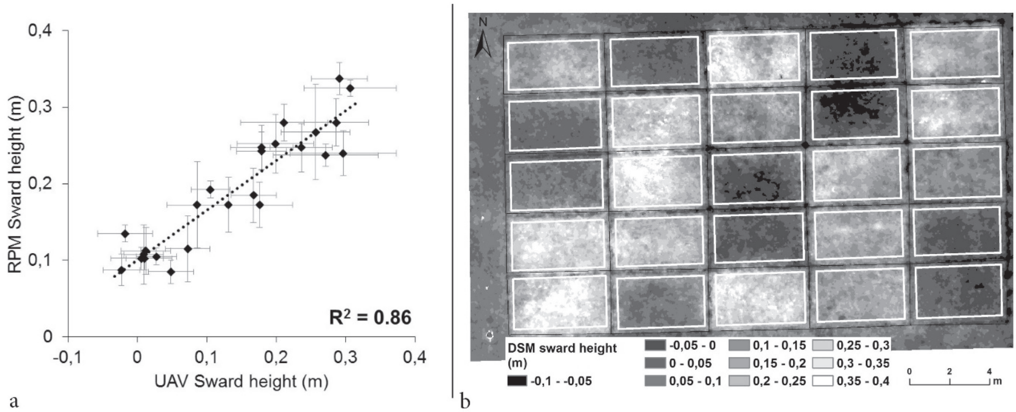

Figure 1. (A) Unmanned aerial vehicle-based sward height prediction vs RPM-based sward height (horizontal error bars indicate standard deviation of UAV-based sward height per plot, vertical error bars indicate standard deviation of RPM-based sward height per plot). (B) Digital surface model with pixel resolution of <2 cm representing sward height (in metres) on 18 May.

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The influence of spent mushroom substrate and cattle slurry on the forage value of alfalfa-grass mixtures

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Abstract

The study was conducted on experimental plots between 2012 and 2015 in a completely randomized arrangement and with three replications. The aim was to evaluate the effect of spent mushroom substrate and cattle slurry on productivity and nutritional value of dry matter of hybrid alfalfa-grass mixtures. In the experiment three plant species: hybrid alfalfa, cocksfoot and perennial ryegrass were used. These species were sown as three legume-grass mixtures and organic fertilizers, spent mushroom substrate and cattle slurry were used in different combinations. We determined dry matter yield of plants, as well as the content of three different fibre fractions. The results showed that slurry application produced the highest dry matter yield of the tested crop mixtures. Fibre fraction concentration in fodder was more affected by spent mushroom substrate than by cattle slurry. The best fodder quality in terms of content of NDF was in the mixture of perennial ryegrass with hybrid alfalfa.

Keywords: spent mushroom substrate, slurry, mixtures, ADF, NDF

Introduction

Poland is a leading producer of mushrooms, which results in large amounts of waste mushroom substrate, which has to be disposed of. The straw of rye and wheat, as well as poultry manure, waste gypsum, phosphogypsum and calcium carbonate are used to produce this substrate (Sakson, 2007). The storage of such hazardous waste can pose a danger to environment. Thus, the law regulates how to rationally store and use it. On the other hand, mushroom substrate is a valuable source of organic matter and plant nutrients, but it can be a problem for both Polish and overseas producers of mushrooms because very often they have no agricultural land to utilise it (Chia-Wei and Vikneswary, 2012; Rutkowska, 2009). Slurry is also a valuable fertiliser, quite different from solid manure, both in physical properties and in nutritional value. The aim of the experiment reported in this paper was to evaluate the effect of spent mushroom substrate and cattle slurry in the field and on the fibre content and yield of different alfalfa-grass mixtures.

Materials and methods

The research was based on a 3-year experiment carried out between 2013 and 2015 in the experimental station. It was set up on soil of the anthropogenic order, the culture-earth soil type and the hortisole subtype, with granulometric composition of loamy sand. The main experimental factors in the study were organic fertilisers (mushroom substrate and cattle slurry, used separately and in combination) and alfalfa-grass mixtures. Treatments were: control without fertilisers (0); spent mushroom substrate (SMS; 30 t ha^{-1}); cattle slurry (S; 60 m^3 ha^{-1}); SMS_{10}+$S_{60,}$ spent mushroom substrate (10 t ha^{-1}) + slurry (60 m^3 ha^{-1}); SMS_{20}+$S_{40,}$ spent mushroom substrate (20 t ha^{-1}) + cattle slurry (40 m^3 ha^{-1}); SMS_{30}+$S_{20,}$ spent mushroom substrate (30 t ha^{-1}) + cattle slurry (20 m^3 ha^{-1}). Three plant species were studied in the experiment: *Medicago sativa* L. (hybrid alfalfa) cv. Tula, *Dactylis glomerata* L. (cocksfoot grass) cv. Bora, and *Lolium perenne* L. (perennial ryegrass) cv. Info. These were sown in equal parts in the following mixtures: M1, hybrid alfalfa-cocksfoot-perennial ryegrass; M2, hybrid alfalfa-cocksfoot; M3, hybrid alfalfa-perennial ryegrass. The crops were harvested three times during each growing season. At each harvest the dry matter (DM) yield and the concentration of neutral detergent fibre (NDF) and acid detergent fibre (ADF) fractions were determined, using near-infrared spectroscopy (NIRS) together with the NIRFex N-500 spectrometer. Mushroom substrate was applied once, before the plants were sown at different seeding rates, and mixed with the soil. Slurry was applied before each harvest. Statistical analysis was performed using analysis of variance (Anova). Detailed comparison of means was done using the Tukey's test at the significance level of α=0.05.

Results and discussion

The DM yield (Table 1) was higher when slurry was applied separately (8.20 t ha^{-1}), but also when the plots were treated with 10 t of mushroom substrate together with 60 m^3 of slurry (8.09 t ha^{-1}). Comparing the yields from plots with all the fertiliser combinations, whenever natural fertilisers were used they significantly increased dry matter yield of hybrid alfalfa-grass mixtures. Barszczewski *et al.* (2011) found similar results in an experiment with grasslands.

Comparing crop mixtures from all experimental years and from plots with all fertiliser combinations (Table 2), the highest concentration of NDF (394 g kg^{-1}) was in the forage from the plot that received 30 t of mushroom substrate together with 20 m^3 of slurry. Mushroom substrate application significantly increased the concentration of NDF in the plants tested in the experiment. Wróbel *et al.* (2013) found contradictory results studying the effects of organic fertilisers on meadow grass, where manure and slurry application resulted in a decrease of the above parameter. As regards the effects of both of the organic fertilisers on ADF concentration in the biomass of the mixtures (Table 2), only the mixtures of ryegrass-alfalfa and cocksfoot-ryegrass-alfalfa had a higher concentration of fibre in the forage harvested in the plot that received mushroom substrate. The highest concentration of the ADF fraction (332 g kg^{-1}) was noted in the forage from the plot that received 30 t of mushroom substrate with 20 m^3 of slurry. In the forage from the plots with mushroom substrate, there was much more ADF than in the plots where slurry was used. Of the alfalfa-grass mixtures (Table 2) the highest concentration of ADF was in the forage from the plots with alfalfa and ryegrass growing together (321 g kg^{-1}).

Sosnowski (2012) found a similar concentration of ADF in a mixture of festulolium and alfalfa, where its amount constituted 31%. This percentage was also confirmed by Grzelak (2010) in an experiment with meadow hay. Tomic *et al.* (2012) found a much higher ADF fraction in cocksfoot (374 g kg^{-1}) than in perennial ryegrass (326 g kg^{-1}). Those results differ from the results found in the present experiment because alfalfa must have affected ADF concentration in the mixtures.

Conclusions

Yields of alfalfa-grass mixtures varied depending on the organic fertiliser applied. Fibre fraction concentration in fodder was affected more by the incorporation of spent mushroom substrate than by applications of cattle slurry. The NDF content was highest in the ryegrass-alfalfa mixture, while the highest ADF was in the fodder from the plots with alfalfa and cocksfoot.

Table 1. Total dry matter yield (t ha^{-1}) of mixtures.[1]

Mixture (B)	Fertilisers applied (A)						Means
	0	SMS	S	$SMS_{10}+S_{60}$	$SMS_{20}+S_{40}$	$SMS_{30}+S_{20}$	
Cocksfoot-ryegrass-Alfalfa	5.76	8.00	8.39	7.44	7.45	7.33	7.39
Cocksfoot-alfalfa	4.04	8.00	8.03	8.68	7.38	7.51	7.27
Ryegrass-alfalfa	3.99	7.31	8.18	8.15	8.20	8.12	7.32
Means	4.60	7.77	8.20	8.09	7.68	7.65	7.33
$LSD_{0.05}$: A=0.43; B=n.s.; A/B=0.52; B/A=0.43;							

[1] SMS = spent mushroom substrate; S = cattle slurry; LSD = least significant difference; n.s. = not significant.

Table 2. Content of neutral detergent fibre and acid detergent fibre (g kg^{-1}) in herbage dry matter of the three tested mixtures.[1]

Mixture (B)	Fertilisers applied (A)						Means
	0	SMS	S	$SMS_{10}+S_{60}$	$SMS_{20}+S_{40}$	$SMS_{30}+S_{20}$	
Neutral detergent fibre							
Cocksfoot-ryegrass-Alfalfa	368.71	397.61	388.50	378.07	383.16	392.57	384.77
Cocksfoot-alfalfa	384.33	384.05	379.69	397.57	396.38	398.33	390.06
Ryegrass-alfalfa	374.81	397.88	378.66	364.10	367.33	390.89	378.94
Means	375.95	393.18	382.28	379.92	382.29	393.93	384.59
$LSD_{0.05}$: A=6.22; B=3.60; A/B=7.62; B/A=6.24;							
Acid detergent fibre							
Cocksfoot-ryegrass-Alfalfa	306.35	327.28	314.32	318.25	313.87	336.01	319.35
Cocksfoot-alfalfa	304.80	307.01	315.51	325.92	328.43	323.50	317.53
Ryegrass-alfalfa	315.73	328.79	315.99	317.87	317.85	335.06	321.88
Means	308.96	321.02	315.27	320.68	320.05	331.52	319.59
$LSD_{0.05}$: A=3.20; B=1.85; A/B=3.92; B/A=3.21;							

[1] SMS = spent mushroom substrate; S = cattle slurry; LSD = least significant difference; n.s. = not significant.

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Improving the resilience of white clover (*Trifolium repens* L.) to environmental stress through interspecific hybridisation

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Abstract

The ability of forage legumes to produce high yields of quality forage while exposed to periods of environmental stress is important if their value to sustainable livestock production systems is to be fully realised. White clover (*Trifolium repens* L.) is a high quality forage species and the most important forage legume of temperate grassland agriculture. Interspecific hybrids between the stoloniferous white clover and the drought-tolerant rhizomatous species Caucasian clover (*T. ambiguum* M. Bieb.) have been produced and, through a backcrossing programme, the rhizomatous growth habit of *T. ambiguum* has been introgressed into a white clover background with a view to improving the drought tolerance and resilience of white clover. The resulting hybrids have formed the basis of the IBERS white clover breeding population. Analysis of the response to drought of genotypes from that population has shown significant variation in the maintenance of leaf relative water content, stolon growth and dry matter yield, indicating the potential for improving the drought tolerance of white clover using this genepool. The implication of these results for exploiting this material in white clover breeding programmes is described.

Keywords: interspecific hybrids, white clover, drought, leaf relative water content, dry matter yield

Introduction

Increasing production to meet global food demand whilst minimising the impact of agriculture on the environment has highlighted the value of nitrogen fixing legume species within crop rotations. White clover (*Trifolium repens* L.) is the most important forage legume of temperate grassland systems and an integral component of sustainable livestock production systems. When grown in mixed swards it can produce high quality forage and contribute 150-250 kg N ha^{-1} yr^{-1} (Humphreys *et al.*, 2005), through N fixation, some of which may be utilised by the companion grass. Although improved forage yield and quality are primary targets for forage plant breeders, increasing the resilience of white clover to abiotic stress, such as periods of intermittent drought, is also important if white clover is to maintain its agronomic performance under potential climate change scenarios. One approach to improving drought tolerance in this species has been to introduce drought tolerance from closely related species within the *Trifolium* genus (Marshall *et al.*, 2015). At IBERS, the rhizomatous growth and drought tolerance traits of Caucasian clover (*Trifolium ambiguum*) have been introgressed into a white clover background through a programme of interspecific hybridisation and backcrossing, and the resulting hybrids have been integrated into the white clover breeding population. As a first step in identifying appropriate drought-tolerant material for further development, this paper describes the results of a glasshouse experiment analysing the response to drought of genotype of the breeding population.

Materials and methods

Six clonal plants of 100 genotypes from the IBERS white clover breeding population (including germplasm derived from interspecific hybrids between white clover and *T. ambiguum*) were grown in 20 cm diameter pots containing Levington potting compost in a heated glasshouse. When fully established, three of the clones were maintained at field capacity and three of the clones were subjected to a 6-week period of moisture stress during which the plants received 70% of the moisture of the well-watered controls for 4 weeks, which was then reduced to 50% for a further 2 weeks. A stolon on each plant was labelled at the beginning of the drought period and scored for leaf development using the scale described by Carlson (1996). This was repeated after 35 days of drought, enabling leaf appearance rate (LAR) and stolon growth rate to be quantified. Leaf relative water content (RWC) was measured after 35 days as described by Marshall *et al.* (2001), on two leaves per plant using the formula: RWC = ((FW – DW) / (RW – DW)) × 100, where FW = leaf fresh weight, RW = rehydrated leaf weight and DW = leaf dry weight. After 35 days all above ground growth was harvested to ground level and dry matter yield per plant calculated after drying overnight in an oven at 80 °C. Value means and standard error of the means was calculated.

Results and discussion

Exploiting interspecific hybrids between white clover and Caucasian clover to improve the drought tolerance of white clover is a major objective of the IBERS breeding programme (Marshall *et al.*, 2015). As part of that programme, hybrids previously identified as having good drought tolerance (Marshall *et al.*, 2015) have been included within the IBERS white clover breeding population. This experiment has analysed for the first time the response of genotypes of this population to drought. The leaf RWC (%) of the plants grown under drought was significantly different (P<0.001) from the control plants maintained at field capacity, averaging 58.8% of the control plants, and ranged from 35.8 to 98.7% (Figure 1). The stolon growth rate, LAR, Carlson score (derived from the sum of Carlson scores of all visible leaves on the marked stolon) and dry matter yield of the plants grown under drought were also significantly (P<0.001) lower than those maintained at field capacity, but the range in response of the plants grown under drought was considerable (Table 1). These data complement other studies on the same genotypes analysing root growth and agronomic performance to identify which genotypes to develop into varieties with improved drought tolerance.

Table 1. Stolon growth, leaf appearance rate (LAR), Carlson score and dry matter (DM) yield of white clover genotypes under well-watered and drought conditions.[1]

Treatment	Stolon growth rate (mm day^{-1})	LAR	Carlson score	DM yield (g plant^{-1})
Control (range)	3.92 (1.24-9.34)	0.13 (0.06-0.289)	5.93(3.53-10.6)	13.98(3.67-22.67)
Drought (range)	2.92 (1.08-8.45)	0.09 (0.05-0.21)	4.63 (2.30-8.46)	9.06 (5.34-12.34)
SED	0.129	0.004	0.142	0.278
Significance	P<0.001	P<0.001	P<0.001	P<0.001

[1] SED = standard error of the difference. Data are presented as mean (and range) of 100 genotypes from the white clover breeding population.

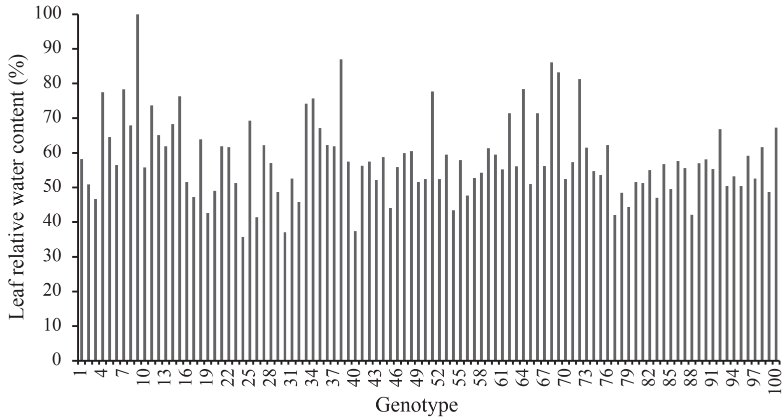

Figure 1. Leaf relative water content (%) of 100 genotypes of the white clover breeding population grown under drought for 5 weeks; standard error of the difference = 12.92 (P<0.001). Data are means of 2 leaves on 3 replicate plants.

Conclusions

The genotypes from the white clover breeding population exhibited significant variation in stolon growth, LAR and dry matter yield when grown under drought in controlled conditions. These results will complement studies on the root growth and field performance of this material to identify genotypes for future development.

Acknowledgements

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Transplanting brush species for the rehabilitation of Sonoran Desert degraded rangelands in Mexico

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Abstract

The study was conducted in 2014 at Santa Ana, Sonora, Mexico. Two hundred plants of iron wood (*Olneya tesota*), palo verde (*Cercidium microphyllum*), mesquite (*Prosopis juliflora*) and zamota (*Coursetia glandulosa*) were sown in the greenhouse during May-July. One hundred plants of each species were transplanted at 10 m intervals along contour furrows in the field. Another hundred plants were transplanted in the flat areas of the field without contour furrows (control). A completely randomized design was used and the information was analysed by ANOVA ($P \leq 0.05$). Precipitation was 20.9% above the mean for 2014 and 39.7% below average for 2015. Plant survival and height was different ($P \leq 0.05$) in areas with and without contour furrows. Plant survival in the contour furrows averaged 48.5% among species with 45, 48, 65 and 35% for ironwood, palo verde, mesquite and zámota, respectively, while in the control, survival averaged 11.3% among species with 12, 5, 20 and 8% for ironwood, palo verde, mesquite and zámota, respectively. The height of species on the contour furrowing plots was higher ($P \leq 0.05$) and ranged from 58-165 cm, whereas in the control plots it was 40-72 cm. It was concluded that rehabilitation of rangelands by transplanting shrubs along contour furrows is an alternative method for restoring productivity on Sonoran Desert ecosystems.

Keywords: land degradation, contour furrows, rehabilitation, productivity, fodder shrubs

Introduction

Large areas of Sonoran Desert Scrub that once were productive are now degraded with scarce vegetation cover and strong erosion problems. Rangelands that required 10 to 20 ha of forage to maintain a cow now require up to twice as much surface. Factors responsible for degradation are: frequent droughts, overgrazing, lack of natural fires, abandonment of farmland, lack of infrastructure in ranches, excessive cultivation, intensive clearing, lack of knowledge and lack of interest among producers, among others. Highly degraded rangelands require partial or total seeding of forage species to recover their productivity (Ibarra *et al.*, 2004). Shrubs and herbaceous plants are important in the rehabilitation of rangelands in poor condition, because they retain and stabilize the soil, improving its fertility and water infiltration, and provide protection for animals and food for livestock and wildlife. Information has been generated for the seeding of native and introduced pasture species, as well as the sowing of forage shrubs in different environments (Martin-Rivera *et al.*, 2008) but there is not enough information on the potential of establishment of shrubs with and without the construction of water retention practices. The objective of this study was to evaluate the adaptation potential of four fodder shrubs with and without the construction of contour furrowing as a practice for rainwater conservation in the Sonora Desert.

Material and methods

The study was conducted during 2014 at Rancho El Águila, Santa Ana (30°19'56.4' N and 110°03'11.8' W), located in the North centre of Sonora, Mexico, at 760 m elevation. The site has less than 3% of slope and soil is of granitic origin and sandy loam texture. The average annual precipitation is 350 mm and the average annual temperature is 23.0 °C. The vegetation corresponds to Desert Shrublands in common conditions (COTECOCA, 1982). The site was seeded with buffelgrass during the summer of 2007 and was in poor condition at the start of the study during 2014. Contour furrows were constructed for retention of rainwater with a plough of 8 discs, each of 81.3 cm in diameter. Two hundred plants of each of 4 species – iron wood, palo verde, mesquite and zamota – were grown in a greenhouse for 90 days from May to July and maintained until reaching a height of 30 to 50 cm. During the summer rains, on 1 August 2014, one hundred plants of each species were transplanted at random in the field, to pits 20×20 cm wide × 30 cm deep, built on the bottom of the contour furrows at 10 m intervals. A further 100 plants were transplanted into pits of the same dimensions, but on the flat ground without furrows (control). Plant height (cm) and survival (%) were evaluated at the end of the growing seasons of 2014 and 2015. Average precipitation at the study site was 410 and 239 mm in 2014 and 2015, respectively, being 17.1% higher and 31.7% below the regional mean value (350 mm). Forage production was not determined to avoid damage to newly established plants by any method. Plant height and plant survival as total number of plants for each species were measured within five 30 m^2 quadrats per plot. A completely randomized design was used with 2 treatments: with and without contour furrows (untreated control) and 100 replicates (transplanted plants). We used ANOVA for the data analysis and the Duncan Multiple Rank Test for the comparison of means. Statistical analyses were run using the SAS package (SAS, 1988).

Results and discussion

The average height and cover of the plants at the time of transplantation were very similar between species, with an average of 19.1 cm (range 12.5-28.5 cm) and 9.1% (range 5.1-14.8%) coverage, respectively. The height of the species in the plots with contour furrows was different and superior ($P \leq 0.05$) to the control areas. This varied in the contour furrowed areas from 58 to 165 cm, whereas in the controls it was from 40 to 72 cm (Table 1). Mesquite was the species with the highest ($P \leq 0.05$) height, while iron wood achieved the lowest growth rates.

Species survival was different ($P \leq 0.05$) in the areas with and without contour furrows (Table 1). Plant survival in plots with contour furrows averaged 48.5% between the species and varied among species from 35 to 65%. Mesquite showed the highest value of survival. Plant survival in the control areas averaged 11.3% and varied among species between 5 and 20%. Mesquite showed again the significantly highest ($P \leq 0.05$) survival, while zamota achieved the lowest survival rates. The retention of water played a very important role in the establishment of transplanted shrubs in this research: there were 2.7, 8.6, 2.2 and 3.4 times more plants established of iron wood, palo verde, mesquite and zamota, respectively, that survived the transplanting in the contour furrowed areas. In arid rangelands of Badia, Jordan, 95% of shrub survival is reported in areas where rainfall was retained with furrows whereas the control obtained only 67% of plant survival (Abu-Zanat *et al.*, 2004). Studies conducted in Sonora, Mexico by Ibarra *et al.* (2004) indicate that the amount of rain occurring during the summer when species transplanting takes place plays a very important role in species survival when using contour furrows and trenches on level curves to ensure plant establishment.

Table 1. Plant height (cm) and survival (%) of four shrub species in two rainy summer seasons after transplanting for the restoration of a Desert Shrublands area in the Sonoran Desert.[1]

Species	Plant height (cm)		Survival (%)	
	Treatments		Treatments	
	Contour furrows	Untreated control	Contour furrows	Untreated control
Olneya tesota (iron wood)	58 a	40 b	45 a	12 b
Cercidium mycrophyllum (palo verde)	96 a	55 b	48 a	5 b
Prosopis juliflora (mesquite)	165 a	72 b	65 a	20 b
Coursetia glandulosa (zamota)	155 a	68 b	35 a	8 b

[1] Mean between treatments followed by the same lowercase letter are not significantly different (*P≥0.05*) Duncan's Test.

Conclusions

Under the conditions in which the study was conducted, it is concluded that the studied shrub species require the construction of soil water retention practices for their establishment. Survival of the species, height and canopy cover of the plants was consistently greater in the contour furrowed areas than in the control areas where water retention practices were not conducted. Rangeland rehabilitation through the transplanting of shrubs upstream contour furrows may be a good alternative for restoring degraded areas into arid ecosystems of the Sonora Desert.

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Linking functional plant traits and forage quality under drought conditions

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Abstract

Drought can strongly impact on forage production. At plant community level, the physiological and ecological mechanisms affecting semi-natural grasslands are being studied more and more but their consequences in terms of forage quality remain little explored. A rainfall manipulation experiment was carried out on three contrasting semi-natural grasslands to explore relationships between plant functional traits and forage quality. Drought was simulated during 8 weeks, either in spring or in summer. Botanical composition and plant functional traits (LDMC: leaf dry matter content, SLA: specific leaf area, vegetative plant height) were assessed just after the simulated drought periods (spring or summer). At the same dates, forage samples were collected for chemical analyses. Drought caused consistent variations in all sites of community-aggregated trait values (CWMs). In contrast, the responses concerning the forage quality were less convergent and characterized by strong site × drought interactions. In the multiple linear models, LDMC was systematically the most important predictor of the nutritive value.

Keywords: functional plant traits, forage quality, drought, semi-natural grasslands

Introduction

Extended drought periods could represent a serious threat for forage production. The effects of water stress mainly concern dry matter (DM) production and, to a lesser extent, forage quality. However, the different impacts are difficult to predict due to the diversity of environmental conditions. It is therefore important to extend the references on the ability of grasslands to cope with drought stress. Measuring functional traits gives insight into the physiological and morphological adaptation mechanisms displayed by plants under resource constraints. In unfavourable conditions (like drought or limited nutrient availability), plants slowdown their metabolism and shift from a resource-acquisition strategy to a more conservative strategy (Reich *et al.*, 2003). Such changes can be reflected in variations of trait values. Previous work has shown that functional plant traits – when aggregated to the community level – provide an interesting basis for studying variation in forage quality (Andueza *et al.*, 2010; Al Haj Khaled *et al.,* 2006). However, the linkages between traits and forage quality under water shortage remain little explored.

In this study we explored the relationships between functional plant traits and forage quality parameters of contrasting grasslands submitted to different water availability treatments. We hypothesized (1) a similar pattern of responses to drought over all sites, for both types of parameters (functional traits and forage quality); and (2) narrow to moderate statistical correlations between functional traits (i.e. leaf dry matter content (LDMC) and specific leaf area (SLA)) and forage quality parameters.

Materials and methods

The experiment was conducted in 2015 on three permanent pastures in the Swiss Jura, located in lowland (540 m a.s.l.); intermediate (940 m a.s.l.) and mountain (1,300 m a.s.l.) areas. In each site, the same complete randomized block design was set up with five replicates, i.e. five rain shelters. Two factors were tested: drought stress (control vs drought) and season (period when the drought stress was applied: spring vs summer). Control plots received 100% of the 30-year precipitation average, whereas drought plots were watered 30% during the period of drought (i.e. spring or summer), and as control for the rest of the time. Vegetation of the plots was cut every 4 weeks during the whole season. Measurements considered in this study were made at the end of each drought periods, corresponding to regrowth cycles 2 and 4.

Botanical composition was determined at the end of each drought period, just prior to the cut, using a pin-point method (Daget and Poissonet, 1971). The functional traits were measured on the dominant species at the same time according to standard protocols (Cornelissen *et al.*, 2003). The functional values at community level (community-aggregated trait values, also called CWMs) have been obtained by weighting the trait values of the dominant plants by their relative abundance in the community. Samples for forage quality were analysed by NIRS, 10% of them being analysed with wet chemistry reference methods.

Statistical analysis was run by means of a linear mixed-effects model (LME) including all three sites. The effects of 'drought' and 'season' (both fixed factors) and their interaction were tested in a nested block design. The model included also the factor 'site' and the interactions 'site × drought' and 'site × season' (all random terms). Due to missing observations/samples, the numbers of data were as follows: n=60 for LDMC and SLA (complete set), n=56 for plant height and n=54 for all forage quality parameters. Models to predict the forage quality from plant traits and botanical composition were obtained using forward stepwise regressions.

Results and discussion

The functional response to drought (Table 1) is mainly a significant increase of LDMC in all sites, with mean values among sites of 227.8±6.1 mg·g^{-1} (control) and 259.3±5.9 mg·g^{-1} (drought). There was also a decrease in SLA (not significant), with a significant drought × season interaction: drought had a greater impact on SLA in summer than in spring. As previously observed on mountain grasslands (Deléglise *et al.*, 2015; Jung *et al.*, 2014), these variations can indicate a slowdown in plant metabolism towards slower tissue turnover and reduced leaf surface, i.e. less transpiration losses. In contrast to the other traits, the response of plant height to drought was not apparent, at least not in this global analysis with all sites.

The results concerning the forage quality parameters were less clear. Cell wall (NDF) was the only fraction that showed a marginally significant increase, from 419±5.6 g·kg^{-1} DM (control) to 453±7.7 g·kg^{-1} DM (drought). The season affected crude protein (CP) and energy (NEL) contents, which slightly increased in summer by 5% and 2%, respectively, compared with spring. For all parameters (except NDF), there

Table 1. Results of mixed effects models (only fixed effects) on community-aggregated trait values (LDMC, SLA and vegetative plant height) and forage quality parameters (CP, NDF and ADF).[1,2]

	Drought			Season			Drought × Season		
	df	F	*P*-value	df	F	*P*-value	df	F	*P*-value
LDMC	1; 2	27.1	0.04	1; 2	1.64	0.33	1; 38	1.64	0.21
SLA	1; 2	6.57	0.12	1; 2	2.81	0.24	1; 38	4.10	0.05
Height	1; 2	2.21	0.28	1; 2	5.78	0.14	1; 34	0.26	0.61
CP	1; 2.0	0.66	0.50	1; 2.5	8.77	*0.07*	1; 32	1.04	0.32
NDF	1; 2.2	9.56	*0.08*	1; 2.1	0.74	0.48	1; 32	1.66	0.21
OM Digestibility	1; 2.0	0.38	0.60	1; 2.2	2.39	0.25	1; 32	1.02	0.32
NEL	1; 2.0	0.10	0.78	1; 3.2	14.4	0.03	1; 32	2.76	0.11

[1] LDMC = leaf dry matter content; SLA = specific leaf area; CP = crude protein; NDF = neutral detergent fibre (cell wall); OM = organic matter; NEL = energy content for lactation. Error terms are constructed using Satterthwaite's method of denominator synthesis. Random effects are not shown in the table.

[2] Significance levels (*P*-values) are given for the effects of drought, season and its interaction. Bold values are significant; italic values denote a trend (P<0.1).

was a strong interaction between site and drought stress (data not shown). In our study, all samples were obtained from the two same growth cycles, at similar regrowth age (4 weeks). The botanical composition did not show any important changes during the growing season. In the end, the nutritive values were comparable among and within the sites, except in the mountain area (1,300 m) where the drought treatment significantly lowered the forage quality. Contrarily to our hypothesis, the experiment did not reveal a general pattern of forage quality response to drought stress.

Plant traits showed to be appropriate for capturing variations in forage quality. Based on functional traits (LDMC, SLA, vegetative plant height) and botanical parameters (proportion of legumes and grasses), between 35 to 60% of the total variation could be explained (Table 2). Among the three evaluated traits, LDMC appeared to be the most important predictor, accounting for at least 67% of the explained variation in the proposed models.

Conclusions

Despite an incomplete comprehension of the links between ecosystem processes and agronomical services (i.e. forage quality parameters), plant traits could represent an interesting tool for identifying responses to drought and are rather good predictors of the effects that these changes might exert on forage quality.

Table 2. Models of prediction of some forage quality parameters. Explanatory variables are botanical characteristics (proportion of legumes (LEG) or grasses (GRA)) and traits (LDMC, SLA and plant height).

		Factor importance (%)			R^2 (adjusted)
Crude protein	$y = a{\cdot}\text{LEG} + m$	100	-	-	0.35
	$y = a{\cdot}\text{LEG} + b{\cdot}\text{LDMC} + m$	67	33	-	0.40
	$y = a{\cdot}\text{LDMC} + b{\cdot}\text{Height} + m$	70	30	-	0.54
Neutral detergent fibre	$y = a{\cdot}\text{LDMC} + m$	100	-	-	0.45
	$y = a{\cdot}\text{LDMC} + b{\cdot}\text{GRA} + m$	82	18	-	0.56
Organic matter digestibility	$y = a{\cdot}\text{LDMC} + b{\cdot}\text{SLA} + m$	71	29	-	0.54
	$y = a{\cdot}\text{LDMC} + b{\cdot}\text{SLA} + c{\cdot}\text{Height} + m$	77	12	11	0.62

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Conservation tillage on forage crops: an opportunity to mitigate climate change

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Abstract

Agriculture is affected by climate change but it also contributes to greenhouse gas emissions. Forage crops represent an important and strategic sector for the European Union. Forage systems are capable of providing satisfactory production while also reducing the amount of total input (i.e. fuels, pesticides, chemical nutrients) as are other agricultural system. Forage systems are also recognised as having potential to help counteract the causes and impacts of climate change. Since 2010 a long-term field experiment which deals with reduced and no tillage techniques has been carried out in Sardinia (Italy) with legumes and cereals. Forage quality and quantity stages (pasture, hay and grain) were measured as well as chemical and physical soil properties. This study is representative of larger areas of the Mediterranean Basin with similar environment. The results determine the feasibility for extending this approach in several agricultural areas characterized by a Mediterranean climate and similar pedological features. This paper focusses on the main changes in soil characteristics obtained so far.

Keywords: forages, conservation tillage, climate mitigation, soil

Introduction

Agriculture is affected by climate change but, at the same time, is the second economic sector in Europe that contributes (9.8%) to emissions of greenhouse gases to the atmosphere (EEA, 2012). Forage crops and their supply chain represent an important and strategic sector for the European Union. The Common Agricultural Policy (CAP) is oriented to the conversion of traditional extensive agricultural systems and cropping techniques with lower inputs and hence more sustainable management (Regulations EU 1305-1306-1307-1308; European Commission, 2013). Tillage techniques with a reduced energy input (reduced tillage and no tillage) do not seem to have the same impact in Europe, especially in Italy, as elsewhere despite of their increasing use in the world (Carboni *et al.*, 2006, 2007; FAO, 2016). Because of these considerations our research unit started a long-term research project in 2010. It is still on-going with the objective to demonstrate that a reduction of total inputs is possible (i.e. fuels, pesticides, chemical nutrients) in a forage system managed with reduced or no tillage techniques without decreasing crop yields and, at the same time, reducing the negative impacts of agriculture on the environment (Mulè *et al.*, 2012). Following the soil evolution under different cultural systems, we tried to answer the need of fertility recovery, compromised by years of a non-sustainable agricultural practices, and to verify its resilience capacity. Finally, we studied also the role of soil as a carbon sink, especially under a correct land-use allowing the accumulation of organic matter.

Materials and methods

The field tests have been carried out in Sardinia since 2010 until now. We adopted a split-plot design in randomised blocks with three replications in plots of 150 m^2. Our unit managed a forage system with leguminous crops and cereals (self-seeding annual alfalfa, barley, close association of vetch, grass and oat) allocated in subplots using both reduced and the no-tillage techniques (seeder blade and disk) on the main plots. The crop rotation was barley-vetch-oat yearly and one year after annual alfalfa self-reseeding it was barley-annual alfalfa. The activity studied the potential impact on the soils in order to determine which were the most important chemical-physical characteristics affected by different agronomic practices. Before starting the trial, soil was classified after some pedological profiles according to Soil Taxonomy (Soil Survey Staff, 2010). Soil samples were taken in each subplots at the same time to set a 'zero-point' of their chemical-physical characteristics. At the beginning of each cropping season, soil samples were collected and analysed in each subplot and their qualitative-quantitative determinations on crop production (forages and grains) were observed. Soil sampling was executed yearly with the purpose of studying the evolution of the organic matter content in the soil, the allotment of principal nutrients, pH modifications and cation exchange capacity (Ministero Risorse Agricole, Alimentari e Forestali, 1994). These sampling were made at two different depths, 0-5 cm and 5-20 cm, to satisfy two different research objectives: (1) to underline the chemical modifications of the soil system along six years, starting from the surface and moving toward the deeper soil layers, and (2) to interpret the relationships between chemical and physical characteristics of the soil and forage quality and quantity under different management (pasture, hay and grain). Data were analysed running REML on GenStat 18th edition (VSN International, 2015) by estimating the treatment effects and variance components in a linear mixed model. Means were compared with VMCOMPARISON procedure (*P*=0.05).

Results and discussion

The organic matter content in the 0-5 cm layer increased according to the tillage technique applied, following this sequence: reduced tillage, disk, seeder blade (Figure 1). Barley contributed more than annual alfalfa and vetch-oat to the increase of the organic matter (Figure 2). The same trend was observed also for nitrogen content, with the exceptions for crops where annual alfalfa was the major contributor to nitrogen increase in the 5-20 cm layer (Figure 2). As observed in other research on conservation tillage (Carboni *et al.*, 2015) at the 5-20 cm layer the increase in the organic matter was greater with reduced tillage. Again, barley helped to increase soil carbon.

Conclusions

The conservation tillage in forage crops allowed the increase of carbon and nitrogen stocks in the soil contributing to a more sustainable agriculture. At the same time these innovative tillage practices could help the farmers improve their grassland with a lower energy input.

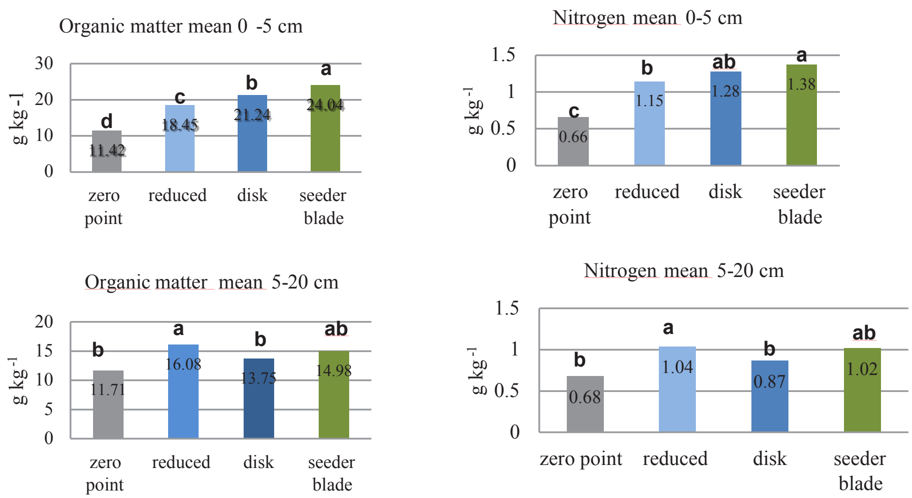

Figure 1. Soil organic matter content and soil nitrogen content in 0-5 cm and 5-20 cm soil layers, following different cultivation techniques (means with the same letter are not different at *P*=0.05).

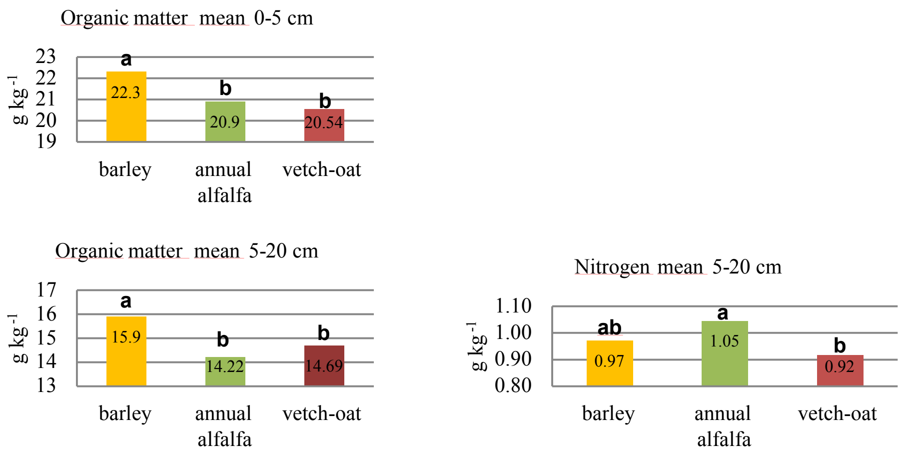

Figure 2. Soil organic matter in 0-5 cm and 5-20 cm soil layers, and soil nitrogen in 5-20 cm layer, following barley, alfalfa and vetch-oat crops (means with same letter are not different at P=0.05).

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The role of re-introducing sheep grazing on protected calcareous xerothermic grasslands

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Abstract

Sheep are farm animals that have accompanied human agricultural activities for many centuries. The tradition of sheep rearing and breeding has played an important role in Poland, particularly in areas dominated by various types of grasslands. Calcareous xerotherms are thermophilic communities of marginal lands, the occurrence of which is dependent on the climatic and soil conditions. In recent years, as a consequence grazing cessation, they have almost disappeared in many areas, and those still existing are at risk. Since preserving such patches of calcareous plants seems essential, some of them are in the Natura 2000 nature conservation network and sheep grazing was re-introduced as a form of active protection. The study was carried out from May to August 2016 to assess the floristic diversity of such grasslands, which occur north of Kraków and are extensively exploited by the Olkuska sheep local breed. The sward of these habitats was classified into *Inuletum ensifoliae* association, within the *Festuco-Brometea* class, and was characterized by large diversity of species, many of which are rare, protected and threatened.

Keywords: Olkuska sheep, calcareous xerotherms, Natura 2000

Introduction

Calcareous xerothermic grasslands are a type of semi-natural grasslands with thermophilic plant species, the occurrence of which is determined by specific climatic and soil conditions: strong sunlight and high air and soil temperatures. Like other grasslands, they have been shaped and maintained by grazing and other agronomic practices for hundreds of years (Dumont *et al.,* 2013) and they display high species richness. Before modern agriculture introduced intensive management, xerothermic grasslands were quite common in the agricultural landscape in Central Europe (Rognli *et al.*, 2013). However, nowadays in Poland they shape the image of marginal lands. Tree and shrub encroachment endanger such patches of calcareous plants. As the landscape values of such grasslands largely depend on their biodiversity, one way to protect them may be to re-introduce sheep grazing (Musiał and Kasperczyk, 2013). The Natura 2000 programme plays an important role in the re-introduction of extensive sheep grazing in agricultural land north of Kraków. The Olkuska sheep is a native and local breed, connected mainly with the areas of Lesser Poland and Silesia, and it is a participating breed in the Polish Genetic Resources Conservation Programme (Sikora *et al.,* 2015). This programme is an important instrument for breed conservation implemented under the EU Rural Development Programme 2014-2020. The use of extensive grazing by Olkuska sheep serves not only to preserve environmentally valuable areas, but it also contributes to the culture of local communities. The aim of the study was to compare the botanical composition of six calcareous xerothermic grasslands, not grazed or grazed by Olkuska sheep.

Materials and methods

The study area was located north of Kraków, within the agricultural land of Miechów Upland. The soils belong to the rendzina intrazonal type and the climate is temperate, without temperature fluctuations, with the average annual precipitation of 600-700 mm. Six Natura 2000 protected areas characterized by the occurrence of calcareous xerothermic grasslands were exploited by Olkuska sheep. The stocking rate was 25 sheep ha^{-1} and animals grazed for 3 days in five rotations from May to September. Three areas were grazed during the last two years: Cybowa Góra (1), Chodów-Falniów (2) and Kalina Mała (3). In Pstroszyce (4), Uniejów Parcele (5) and Widnica (6) grazing stopped about 20 years ago, but the General Directorate for Environmental Protection, which is responsible for nature conservation in Poland, plans to re-introduce it. The surface of the areas ranged between from 3 ha (Uniejów Parcele) to 25 ha (Kalina Mała). The research was carried out during May-August 2016 when 60 phytosociological relevés (10 in each area) were carried out using the Braun-Blanquet method (1964) over a surface of 100 m^2. The surveys were classified according to Matuszkiewicz (2008) and plant species names follow Mirek *et al.* (2006).

Results and discussion

All the swards of the six areas were classified into *Inuletum ensifoliae*, within the *Festuco-Brometea* class. They were characterized by a large diversity of species, many of them rare, protected and threatened (Table 1). The most species-rich community was Chodów-Falniów (2), with 34 species on average. On the other hand, a lower number of species occurred in the ungrazed area of Uniejów Parcele (5), where 23 species were detected. The mean number of species for all areas was 28. According to Dumont *et al.* (2013) and Musiał *et al.* (2015), the lack of land usage is an important factor that curbs the number of vascular plant species of different types of grasslands, as the relevés of areas 4, 5 and 6 showed. Some differences in botanical composition between grazed and ungrazed vegetation were noted. One of them concerned the cover-abundance of grasses like *Festuca ovina* and *Festuca rubra*, which was slightly higher for grazed vegetation (1 in Braun-Blanquet scale corresponding to 5% cover on average). Moreover, some steppe species from South-Eastern Europe were noted (e.g. *Aster amellus, Campanula sibirica* and *Cirsium pannonicum*), which are nowadays rare in the Polish flora and whose presence is limited to xerothermic grasslands. Although their abundance was low, they occurred more frequently in grazed swards. However, small differences in flora were observed in general, since grasslands were grazed only for two years. Shrubs and trees, mainly *Cornus sanguinea, Crataegus monogyna, Frangula alnus, Juniperus communis, Ligustrum vulgare, Prunus spinosa* and *Viburnum opulus*, occurred in both the grazed and ungrazed grasslands, but their cover-abundance was lower in the former (Table 1).

Conclusions

Xerothermic grasslands are often found on hard-to-reach steep slopes, which are suitable for sheep grazing. Olkuska sheep may control effectively the tree and shrub vegetation and maintain the valuable elements of local flora, like steppe vegetation. It also seems important to introduce, in the future, grazing of calcareous grasslands at a large scale, as grazing introduction would not only reduce woody vegetation, but also enable increase in the population of this local sheep breed, which is rare in Poland today.

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Table 1. Flora composition of grazed and ungrazed swards of the six areas.

Areas of Natura 2000	1	2	3	4	5	6
No of relevés	10	10	10	10	10	10
Mean no of species	29	34	30	26	23	27

Species	grazed			ungrazed			Species	grazed			ungrazed		
	1	2	3	4	5	6		1	2	3	4	5	6
	cover-abundance scale							cover-abundance scale					
Allium rotundum	·	+	·	·	·	·	*Galium verum*	1	+	1	1	1	1
Anemone silvestris	·	·	·	·	·	1	*Gentiana cruciata*	·	·	·	+	·	·
Anthericum ramosum	1	2	2	·	·	·	*Helianthemum nummularium*	·	1	·	·	·	·
Anthylis vulneraria	+	+	+	+	+	+	*Hieracium bauhinii*	+	+	+	+	+	+
Asperula cynanchica	+	+	+	·	·	·	*Hypericum perforatum*	+	+	1	+	1	+
Aster amellus	+	+	·	+	·	+	*Hypochoeris maculata*	+	+	+	+	+	+
Astragalus glycyphyllos	+	+	+	·	·	·	*Inula ensifolia*	1	1	1	+	1	2
Brachypodium pinnatum	3	3	3	3	4	4	*Juniperus communis*	1	·	·	1	1	1
Campanula glomerata	+	+	+	·	+	+	*Libanotis pyrenaica*	·	2	·	·	·	·
Campanula persicifolia	·	·	·	·	+	·	*Ligustrum vulgare*	·	·	+	+	1	+
Campanula sibirica	·	+	·	+	·	+	*Linum hirsutum*	+	·	·	·	·	·
Carlina acaulis	1	1	1	+	·	+	*Melampyrum arvense*	+	+	+	+	+	+
Centaurea stoebe	·	+	+	·	·	+	*Odontites lutea*	+	+	+	·	·	·
Centaurea scabiosa	1	1	1	+	1	+	*Ononis spinosa*	·	+	+	·	·	·
Centaurium erythrea	·	+	·	·	·	·	*Picris hieracioides*	+	+	+	+	+	+
Cerinthe minor	+	+	·	·	·	·	*Polygala comosa*	+	+	+	+	+	+
Chamaecytisus ruthenicus	+	·	·	·	·	·	*Prunella grandiflora*	·	·	1	·	·	+
Cirsium pannonicum	·	+	·	·	·	·	*Prunus spinosa*	+	·	+	1	1	1
Cornus sanguinea	·	·	·	1	1	+	*Salvia pratensis*	·	+	+	·	·	·
Coronilla varia	+	+	+	+	+	+	*Salvia verticillata*	+	+	+	+	+	+
Crategus monogyna	+	·	·	+	+	1	*Sanguisorba minor*	·	+	+	·	+	+
Cruciata glabra	+	+	+	+	+	+	*Scabiosa canescens*	+	+	+	+	+	+
Echium vulgare	+	+	+	+	+	+	*Scabiosa columbaria*	+	+	+	+	+	+
Euphrasia stricta	·	+	·	+	·	+	*Thymus marshallianus*	+	+	·	·	·	+
Festuca ovina	1	1	1	+	·	+	*Trifolium montanum*	·	·	+	·	·	·
Festuca rubra	1	1	1	+	+	+	*Verbascum lychnitis*	+	+	+	+	+	+
Fragaria viridis	·	+	+	·	·	·	*Veronica spicata*	·	+	·	·	·	·
Frangula alnus	·	+	·	+	·	·1	*Viburnum opulus*	+	·	·	1	1	1

Synergy between *Axonopus catharinensis* and *Medicago sativa* on *in vitro* rumen fermentation characteristics

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Abstract

The aim of this study was to evaluate the associative effects between *Axonopus catharinensis* (a tropical grass) and *Medicago sativa* (a temperate legume), two species which can be grown in South Brazil, on rumen digestion. We conducted an *in vitro* rumen fermentation assay in which we tested different proportions of these two plants on a % dry matter (DM) basis, i.e. 0:100, 25:75, 50:50 75:25 and 100:0. Positive quadratic effects were detected on DM disappearance (P=0.025), total gas production (P=0.001), and on total and *iso*-volatile fatty acids (VFA) production (P=0.001) indicating a synergy between the plants, with an optimum when the proportion of *M. sativa* was 25%. At this proportion, the DM disappearance was 52.6% (50.7 and 45.8% for pure *A. catharinensis* and *M. sativa*, respectively) and the VFA production was 8.52 mmol g^{-1} DM (8.33 and 8.02 mmol g^{-1} DM for pure *A. catharinensis* and *M. sativa*, respectively), suggesting a better microbial protein synthesis when the plants were mixed, compared to pure grass. We conclude that complementarities between the two plants in terms of energy and protein contents can create favourable conditions for rumen digestion when they are mixed together.

Keywords: grass-legume mixtures, *Axonopus catharinensis*, *Medicago sativa*, tropical forage, *in vitro* rumen fermentation, associative effects

Introduction

Some recent studies have evaluated the associative effects in grass-based diets combining a temperate legume with a temperate grass (Niderkorn *et al.*, 2011, in press) or a tropical legume with a tropical grass (Dall-Orsoletta *et al.*, in press), but mixtures between tropical and temperate species deserve to be better investigated. Indeed, certain temperate species, such as alfalfa (*Medicago sativa*), are adapted to a wide range of pedoclimatic conditions (Moreira *et al.* 2011). Thus, it is possible to find temperate plants growing in tropical or subtropical regions, such as northeastern Brazil (Pompeu *et al.*, 2003) and South Africa (Theron and Snyman, 2015). Additionally, the average temperature of several regions of the planet has been increasing, which may increase the occurrence of tropical pastures in subtropical and temperate regions of the world. We hypothesize that a wide range of associative effects may occur when mixing different temperate and tropical grass and legume species. Thus, the aim of this work was to assess the ruminal digestive parameters when a temperate legume (*M. sativa*) was mixed with a tropical grass (*Axonopus catharinensis*).

Materials and methods

Axonopus catharinensis and *M. sativa*, taken from plant collections of UDESC and INRA, were grown in the subtropical humid climate of southern Brazil (27°25′S, 49°38′W) and in the temperate climate of Central France (45°46'N, 3°08'E) respectively, and harvested at a vegetative stage. Ground freeze-dried samples (0.6 g) of *A. catharinensis* (in g kg^{-1} of dry matter (DM), neutral detergent fibre (NDF): 617, acid detergent fibre (ADF):280, acid detergent lignin (ADL):12, crude protein (CP): 104) and *M. sativa* (in g kg^{-1} of DM, NDF: 496, ADF:37, ADL:74, CP: 279) were incubated in different proportions (in %, 100:0, 75:25, 50:50, 25:75 and 0:100) in anaerobic conditions at 39 °C in culture bottles containing 40 ml of buffered rumen juice from sheep (Niderkorn *et al.* 2011). The total gas and methane (CH_4) production, the *in vitro* dry matter disappearance (IVDMD), and the concentrations of total volatile fatty acids (VFA), *iso*-VFA and ammonia in the fermentation medium were measured after 24 h of fermentation. The treatments were tested in triplicate by conducting three runs of fermentation over two weeks. Values were adjusted by subtracting the values from blanks without plant substrate. Data were submitted to analysis of variance using the MIXED procedure of the SAS suite (SAS Institute, Inc., Cary, NC, USA) considering the proportion of *M. sativa* as fixed effects and the repetition as random effect. Orthogonal contrasts were used to evaluate the fermentation parameters for linear and quadratic effects of the proportion of *M. sativa* in the mixture using PROC MIXED. PROC IML was used to adjust the contrast coefficients.

Results and discussion

Positive quadratic responses were observed on all the parameters measured, namely IVDMD (P=0.025), and productions of total gas (P=0.001), CH_4 (P=0.002), VFA (P=0.001), *iso*-VFA (P=0.001) and ammonia (P=0.014) (Figure 1). These quadratic effects indicate that a synergy occurs between *A. catharinensis* and *M. sativa* on the rumen digestion efficiency. Considering the IVDMD and the total VFA production, the optimal proportion of *M. sativa* was 25%. At this proportion, the IVDMD was 52.6% (50.7 and 45.8% for pure *A. catharinensis* and *M. sativa*, respectively), the VFA production was 8.52 mmol g^{-1} DM (8.33 and 8.02 mmol g^{-1} DM for pure *A. catharinensis* and *M. sativa*, respectively).

It is known that the CP content of medium or low quality forages (<130 g kg^{-1} DM) is not sufficient to meet the requirements of rumen microorganisms, with negative impact on DM digestibility (Vérité and Peyraud, 1989). In the present study, the CP content of *A. catharinensis* was 104 g kg^{-1} DM and 279 g kg^{-1} DM in *M. sativa*. Thus, our results suggest that nitrogen from *M. sativa* allows a synergy on ruminal digestion efficiency when the plants were mixed, likely through a better microbial protein synthesis compared with pure plants.

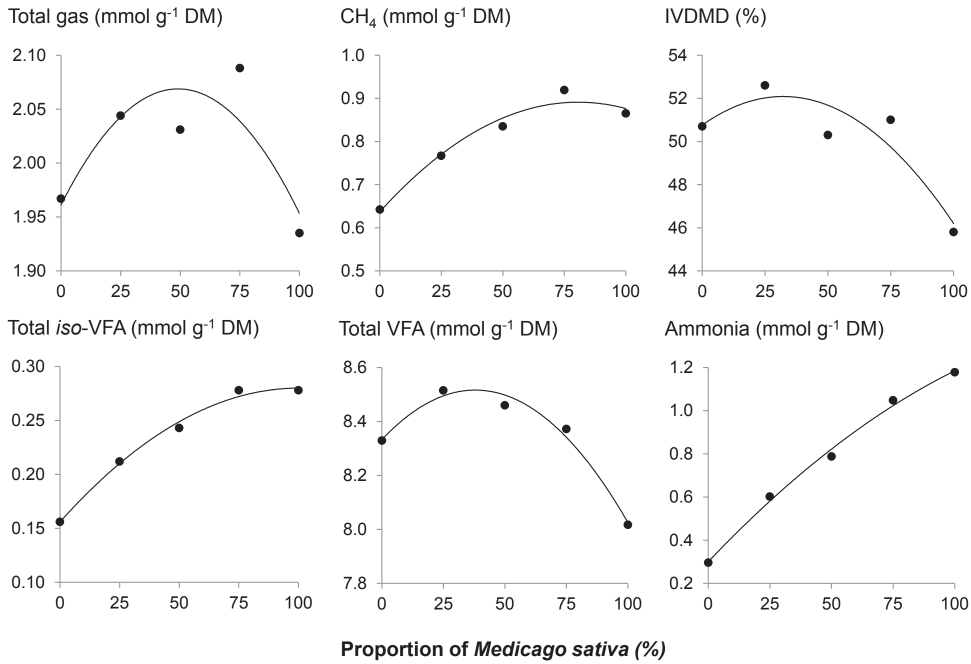

Figure 1. *In vitro* dry matter disappearance (IVDMD), total gas, methane (CH_4), total volatile fatty acids (VFA), total *iso*-VFA and ammonia productions after 24 h-rumen fermentation of *Medicago sativa* mixed in different proportions with *Axonopus catharinensis*.

On the other hand, the lignin (ADL) content was rather high in *M. sativa* (74 g kg^{-1} DM) and was 12.5 g kg^{-1} DM in *A. catharinensis* in this study. As lignin is the main barrier to the fibre digestion (Jung *et al.*, 1989), dilution of the lignin content in mixtures may have resulted in a better energy supply to ruminal microorganisms, increasing fibrolytic bacteria activity and digestibility of less degradable fibres. The extent of fermentation of fibres increases as ammonia concentration increases in the rumen, but the rate of fermentation also depends on the quantity of digestible NDF present in the substrate (Nagadi *et al.*, 2000). Our results indicate that positive associative effects may be expected not only when a legume with high nitrogen content is mixed with a grass with low nitrogen content, but also when a grass with low lignin content is mixed with a legume with rather high lignin content.

Conclusions

Synergistic effects were observed on ruminal digestive efficiency when *M. sativa* was mixed with *A. catharinensis*. This result highlights some complementarities between the two plants, in particular in terms of energy and protein contents, which can create favourable conditions for rumen digestion when they are mixed together. Although the optimal proportions of species to maximize total forage yield remains to determine, our study suggests that synergism can occur between tropical and temperate plants with a potential of use in some geographic areas and under climate change.

Acknowledgements

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Indicator species of fertilization intensity in mountain grasslands

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Abstract

In Romania specialists in grassland science are trying to evaluate the management of grasslands by using indicator species. They also intend to develop a list of indicator species considering stational conditions. The goal of our research is to track some species indicators of the intensification of organic fertilization in mountain grassland. Experimental trials were installed in 2001 in Romania and were designed to assess the effect of small and large inputs of manure on grassland productivity and floristic composition. In order to identify the indicator species we used blocked indicator species analysis. Species usable as indicators were highlighted both for control experiments and for the treatments fertilized with manure. Blocked indicator species analysis of the data recorded from long-term experiences highlighted species with real indicator value for the intensity of the management applied.

Keywords: indicator species, fertilization, mountain grassland

Introduction

The identification of indicator (or characteristic) species is a traditional question in ecology and biogeography (Legendre and Legendre, 2012). Nowadays there is a general concern about the identification of indicator species, particularly in the field of monitoring, conservation and management of grassland ecosystems. Decision-making for nature-friendly land use in farming landscapes requires methods and indicators for assessing effects on biodiversity (Jeanneret *et al.*, 2014). In an inverse approach, species associations may be used to predict environmental characteristics or as indicators of environmental quality (Legendre, 2005). Currently, in Romania, specialists are trying to evaluate the management of grasslands by using indicator species, planning also to compile a list of species considering stational conditions. However, defining those species requires long-term scientific experiments which, in our country, are rather rare. The goal of our research is to identify some indicator species of the intensification of organic fertilization in mountain grasslands.

Materials and methods

Experimental trials were established in 2001 in the Gheţari village (Gârda de Sus municipality, Romania) and were designed to assess the effect of small and large inputs of manure on grassland productivity and floristic composition. The experimental design was a randomized block with four replicates and four treatments: T1: unfertilized control; T2, 3 and 4: received 10, 20 and 30 Mg ha^{-1} manure, respectively. The manure contained 3.04 kg Mg^{-1} N, 2.90 kg Mg^{-1} P and 2.47 kg Mg^{-1} K. In all treatments, one cut per year was applied. The harvesting period was chosen according to the particular conditions of the experimental site, located on an altitude of 1,130 m a.s.l. and characterized by average annual temperature of 5.2 °C and precipitation of 1,123 mm. The floristic composition was determined by the Braun-Blanquet method as modified by Păcurar and Rotar (2014). Scientific results derive from a 15-year observation period. The nitrogen fertility index (I_N) of plants (ranging from 1 to 9, where 1 represents oligotrophic species and 9 represents eutrophic species) was taken from Ellenberg *et al.* (1991). Floristic data processing was performed by PC-ORD, version 6, which uses the multivariate analysis of botanical data (McCune and Grace, 2011). Cluster analysis was applied to distinguish the effect of organic fertilization on floristic composition. The clustering of species usually has the objective of identifying groups of species with similar responses to environmental gradients expressed across the sample units in a study (Peck, 2010). Cluster analysis was carried out using the Euclidean distance to compute similarity and Ward as agglomeration method. To identify indicator species we used the 'indicator value indices' (INDVAL), which are used for assessing the predictive values of species as indicators of the conditions prevailing in site groups, e.g. for field determination of community types or ecological monitoring (Cáceres *et al.*, 2012). We used blocked indicator species analysis (Dufrêne and Legendre, 1997). This analysis calculates indicator values based on proportional representation of a species within a single group, compared with the species prevalence across all groups (McCune *et al.*, 2002).

Results and discussion

Three clusters, identifying different grassland types, were distinguished (Figure 1): cluster 1: *Festuca rubra* L. and *Agrostis capillaris* L. type; cluster 2: *Agrostis capillaris* L. and *Festuca rubra* L. type; cluster 3: *Trisetum flavescens* (L.) P. Beauv. and *Agrostis capillaris* L. type.

Cluster 1 identified the vegetation of the control treatment. Five species with indicator value were observed (Table 1). These indicator species were oligotrophic (I_N: 2) and oligomesotrophic (I_N: 3). They indicate a reduced soil trophic level and implicitly the absence of fertilization. Cluster 2 identified the vegetation of the 10 Mg ha^{-1} manure treatment. Other specific indicator species were present (Table 1). Most of the indicator species for this grassland type were oligomesotrophic (I_N: 3, 4). But there were also two mesotrophic species (I_N: 5, 6) preferring the fertilization with 10 Mg ha^{-1} manure. Even if according to Ellenberg *et al.* (1991), *Festuca rubra* and *Plantago lanceolata* are indifferent, according to our results they are indicator for *Agrostis capillaris* L. – *Festuca rubra* L. grassland type and implicitly for treatment with 10 Mg ha^{-1} manure. Cluster 3 identified the vegetation of the 30 Mg ha^{-1} manure treatment. Application of 20-30 t ha^{-1} manure determined the establishment of *Trisetum flavescens* (L.) P. Beauv. – *Agrostis capillaris* L. grassland type, which shows a series of indicator species (Table 1), mainly mesotrophic (I_N – 6) and just a single one eutrophic (I_N – 7, *Poa trivialis* L.).

Conclusions

Species with indicator values were highlighted for both the control (T1) and T2 treatments. No indicator species were observed specifically for T3 and T4 treatments. Blocked indicator species analysis of the data recorded from long-term trials highlighted species with real indicator value for the intensity of the management applied.

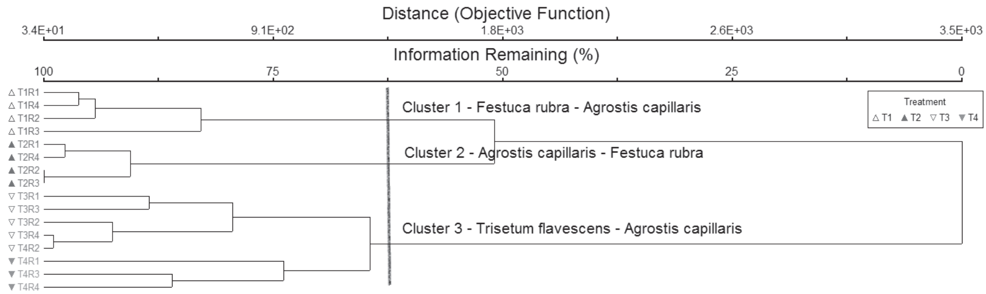

Figure 1. The influence of organic fertilization on floristic composition (T: treatment, R: replicate).

Table 1. Indicator species characteristic of the grassland types.[1]

I_N	Species	T	INDVAL	Mean	SD	*P*-value
Cluster 1: *Festuca rubra* and *Agrostis capillaris* grassland type – T_1: control						
3	*Gentianella lutescens* (Velen.) Holub	1	75.0	23.1	15.15	0.0284
3	*Gymnadenia conopsea* (L.) R. Br. s. l.	1	75.0	23.4	15.26	0.0300
2	*Polygala vulgaris* L. s. l.	1	75.0	23.6	15.24	0.0302
2	*Potentilla erecta* (L.) Raeuschel	1	96.8	28.9	13.29	0.0020
2	*Scabiosa columbaria* L.	1	100.0	30.0	15.24	0.0020
Cluster 2: *Agrostis capillaris* and *Festuca rubra* grassland type – T_2: 10 t ha^{-1} manure						
x	*Festuca rubra* L.	2	75.3	58.5	6.13	0.0258
4	*Carex pallescens* L.	2	100.0	41.0	18.2	0.0258
3	*Luzula multiflora* (Ehrh.) Lej.	2	100.0	41.0	18.12	0.0258
4	*Lotus corniculatus* L.	2	100.0	41.3	18.31	0.0280
6	*Trifolium pratense* L.	2	69.7	57.3	4.3	0.0258
5	*Leontodon autumnalis* L.	2	100.0	41.0	18.12	0.0258
x	*Plantago lanceolata* L.	2	100.0	41.0	18.12	0.0258
3	*Rhinanthus minor* L.	2	100.0	41.0	18.12	0.0258
Cluster 3: *Trisetum flavescens* and *Agrostis capillaris* grassland type – T_{3-4}: 20-30 t ha^{-1} manure						
6	*Dactylis glomerata* L. s. str.	3	63.2	31.7	13.05	0.0484
6	*Festuca pratensis* Huds. s. l.	3	100.0	45.5	16.06	0.0258
7	*Poa trivialis* L.	4	77.8	30.5	13.7	0.0160
	Trisetum flavescens (L.) P. Beauv.	4	72.9	57.8	5.67	0.0280
6	*Veronica chamaedrys* L. s. str.	4	84.7	63.3	8.15	0.0280

[1] I_N = Ellenberg nitrogen fertility index; T = treatment; INDVAL = indicator value; SD = standard deviation.

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Temporary night penning as an effective tool to improve plant diversity in nutrient-poor dry grasslands

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Abstract

The socio-economic transformations that have occurred in Europe over the last decades have led to changes in land use intensity, with broad abandonment of land use in mountain areas. In semi-natural grasslands especially, the decline in the workforce and in the number of stocked animals has resulted in long-term loss of plant diversity, often with standing-dead litter accumulation, which is known to change the competitive relations among plant species. To restore plant diversity and vegetation structure by reducing the standing-dead litter, we arranged five temporary night pens (TNP) over nutrient-poor dry grasslands of the Natura 2000 site IT1110030 (Western Italian Alps). Within each TNP, we fenced 250 sheep for 2-3 nights and we surveyed vegetation along permanent transects before the arrangement of TNP (2015) one year after the treatment. We evaluated the effects on herbaceous, rock and bare ground cover, and we computed biodiversity indices (species richness and Shannon diversity index (H')). Temporary night penning reduced the abundance of standing-dead litter and herbaceous cover ($P<0.01$), and increased bare ground ($P=0.004$). Furthermore, the number of plant species increased ($P<0.001$), whereas H' did not change significantly. These short-term findings highlight that plant-diversity restoration in abandoned dry grasslands may benefit from temporary night penning practice.

Keywords: abandoned grasslands, sheep, species richness, standing-dead litter, vegetation structure

Introduction

The reduction of agro-pastoral practices in the European mountains in the last decades has affected the ecosystem services provided by semi-natural grasslands. In particular, the accumulation of standing-dead litter, which changes competitive relations among plant species, may result in a progressive loss in biodiversity. Temporary night camp areas for cattle are known to enhance vegetation structure and increase biodiversity in sub-alpine grasslands (Pittarello *et al.*, 2016; Tocco *et al.*, 2013), but the information available in literature refers to implementation of temporary night penning (TNP) of sheep. Sheep are better suited for grazing on steep slopes and rugged areas than cattle, as they are lighter and more agile (Crofts and Jefferson, 1999). The objective of this research was to assess the effects produced by TNP on vegetation structure and botanical composition over a short-term period in abandoned dry grasslands (*Festuco – Brometea* class), which represent one of the most threatened habitats in Europe.

Materials and methods

The research was conducted within the Natura 2000 site IT1110030 (Western Italian Alps – 45°08'N, 7°06'E). The study area was a pasture of 120 ha ranging between 510 and 1,260 m a.s.l. and abandoned since the 1950s. Dry grasslands were mainly dominated by *Stipa pennata* L., *Bromus erectus* Hudson, and *Festuca ovina* s.l. (habitats 6210* and 6240*, Annex 1 of Directive 92/43/CEE). Within the pasture, which was grazed for the first time by a flock of 250 Bergamasca sheep for 32 days (15 April to 16 May 2015), five temporary night pens (TNP) were arranged over nutrient-poor herbaceous areas. The flock was confined for 2-3 consecutive nights within each area (820 m^2, on average) delimited with electric fences. Within each TNP, botanical composition was determined using the vertical point-quadrat method along five to six permanent linear transects (Pittarello *et al.*, 2016). Each transect was 12.5-m long and at every 25-cm interval plant species touching a steel needle were identified and recorded. For each plant species recorded in each transect, the frequency of occurrence (f_i= number of occurrences/50 points of vegetation measurement) was calculated. Within 1-m buffer around the linear transect (vegetation plot), the percentage of herbaceous, rock, and bare ground cover was visually assessed to give an estimate of the basal cover. Moreover, all other plant species not recorded along the transect were listed. The abundance of standing-dead litter and live biomass were estimated using four abundance categories: 1 = Null; 2 = Scarce; 3 = Medium; and 4 = Abundant. Surveys were carried out during spring 2015, before fencing the flock into night pens (pre-treatment), and in spring 2016, one year after treatment. Biodiversity was expressed as species richness (number of plant species within each vegetation plot) and by Shannon diversity index (H') (computed for each vegetation transect as described by Pittarello *et al.*, 2016). Paired-samples *t*-tests were used to evaluate the differences between year 2015 and 2016 for vegetation structure variables (percentage of herbaceous, rock, and bare ground cover) and biodiversity indexes. Shapiro-Wilk test for normality and Levene test for homogeneity of variance were used to evaluate the variable distribution. The sequence of transition of standing-dead litter and live biomass abundance categories from 2015 to 2016 was calculated for each vegetation plot and the dyads of the preceding and following scales were evaluated with a transition matrix. The transition matrix was compared to a random model using Chi-square analyses to assess changes in standing-dead litter and live biomass one year after treatment.

Results and discussion

One year after the implementation of TNP vegetation structure changed and species richness increased. Trampling by sheep caused a moderate reduction of herbaceous cover (about -6%) and an increase of bare ground (+6.4%), without compromising the sward excessively or inducing soil erosion (Table 1). As expected, rock cover did not change after treatment.

The standing-dead litter reduced in abundance in 20 vegetation plots out of 27 (Table 2): in ten vegetation plots it shifted from medium to scarce, in three from abundant to medium, and in two from abundant to scarce. An increase was observed in only one vegetation plot (from scarce to medium) whereas in the remaining six plots abundance did not change. Conversely, one year after treatment, the live biomass did not show variation in most of the vegetation plots (15) (Table 2). The general enhancement of vegetation structure had positive effects also on the species richness, which increased by about seven plant species in each vegetation plot, on average (Table 1). It is worth mentioning that many of these species were annual plants, which probably took advantage of the gaps created by sheep trampling. Such gaps also favoured some perennial plant species (e.g. *Briza media* L.*, Trifolium montanum* L. and *Brachypodium sylvaticum* Hudson Beauv.) which probably germinated by transient seeds in the soil. Moreover, sheep may have acted as vectors for herbaceous seeds from adjacent grazed pastures, fostering the recolonization process of bare ground gaps. Instead, H' did not change significantly, as the abundance of plant species in 2015 and 2016 had similar distribution.

Table 1. Effects produced by the arrangement of five Temporary Night Pennings (TNP) on vegetation structure and biodiversity indexes.[1]

		2015 mean ± SE	2016 mean ± SE	t	d.f.	*P*-value
Vegetation structure	Herbaceous cover (%)	81.8±2.17	75.8±1.82	2.94	26	**
	Bare ground cover (%)	16.0±1.94	22.4±1.6	-3.17	26	**
	Rock cover (%)	2.2±0.57	1.8±0.29	1.05	26	ns
Biodiversity indexes	Number of species	37.9±1.11	44.8±1.15	-6.10	26	***
	Shannon diversity index (H')	3.0±0.05	3.1±0.05	-0.84	26	ns

[1] Values are means and mean standard errors (SE). *** = $P<0.001$; ** = $P<0.01$; * = $P<0.05$; n.s. = not significant ($P>0.05$).

Table 2. Transition matrix and Chi-square analyses for the period 2015-2016 of the abundance categories for standing-dead litter and live biomass.

		Standing-dead litter 2016				Live biomass 2016			
2015		1	2	3	4	1	2	3	4
1 – Null	Observed	0	0	0	0	0	0	0	0
	Expected	0	0	0	0	0	0	0	0
	Chi-square	-	-	-	-	-	-	-	-
2 – Scarce	Observed	3	1	1	0	0	0	1	0
	Expected	0.9	2.4	1.7	0	0.0	0.0	0.9	0
	Chi-square	4.6	0.8	0.3	-	-	0.0	0.0	0.1
3 – Medium	Observed	1	10	5	0	0	1	15	2
	Expected	3.0	7.7	5.3	0	0.0	0.7	16.0	1
	Chi-square	1.3	0.7	0	-	-	0.2	0	0.3
4 – Abundant	Observed	1	2	3	0	0	0	8	0
	Expected	1.1	2.9	2	0	0.0	0.3	7	1
	Chi-square	0	0.3	0.5	-	-	0.3	0.1	0.6
		Overall Chi-square= 8.53				Overall Chi-square= 1.69			
		df = 9				df = 9			
		P=0.074				P=0.793			

Conclusions

The implementation of TNP was an effective tool to enhance vegetation structure and to increase species richness in semi-natural dry grasslands even in the short-term period. However, these results represent a starting point for a longer monitoring period, necessary to obtain a better evaluation of the effectiveness of TNP.

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Topsoil organic carbon content in three representative silvo-pastoral areas at different elevation in Sardinia

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Abstract

Sardinia is an ancient island in the centre of Mediterranean Sea. It has complex geology from 400 million years and few zones in the world exhibit such a large variety (igneous, sedimentary and metamorphic) of rocks in a restricted area. Plant residues and microorganism affect microbial growth, and decomposition contributes to soil organic matter. Soil organic carbon (SOC) is a major component of soil organic fraction, positively affecting soil properties. The aim of this work was to determine organic carbon contents in topsoil in three regions of Sardinia (Italy) representative of Mediterranean environments and the variation in SOC of sites along an elevation gradient. The SOC concentration in the topsoil layer ranged from 8.8 to 47.7 g kg^{-1} in Arci-Grighine, from 30.0 to 71.0 g kg^{-1} in Montepisanu and from 13.2 to 73.4 g kg^{-1} in Ogliastra, differences were proportional with elevation. The results highlight that elevation is strongly correlated to SOC concentration in the topsoil.

Keywords: soil organic carbon, SOC, elevation, natural grazing

Introduction

Soils are the largest carbon sink in terrestrial ecosystems (Muňoz-Rojas *et al.*, 2012) and the primary terrestrial reservoirs of carbon (C). In fact, more than 71% of the terrestrial organic carbon pool is found in soils (Lal, 2010). Soils consist of partially decayed plant residues, microorganisms and by-products of microbial growth. As decomposition plays an important role in the global carbon cycle, changes in its stability may alter carbon release from the soil and, consequently, the atmospheric CO_2 concentration (Tian *et al.*, 2016). Soil organic matter (SOM) is a key component of any terrestrial ecosystem and any variation in abundance and composition has important effects on many of the processes that occur within the system (Batjes, 2014). SOC is a major component of the soil organic fraction; it positively affects many soil properties and, consequently, soil functions (Francavilla *et al.*, 2014). SOM is generally recognized to contain 58% of organic carbon (SOC), and in most cases, it is effectively measured as organic carbon (de Brogniez *et al.*, 2015). Variation in SOM along elevation gradients is related to climate, organic matter input and decomposition rate. There is about 1500 Pg of soil organic carbon to a depth of 1 m in the World's soil, with approximatively 50% in the upper 30 cm (Batles, 2014; Lal, 2008; Parras-Alcántara *et al.,* 2015). SOC varies among environments and management systems; it increases with high mean annual precipitation and lower mean annual temperature, and is maintained in dynamic equilibrium by factors such as vegetation and soil texture (Tsui *et al.*, 2013). The mean annual temperature can change soil physiochemical properties and alter quality; and as temperatures decrease by 0.6 °C every 100 m (Tsui et al 2013), soil carbon mineralization generally reduces at increasing elevation. As CO_2 exchange between soil carbon and atmospheric CO_2 varies strongly along climate gradients, scientists focus on whether there are enhanced response patterns in SOC stability along increasing latitudinal or altitudinal gradients (Tian *et al.*, 2016). As the estimation of soil carbon content is important for soil protection and in mitigation strategies for global warming, Jones *et al.* (2005) proposed an approach that considered SOC estimates based on topsoil (0-30 cm) organic carbon content. The aim of the work reported in this paper was to determine the organic carbon contents (%) in topsoil in three regions of Sardinia (Italy) representative of the Mediterranean environment, and the variation in SOC along site elevation.

Materials and methods

This study was carried out in three different areas of Sardinia along the minor axis of the island from west to east. The areas were different in terms of vegetation, altitude, pedological and lithological characteristics. Our studies were restricted to 250 km^2 for both Ogliastra (Ogliastra mountain chain, OG) and Marmilla-Sarcidano-Barigadu (Arci-Grighine mountain, ARC_GRIG) and to 30 km^2 for Goceano (Montepisanu forest, MP). Vegetation was characterized by *Quercus ilex*, *Quercus pubescens*, *Quercus suber*, *Taxus baccata*, *Ilex aquifolium* and *Castanea sativa* in MP and ARC_GRIG, while mainly pastures and reforestation species (*Pinus* spp.) occured in OG. Soils were derived from Metamorphic rocks (schists, arenaceous and shales), effusive rocks (basalts) and acid-effusive rocks (andesites and rhyolites) in ARC_GRIG; intrusive rocks (granites, granodiorites and leucogranites) and metamorphic rocks (schists, arenaceous and shales) in OG; effusive rocks (basalts), acid-effusive rocks (andesites and rhyolites) and metamorphic rocks (schists, arenaceous and shales) in MP (Aru *et al.*, 1989). The reaction of topsoils ranged from pH 5.3 to 6.6 in MP, from 5.5 to 8.1 in ARC_GRIG and from 4.8 to 7.8 in OG. Average elevations were higher in OG (1,013 m a.s.l. range 291-1,366 m) and lowest in ARC-GRIG (307 m, range 38-495 m). Average elevation in MP was 790 m (601-1,195 m). Topsoil sampling was taken to a depth of approximately 0-30 cm on 150 georeferenced points, vegetative residues were removed and samples air-dried. The analyses were made on the <2mm soil fraction after sieving. Samples were grouped by elevation gradient into 100 m classes. SOC was determined with the Walkley-Black method. Statistical analysis was performed by Statgraphics Centurion XVI.

Results and discussion

SOC concentration in the topsoil layer ranged from 8.8 to 47.7 g kg^{-1} in ARC_GRIG, from 30.0 to 71.0 g kg^{-1} in MP and from 13.2 to 73.4 g kg^{-1} in OG, proportionally with elevation (Figure 1). There was a statistically significant relationship in the ANOVA ($P<0.05$) between elevation and SOC, indicating a relatively strong relationship between the variables. Results are in line with findings of Muňoz-Rojas *et al.* (2012) and Tsui *et al.* (2013); the latter reported mean SOC values of 69.3 and 67.3 g kg^{-1} in topsoil (0-30 cm) in andisols and inceptisols, respectively, at an average elevation of 750-780 m in Taiwan. Costantini and Dazzi (2013) reported, on the basis of Jones (2005), that 23% of Italian soils contain less than 1% organic carbon. These areas are concentrated mainly in Sicily, Sardinia and Southern regions. In North Sardinia, Francaviglia *et al.* (2014) found SOC concentrations of 18.7 g kg^{-1} in pastures and 21.8 g kg^{-1} in cork forest at 285 m. a.s.l., which are comparable to our results at the same elevation. In contrast, Tian *et al.* (2016) in China reported that SOC was not correlated with the altitudinal gradient,

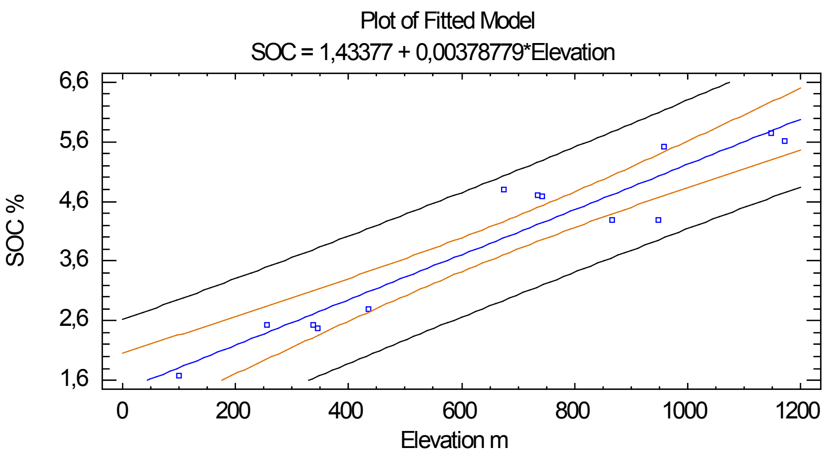

Figure 1. Relation between elevation and soil organic carbon (SOC).

pointing out that mean annual temperature can change soil physicochemical characteristics. The same author concluded that the stability of SOC along the altitudinal gradient was mainly dependent on the intrinsic process of SOC formation and nutrient status.

Conclusions

Soil is an important source and sink of atmospheric carbon. The land examined in our study was always grazed extensively. The highly significant linear relationship between SOC and elevation observed in this study suggests that elevation is an effective factor that may be used to predict the SOC stock in Mediterranean areas. Moreover, this paper provides further information for implementing the LUCAS (Land Use/Cover Area frame Statistical Survey by ESDAC) approach.

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Ecosystem services provision: how compatible are species conservation and livestock rearing?

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Abstract

Mediterranean silvopastoral systems have traditionally provided a wide diversity of ecosystem services (ES), especially in low productivity sites where intensive management and high yield are not possible. In this paper we analyse the trade-offs between two ES: pasture yield for livestock rearing and habitat for a protected species, white stork (*Ciconia ciconia* L.) in the Sierra de Guadarrama National Park (Spain). The research was developed in an ancestral silvopastoral landscape of ash dehesa and meadows. Its main goal was to quantify the effects of white stork occupied nests on pasture yield, quality and diversity. We selected 40 trees (20 occupied and 20 without nest) and three positions/distances to the stem; we analysed the influence of the presence of an occupied nest through the comparison of biomass and species diversity of herbaceous pastures. The influence of nest presence is limited to the subcanopy microsite where species richness and nitrophilous species abundance are reduced. The results allow the quantification of losses in livestock grazing due to the habitat use by white stork and contribute to multifunctional management decision-making.

Keywords: protected species, biodiversity, richness, pasture yield, meadows

Introduction

Protected species and spaces conservation, which are priority management objectives in many Spanish silvopastoral systems, could show some incompatibilities with other ecosystem services provision (Jose, 2009). Livestock rearing is an economic activity that, in these highly diverse and complex ecosystems of the Mediterranean basin, contributes to sustainable rural development. In this work we analyse the possible trade-offs between two ES in the Sierra de Guadarrama National Park (Spain): pasture yield for livestock rearing and habitat for a protected species (white stork, *Ciconia ciconia* L.), whose colonies have increased greatly during the last decades.

Materials and methods

The study was carried out in an ancestral silvopastoral landscape in Madrid region formed by a *dehesa* of low-density ash trees (*Fraxinus angustifolia* Vahl; 21 trees ha^{-1}) and semi-natural meadows. Its objective was to quantify the effects of white stork occupied nests on pasture biomass, quality and diversity. We sampled 40 trees (20 occupied and 20 without nest) on an area with a high-density breeding colony of white stork (100 pairs 50 ha^{-1}). At each tree, we surveyed 50×50 cm^2 sample areas (n=120) at three different microsite/distances from the stem: subcanopy (beneath the nest and at equal distance to those trees without nest), ecotone (at the edge of crown projection) and intercanopy (beyond the influence of the canopy). Surveys were carried out in spring 2016. Stork breeding pairs and their chicks (4-6 birds per nest during 90 days) occupied the nests. Data processing and statistics were performed using R 3.2.4 (R Core Team, 2016). We used Generalized Linear Mixed Models (GLMMs) to analyse three response variables. The following variables determined in each subplot were included in the models as response variables: number of herbaceous species, nitrophilous species abundance and herbage dry matter. Tree nested within nest presence treatment was set as a random effect. In the models with species richness and nitrophilous species abundance as response variables, the following variables were included as fixed effects: microsite (subcanopy, ecotone and intercanopy) and nest presence (nest and no nest). Interactions between microsite and nest presence were also included in the model. The number of herbaceous species was fitted to a Poisson error distribution with a log link function. In the models with dry matter as response variable, in addition to the above fixed effects, plant functional group (grasses, legumes and forbs) were also included. We used the model-averaging approach in all models. First, we fitted the maximal model, containing all the predictors. Then, we performed model comparison of all possible models by using the AIC weights. For model comparison, we used the 'dredge' function within the 'MuMIn' package of R. Finally, we obtained the model-averaged coefficients as well as the relative importance of each predictor (from 0 to 1) by using 'model.avg' function of MuMIn. We calculated the explained deviance as well as the dispersion parameter of each model to evaluate its fit and ensure no overdispersion.

Results and discussion

Herbaceous plant diversity

A total of 64 different plant species linked to meadows were recorded on the study plots. We found that species richness significantly differed depending on microsites generated by trees (Table 1, Figure 1A). In areas beneath the canopy influence, species richness was significantly lower (6 species per 0.25 m^2) than in areas located within the ecotone or beyond the canopy influence (11 and 10 species, respectively). There were no significant differences in species richness between trees with active nests and without nests across all microsites. However, we found significantly lower species richness (3 species per 0.25 m^2) in trees with occupied nests beneath the canopy influence than in trees without nest for the same microsite (9 species per 0.25 m^2; Figure 1A).

Nitrophilous species abundance

Nitrophilous species abundance significantly differed depending on microsite generated by trees (Table 1, Figure 1B). In areas beneath the canopy influence, nitrophilous species abundance (*Hordeum, Lolium, Echium* and *Rhinanthus* sp) was significantly greater (53%) than in areas located within the ecotone canopy influence (22%), which in turn was significantly higher than in areas located beyond the canopy influence (16%). We did not find significant differences in this variable between trees with nest and without nest across all microsites. However, we found significantly lower (35%) nitrophilous species

Table 1. Summary of the Generalized Linear Mixed Models fitted to analyse the factors affecting species richness and nitrophilous species abundance.

Response variable	Predictors	Relative Importance	Factors	Coeff.	SE[1]	*z*-value	*P*-value
Species richness	Intercept			2.377	0.120	21.118	<0.001
	Microsite (M)	1.00	Subcanopy	-1.134	0.195	5.686	<0.001
			Intercanopy	-0.057	0.137	0.403	0.687
	Nest presence (N)	1.00	No nest	-0.005	0.155	0.034	0.973
	M×N	1.00	Subcanopy×No nest	0.922	0.241	3.738	<0.001
			Intercanopy×No nest	0.029	0.193	0.145	0.885
Nitrophilous species abundance	Intercept			2.940	0.258	11.141	<0.001
	Microsite (M)	1.00	Subcanopy	1.237	0.079	15.313	<0.001
			Intercanopy	-0.265	0.106	2.457	0.014
	Nest presence (N)	1.00	No nest	-0.168	0.366	0.449	0.653
	M×N	1.00	Subcanopy×No nest	-0.849	0.115	7.193	<0.001
			Intercanopy×No nest	-0.155	0.148	1.028	0.304

[1] SE = standard error.

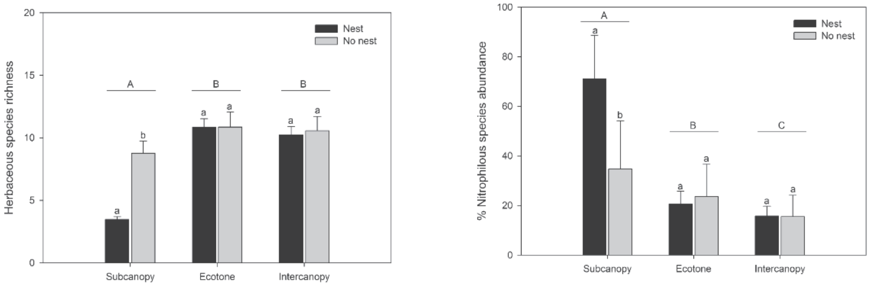

Figure 1. (A) Predicted herbaceous species richness (left) and (B) predicted nitrophilous species abundance (right). Predicted species (bars) depending on the microsites (y-axis). Error lines are 95% confidence intervals. Same lower case letters (a, b) above bars indicate no significant differences in species richness or nitrophilous species abundance within each microsite. Same capital letters (A B C) above group of bars indicate no significant differences among microsites.

abundance in areas beneath canopy influence in trees without nest than in trees with nest for the same microsite (71%; Figure 1B).

Herbaceous biomass

We did not find significant differences in dry matter yields between trees with nest and without nest across all microsites and plant functional groups (Figure 2). We found significantly higher grass yields than legume and forb yields across all microsites.

Conclusions

The two activities developed in the dehesa (nest protection and livestock grazing) showed quite good indicators of compatibility, even with such a high occupation nest density (Tryjanowsku *et al*, 2005). In this study, the presence of white stork nests affected only the subcanopy area, especially by increasing the abundance of nitrophilous species. Therefore, grazing livestock lose some herbage quality and diversity just underneath the active nests.

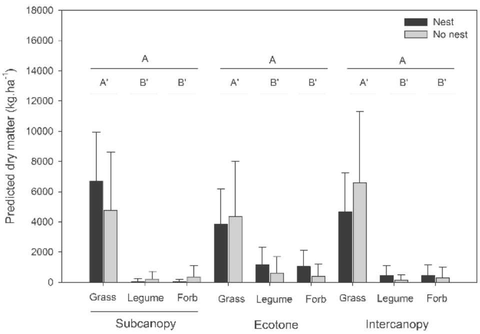

Figure 2. Predicted dry matter yield depending on the microsites and functional group. Error lines are 95% confidence intervals. Capital letters ($A^{/}$ $B^{/}$) above bars indicate significant differences within plant functional group, and same capital letters (A) above group of bars indicate no significant differences between different microsites.

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Fertilization with UAN on a natural grassland dominated by *Festuca rubra* L. and *Agrostis capillaris* L.

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Abstract

A field experiment was established in 2014, in the eastern part of Apuseni Mountains, with the aim of evaluating the effects of the fertilization with UAN (a liquid fertilizer containing 32% of nitrogen) on the dry matter (DM) yield and floristic composition of a natural grassland. The treatments were 0, 50, 75 and 100 kg ha^{-1} of UAN. The grass was cut once in 2014 and in 2015, and DM yield and botanical composition were determined. The natural meadow of *Festuca rubra* L. and *Agrostis capillaris* L. responded very well to the application of the liquid fertilizer. In situations where increased production is required the use of liquid nitrogen fertilizers may be advisable because of their positive effect on the DM yields of natural grasslands.

Keywords: *Festuca rubra* L., *Agrostis capillaris* L., liquid fertilizer, natural grassland, productivity

Introduction

Mountain farming in Europe is based mainly on ruminant livestock and utilization of locally produced forage resources by grazing and hay (Hopkins, 2011). Semi-natural grasslands are particularly important in mountainous areas of Romania, and for many farms, they are the only source of forage. An important part of efficient livestock production is ensuring sufficient grass for hay and pasture. However, low soil nutrient levels often limit forage production. In Romania, grasslands are an important forage resource, but irrational management systems during recent years have led to their present state of degradation (Vintu *et al.*, 2011). A deeper understanding of how agricultural practices have altered the landscape is essential for the management of biodiversity and conservation of semi-natural grasslands (Aune and Hovstad, 2016). With a good management of soil fertility, the productivity of many hay meadows and pastures can be greatly improved. The use of liquid mineral fertilizers (especially nitrogen) represents a measure for improving the productivity of semi-natural grasslands.

Materials and methods

The experiment was established in 2014, in the eastern part of Apuseni Mountains, at Baisoara (Cluj county), at an altitude of 1240 m. The experimental design was randomized block with three replicates of four treatments: V1 (0 kg ha^{-1}, control), V2 (50 kg ha^{-1}), V3 (75 kg ha^{-1}) and V4 (100 kg ha^{-1}) of UAN, a liquid fertilizer containing 15.5% of ammonium nitrate and 16.5% of urea, with chemical formula NH_4NO_3 NH_2-CO-NH_2.

Floristic surveys were performed using the Braun-Blanquet method before mowing, when *Agrostis capillaris* L and *Festuca rubra* L. were at flowering stage. Statistical analysis of results for dry matter (DM) yield was conducted with an analysis of variance and Duncan test for polyfactorial experiments. Floristic data were processed using the PC-ORD, Version 6, with Nonmetric Multidimensional Scaling (NMS).

Results and discussion

The phytocoenosis of the grassland was dominated by *Festuca rubra* L. and *Agrostis capillaris* L. as co-dominant species. The composition was modified by the application of more than 50 kg ha^{-1} of UAN, since the treatments V3 and V4 increased the proportion of *Agrostis capillaris* L., which became the dominant species.

Cyperaceae and *Juncaceae* species were linked to the lowest level of fertilization ($P \geq 0.05$), while the *Fabaceae* were associated with the untreated control and were reduced by the fertilization ($P \geq 0.05$). Species belonging to other botanical families were more abundant in the unfertilized or slightly fertilized plots. The floristic diversity was reduced by the fertilizer application, the greatest diversity being recorded in the unfertilized parcels ($P \geq 0.001$).

As shown in Figure 1, most species were related to lower fertilization: *Anthyllis vulneraria* (Anthvuln), *Achillea millefolium* (Achimill), *Briza media* (Brizmedi), *Campanula glomerata* (Campglom), *Cerastium holosteoides* (Ceraholo), *Cynosurus cristatus* (Cynocris), *Festuca rubra* (Festrubra), *Gentiana asclepiadea* (Gentascle), *Genista sagittalis* (Genisagi), *Genista tictoria* (Genitinc), *Gymnadenia conopsea* (Gymncono), *Holcus lanatus* (Holclana), *Leontodon autumnalis* (Leonautu), *Lotus corniculatus* (Lotucorn), *Polygala vulgaris* (Polyvulg), *Potentilla erecta* (Poteerec), *Rhinanthus minor* (Rhinmino), *Ranunculus acris* (Ranuacri), *Salvia officinalis* (Salvoffi), *Stellaria graminea* (Stelgram), *Trifolium pratense* (Trifprat), *Trifolium pannonicum* (Trifpanon), *Veronica chamaedrys* (Verocham).

Other species preferred higher amounts of fertilizer (75-100 kg ha^{-1} UAN): *Agrostis capillaris* (Agrocapi), *Anthoxanthum odoratum* (Anthodor), *Centaurea pseudophrygia* (Centpseu), *Carex pallescens* (Carepall), *Carum carvi* (Carucarv), *Galium verum* (Galiveru), *Hypericum maculatum* (Hypemacu); *Knautia dipsacifolia* (Knaudips); *Peucedanum palustre* (Peucpalu); *Phleum pratense* (Phleprat), *Plantago media* (Planmedi), *Rumex acetosa* (Rumeacet).

Regarding the DM yield, this was directly proportional especially to the large quantities of applied fertilizer (V3 and V4). This increase of DM was obtained mostly on account of *Poaceae* family plants ($P \leq 0.001$). In 2015, the DM yield ranged from 2.33 t ha^{-1} DM, for the control variant, to 4.85 t ha^{-1} DM for the most-fertilized variant, V4 (Table 1, Rotar *et al.*, 2016).

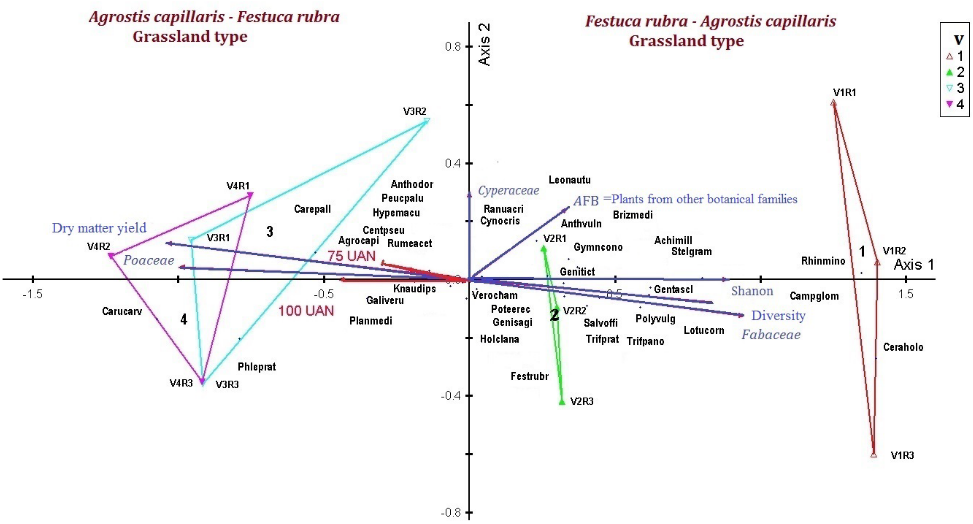

Figure 1. Influence of the treatments on plant species composition (Axis 1 and Axis 2). V1 = unfertilized control; V2 =50 kg ha^{-1} UAN; V3 = 75 kg ha^{-1} UAN; V4 = 100 kg ha^{-1} UAN.

Table 1. Influence of fertilization on DM yield in 2015 (t ha^{-1}).

Fertilization	Yield (t ha^{-1})	Significance[1]
V1-0 kg ha^{-1} UAN	2.33	(unfertilized control)
V2-50 kg ha^{-1} UAN	3.30	***
V3-75 kg ha^{-1} UAN	4.26	***
V4-100 kg ha^{-1} UAN	4.85	***

[1] Significance: *** $P<0.001$.

Conclusions

The *Festuca rubra* L. and *Agrostis capillaris* L. grassland type has changed in the phytodiversity, especially when 75-100 UAN ha^{-1} liquid fertilizer were applied. Results show that fertilization with UAN leads to some species increasing productivity at the expense of floristic diversity.

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Scorpiurus muricatus L.: an interesting legume species for Mediterranean forage systems

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Abstract

Scorpiurus muricatus L. (prickly scorpion's tail) is a legume species widely distributed as a spontaneous plant in Mediterranean pastures. In Sicily, farmers ascribe to this species a very high palatability and galactogogue effect, so that its abundance increases the value of the pasture. However, despite its worthy traits, the use of *S. muricatus* as a forage within cropping systems has not been well investigated. A field experiment was performed during two growing seasons in a semiarid Mediterranean environment to acquire information on the productivity of *S. muricatus* in comparison with other forage species grown in Mediterranean areas (e.g. berseem clover, burr medic, subterranean clover) and on its response to different cutting managements (cuts made in different phenological stages). Results showed that *S. muricatus* can provide biomass yield similar to, and in some cases higher than, that of the other forage legumes evaluated, differing from these species in its temporal distribution of the biomass accumulation. The findings contribute to define the role that *S. muricatus* could play in improving the productivity sustainability of the Mediterranean forage systems.

Keywords: biomass, cutting management, berseem clover, burr medic, subterranean clover

Introduction

Scorpiurus muricatus L. (common name: prickly scorpion's tail) is a self-reseeding annual legume fairly common in native pastures throughout the Mediterranean regions, particularly in Morocco, Algeria, Portugal, Spain, southern France and southern Italy. The species shows broad tolerance to soil type and pH and provides considerable biomass of good quality (Di Giorgio *et al.*, 2009). In Sicily, farmers ascribe to *S. muricatus* a very high palatability and a high galactogogue effect; so much so that when it is abundant the economic value of the pasture generally increases. Although *S. muricatus* could represent a very interesting species to be used as forage in Mediterranean cropping systems, the use of this legume within such systems is not well documented. Very few studies have been conducted to evaluate the effects of different cutting managements on the productivity and regrowth ability after defoliation of *S. muricatus*, and even fewer have investigated the behaviour of this species in comparison with other annual legumes utilized or utilizable for forage production in the Mediterranean areas. To address this knowledge gap, we performed a field experiment by growing, in pure stands, *S. muricatus* and other three legume species (burr medic, *Medicago polymorpha* L.; berseem clover, *Trifolium alexandrinum* L.; and subterranean clover, *Trifolium subterraneum* L. subsp. *Brachycalycinum*) commonly grown (or potentially utilizable) in the Mediterranean forage systems.

Materials and methods

A field experiment was conducted during the 2008-2009 and 2009-2010 growing seasons at the experimental farm Pietranera (37°30' N, 13°31' E; 178 m a.s.l.). In both growing seasons, the experiments were on a Vertic Haploxerept soil (525 g kg^{-1} clay, 227 g kg^{-1} silt, and 248 g kg^{-1} sand; pH 8.2; 16.8 g kg^{-1} total C and 1.78 g kg^{-1} total N). The experiment was set up in a split-plot design with four replications. Treatments consisted of four forage legume species (*S. muricatus*, berseem clover, burr medic, and subterranean clover) grown in pure stands, and four different cutting managements: T1 = first cut at 100 days after sowing (DAS) and cuts of regrowth every 28 days; T2 = first cut at 128 DAS and cuts of regrowth every 28 days; T3 = first cut at 156 DAS and cut of regrowth after 28 days; T4 = cut at 184 DAS. In both years, the previous crop was wheat. Soil was ploughed in August and harrowed after the first autumn rainfalls; before harrowing, 69 kg P_2O_5 ha^{-1} was applied in all treatments. Crops were hand-sown in both seasons in the last 15 days of December using, for each species, the seeding rate ordinarily adopted by farmers in the area (900, 400, and 900 viable seeds m^{-2} respectively for burr medic, subterranean clover, and berseem clover); on the basis of previous experiments performed on *S. muricatus* in the same area, a density of 400 viable seeds m^{-2} was used for this species. Plots were 4.0×7.0 m (16 rows, 0.25 m apart and 7 m long). Seeds were not inoculated with *Rhizobium* before planting because prolific nodulation occurs naturally at the experimental site. All plots were hand-weeded. Each plot was harvested by hand at the scheduled dates, and all plants were cut at 5-cm stubble height. At crop harvesting, total fresh weight was determined and a sample of plant material taken from the centre of the subplots was dried at 60°C for 36 h and then weighed to determine the dry matter yield.

A mixed model according to the experimental design was used on the combined 2-year data set, with year as a random factor. Treatment means were compared using Fisher's protected least significant difference (LSD) test at the 5% probability level. During the experimental period total rainfall was 715 mm in 2008-2009 and 810 mm in 2009-2010 (+30 and +47% than the long-term average for the area, respectively).

Results and discussion

Treatments T1 and T2 allowed us to harvest, respectively, 4 cuts and 3 cuts for all the species except for burr medic (3 and 2 cuts respectively); T3 provided 2 cuts only for berseem and subterranean clover; T4 consisted of only one cut. On average, *S. muricatus* had a dry matter production similar to, or in some cases higher than, that of the other annual legumes included in the study (Table 1).

The mean value observed for *S. muricatus* (850 g DM m^{-2}) is higher than the value found by Abbate and Maugeri (2007) for the species, and in line with the findings of Di Giorgio *et al.* (2009). *Scorpiurus muricatus* showed the slowest initial growth rate when compared with the other legumes, its biomass production, at the first cut of T1, being equal to 39, 55 and 77% of the biomass productions of berseem clover, burr medic and subterranean clover, respectively (Figure 1). On the whole, *S. muricatus* exhibited a high regrowth ability after defoliation, showing a good aptitude to utilizations based on intense, but spaced in time, defoliations. This ability was lower compared with that of berseem clover (which is reputed to be a species with a very high aptitude to this kind of utilization) but markedly higher than burr medic and subterranean clover, both species having a high capacity for regrowth but particularly suited for grazing (based on light but frequent defoliations). Moreover, *S. muricatus* showed a good productivity potential in the late growth stages, as shown by the dry matter yields in the last cuts of T1 and T2, where *S. muricatus* proved to be the most productive species. Thanks to this characteristic, *S. muricatus* could be integrated with other forage resources in the Mediterranean cropping systems, giving an important late contribution to forage supply and, therefore, extending the grazing season.

Table 1. Total biomass yield (g DM m^{-2}) by legume species and cutting management strategy. Data are means of two years.[1,2]

Cutting management	*Scorpiurus muricatus*	*Medicago polymorpha*	*Trifolium alexandrinum*	*Trifolium subterraneum*	Average
T1	698	354	928	583	641 c
T2	754	612	964	648	744 b
T3	934	702	965	965	891 a
T4	1,015	733	791	1,050	897 a
Average	850 b	600 c	912 a	811 b	

[1] See Materials and methods for details.

[2] The interaction 'Cutting management × Legume species' is significant at $P<0.001$ (least significant difference = 81). Different letters denote significant differences at $P<0.05$.

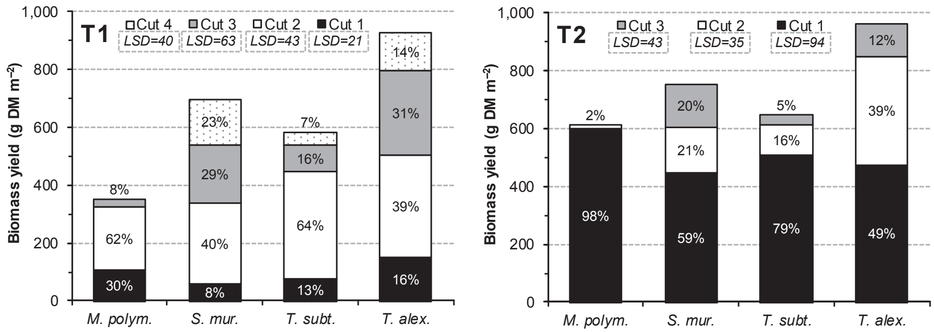

Figure 1. Biomass yield (g dry matter (DM) m^{-2}) by legume species and cutting management strategy (T1 and T2). Each histogram is divided into sub-histograms representing the biomass yield at each cut within each cutting management strategy. The proportion of each cut to the total biomass accumulated by each species is reported within each histogram. To compare the biomass yields given by the legume species at each cut, the significance and the LSDs are reported under the legend of each cut.

Conclusions

Results from the present study represent a contribution to the evaluation of the potential of *S. muricatus* to be used as a forage crop in the Mediterranean cropping systems. In particular, our research has shown that *S. muricatus*:

- has high dry matter productivity similar to, or in some cases higher than, that of other annual legumes commonly grown (or potentially utilizable) in the Mediterranean forage systems;
- is well-suited to cutting management strategies based on repeated defoliations; nevertheless, it is necessary to identify the timing for the first utilization in order to fully exploit the potential of the species, being the latter quite slow in the initial plant growth.

Moreover, since in both years total rainfall was markedly higher than the long-term average for the area, it will be necessary to carry out further research to test the behavior of *S. muricatus* under more typical rainfall conditions.

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Vegetation evolution in Mediterranean oak wood grazed by cattle

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Abstract

The aim of this work was to evaluate, in an oak woodland in Sardinia grazed by cattle, vegetation evolution observed in different areas located at increasing distance from animal water tank and hay feeder area. Between 2011 and 2015 at a 70 ha wooded study area (NW Sardinia; 670 m a.s.l.), dominated by *Quercus pubescens* L. and managed with decreasing cattle grazing intensity over the period, six circular plots (1,256 m^2) were studied. Herbaceous layer and tree canopy were evaluated within each plot. Species richness, contribution of each species to presence, and grazing value were assessed annually. Overall, the areas characterized by both low tree canopy density and basal area showed a significantly higher species richness and grazing value with greater percentage contribution of legume species. Grazing value highlighted a decreasing trend over the experimental period in four plots out of six, whereas species richness showed variability between the years. A trend towards forest encroachment was observed in the plots furthest from the water tank and hay feeder area.

Keywords: species richness, grazing value, cattle grazing, pasture-based livestock system

Introduction

Knowledge about how stocking rate and grazing management influence plant community structure is of critical importance for the sustainability of grassland ecosystems. In oak woods of Sardinia, under 25 years of constant grazing, stocking rate of 0.5 and 0.9 livestock units (LU) ha^{-1} have been shown to preserve biodiversity, avoid forest encroachment and favour tree growth (Scotti *et al.*, 2005). The aim of this work was to evaluate in an oak woodland in Sardinia grazed by cattle, the vegetation evolution in different areas located at increasing distance from animal water tank and hay feeders.

Materials and methods

The research was carried out between 2011 and 2015 in a 70-ha wooded area (NW Sardinia; 670 m a.s.l.) dominated by *Quercus pubescens* L. and grazed by cows for 40 years. The studied area is characterized by Mediterranean climate (mean annual rainfall 850 mm; average temperature 12.6 °C). Over the period, rainfall averaged about 982, 893, 1,253, 1,021 and 761 mm in 2011, 2012, 2013, 2014 and 2015, respectively. The predominant soils are clay-sandy (5.5 pH). Six circular plots (1256 m^2) were located from the farthest point (F1, F2, F3) to the nearest one (C1, C2, C3) to the water tank and hay feeder area. Within each plot, tree canopy was characterized by species and number of trees and by their basal area (BA) (FAO, 2010). The herbaceous layer was surveyed each year by inventorying plant species in spring using the point quadrat method (Daget and Poissonet, 1971). Total species richness (SR), percentage contribution of each species to presence (CSP) and grazing value (GV) per plot were calculated (Roggero *et al.*, 2002). The area was continuously stocked by cattle; the stocking rate (LU ha^{-1} year^{-1}) decreased over the five years observed (0.5±0.12; 0.31±0.06; 0.31±0.06 and 0.22±0.02 in 2011/12, 2012/13, 2013/14 and 2014/15, respectively). SR, CSP and GV were analysed with GLM procedure of SAS using year, plot and their interaction as fixed effects. The Cluster analysis based on CSP data was performed to investigate the similarity between plots. Clusters were separated using Silhouette analysis (Cluster packages of RStudio; http://www.rstudio.com).

Results and discussion

On the whole experimental area, over five years, a total of 90 species were recorded. Vegetation was dominated by *Quercus pubescens, Brachypodium sylvaticum* and *Hedera helix*. Shrub vegetation was dominated by *Pyrus spinosa, Rubus ulmifolius, Crateaegus monogyna* and *Prunus spinosa*. The overall tree density was high, whereas the difference in BA pointed out dissimilarity in the wood structure that significantly influenced vegetation characteristics (Table 1). In particular, C3, characterized by the lowest BA, which resulted in wide pasture patches outside of the tree crowns, had the highest SR and GV (Table 2).

SR significantly differed between plots and showed variability between years probably linked to the inter-annual rainfall variability. Overall, GV indicated a medium quality pasture (Argenti, 2006) and it showed decreasing trend over the experimental period in four plots out of six.

High CSP of legume and low CSP of *Pterridium aquilinum* and of *R. ulmifolius* characterized the C3 plot, whereas *P. aquilinum* (CSP from 10.1 to 13.5) and *R. ulmifolium* (CSP from 19.8 to 25.5) encroached on the F1 plot that was completely excluded from grazing since 2013 (Table 3). The tendency to encroachment was also observed in F2, where CSP of *R. ulmifolius* increased from 4.4% in 2011 to 10.1% in 2015, and in C1 where the GV decrement was due to the increase of *P. aquilinum* (7.9% to 12.2% CSP from 2011 to 2015). Cluster analysis (Figure 1) separated the six plots into three clusters, regardless the year, by finding similarities between F1, F2 and F3 (cluster1) and between C1 and C2 (cluster2). The C3 vegetation was different from the others (cluster3).

Conclusions

Wood structure significantly influenced vegetation characteristics with an improvement of the pasture quality in plots with wide patches outside the tree crowns. The decreasing GV and the increasing

Table1. Tree density and basal area of the experimental areas recorded at the beginning of observation period.

		F1	F2	F3	C1	C2	C3
Tree density	n ha^{-1}	836	3,073	1,162	2,237	2,794	1,058
Basal Area	m^2 ha^{-1}	26.3	30.3	31.1	19.1	27.1	10.4

Table 2. Species richness, grazing value and their evolution during the experimental period.[1]

	F1	F2	F3	C1	C2	C3
Species richness						
2011	25 AB a	21.8 B a	21.0 B a	27.0 AB a	26.3 AB ab	29.5 A bc
2012	20.8 C a	22.0 BC a	26.0 BC a	27.3 B a	26.3 BC ab	35.0 A ab
2013	20.3 BC a	17.3 C a	21.5 BC a	24.8 AB a	23.8 AB b	28.8 A c
2014		21.0 C a	25.8 BC a	26.8 BC a	30.3 AB a	36.3 A a
2015		22.0 B a	13.8 C b	25.5 B a	25.5 B ab	33.5 A abc
Grazing value						
2011	20.2 CD a	22.2 BC a	15.7 E a	23.4 B a	18.8 D a	36.5 A a
2012	16.7 D b	21.1 B a	16.6 D a	20.1 BC b	17.5 CD a	31.9 A b
2013	15.1 C b	21.0 B a	14.4 C a	21.0 B ab	20.1 B a	31.8 A b
2014		17.8 B b	14.0 C a	19.3 B b	18.5 B a	32.6 A b
2015		19.8 B ab	16.7 C a	20.1 B b	20.2 B a	27.0 A c

[1] Means followed by different uppercase letters within rows or lowercase letters within columns are significantly different at $P<0.05$ (protected Fisher's test).

Table 3. Average species to presence of the botanical families recorded in the plots.[1]

Family	F1	F2	F3	C1	C2	C3
Asteraceae	2.1 C	10.5 A	3.4 C	6.4 B	1.0 C	6.2 B
Caryophyllaceae	3.4 AB	2.8 B	5.3 A	0.4 C	2.1 BC	0.5 C
Graminacee	7.4 C	15.7 B	19.4 AB	7.3 C	20.8 A	17.9 AB
Leguminosae	0.9 D	0.6 D	0.9 D	10.5 B	6.4 C	15.7 A
Liliaceae	0.2 D	1.5 D	1.0 D	11.1 B	6.9 C	18.3 A
Pteridium aquilinum	13.3 B	8.7 C	18.9 A	14.4 B	8.5 C	0.4 D
Rubus ulmifolius	23.6 A	8.9 C	13.0 B	3.2 DE	4.9 D	0.8 E

[1] Means followed by different letters within rows are significantly different at $P<0.05$ (protected Fisher's test).

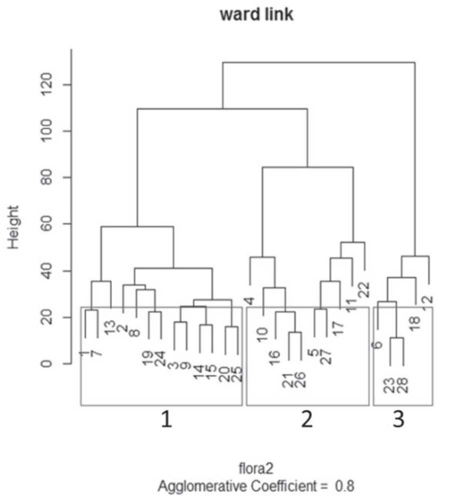

Figure 1. Cluster dendrogram reveals three groups of plots: cluster1 with F1 (2011-2013) and F2, F3 (2011-2015), cluster2 with C1 and C2 (2011-2015) and cluster3 with C3 (2011-2015).

encroachment could be linked to the reduction of stocking rate over the period. These was more evident in plots located furthest from water and feeders than the others, probably because they were less visited by the cattle. Precision-grazing approach, allowing the estimation of time spent from herd in a specific area (GPS-GIS system), could be a helpful tool in sustainable management of forest-based grazing system.

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Biodiversity and ecosystem services in Mediterranean cork oak wooded grasslands as affected by tree canopy

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Abstract

Mediterranean wooded grasslands are multipurpose systems that support high biodiversity levels and offer several ecosystem services (ES) such as forage production, soil carbon sequestration, nutrient recycling and soil protection. In a long-term observatory located in NE Sardinia (Italy), ten grazed wooded grassland fields were monitored over three years for plant biodiversity and selected ES below (BT) and outside (OT) the tree crowns. Botanical composition and seasonal herbage production, pastoral value and pasture utilization rate, biodiversity indicators (Shannon index and plant species richness), and the content of soil organic carbon (SOC) were assessed. In spring, dry matter (DM) yield and legume content were higher in the OT than in BT. In autumn and winter no differences were observed between OT and BT areas in terms of DM production. In winter, legume abundance and animal utilization rate were higher in OT than BT. The Shannon index was not influenced by the tree crown, while the plant species richness was slightly higher in OT than in BT. Top SOC content was lower in OT than in BT, but it showed a more uniform distribution along the soil profile.

Keywords: forage legumes, grassland production, pastoral value, soil organic carbon, tree crown

Introduction

Mediterranean wooded grasslands support high biodiversity levels and provide important ecosystem services (ES), including forage production for grazing animals and soil carbon sequestration. The specific microclimate and management conditions of the pasture under tree canopies result into peculiar features in terms of herbaceous dry matter (DM) yield, plant biodiversity and soil quality (Lòpez-Sànchez *et al.*, 2016; Rossetti *et al.*, 2015). Lòpez-Sànchez *et al.* (2016) in a typical dehesa ecosystem of central Spain found higher Shannon indices and species richness in the open grassland than under the tree canopies. The soil organic carbon (SOC) storage under the tree canopies is generally higher compared with open grassland (e.g. Seddaiu *et al.*, 2013). The aim of this study was to assess the tree canopy influence on plant biodiversity, DM production, pastoral value (PV) and SOC content in Mediterranean wooded grasslands.

Materials and methods

The study was conducted in the Berchidda-Monti long-term observatory in north-eastern Sardinia, characterized by a variety of land uses (Bagella *et al.*, 2014) with a dominance of wooded grasslands (30% of the study area). Average annual precipitation is 632 mm and mean annual temperature is 14.2 °C. The soil, developed from granitic rock, was classified as Typic Dystroxerept (USDA, 2010). Soil texture in the Ap horizon is sandy loam (USDA, 2010) with average pH 5.7 and SOC 2.3% (Seddaiu *et al.*, 2013). Ten grazed wooded grasslands were monitored from 2013 to 2015. In each field, three cork oak trees were randomly selected to assess outside (OT) and below (BT) the tree canopies, DM yield, pastoral value, plant biodiversity and SOC content. Pasture DM yield and herbage growth rate were evaluated monthly using the movable grazing exclusion cage (1×1 m) method (Frame, 1993). The

DM contribution of grasses and legumes and the pasture utilization rate by animals (UR, calculated in the cages as the ratio: (internal-external)/internal pasture DM) were assessed monthly when herbage was harvestable. Vegetation surveys were carried out in spring to assess Species Richness (S), Shannon's Index (H') (Shannon and Weaver, 1949) and PV (Daget and Poissonet, 1971; Roggero *et al.*, 2002). Soil samples were collected in April 2014 at 0-20, 20-40 and 40-60 cm depth and the SOC content was determined with a LECO CHN 628 elemental analyzer. Tree canopy effects (OT vs BT) were tested within sampling date with a one-way ANOVA (SAS Institute, 2002).

Results and discussion

During the three-year study, the cumulated autumn and spring rainfall was lower and the cumulated winter rainfall higher than the long-term average (196±148, 88±49 and 249±74 mm, in autumn, spring and winter, respectively, mean ± standard deviation). Mean seasonal temperature did not differ from long-term average values (12.4±0.5, 17.3±0.5 and 8.8±0.5 °C in autumn, spring and winter, respectively). In spring, OT produced more DM than BT, whereas in autumn and winter no differences were observed (Table 1). Autumn herbage production was negligible (≤0.1 Mg ha^{-1}). Legumes were more abundant in OT than in BT in winter and spring whereas no differences were detected for grasses. The PV and pasture UR were higher in OT (40.7) than in BT likely because of a higher legume content. The lower pasture UR in winter in BT resulted into a lower herbage spring growth rate in BT than in OT (17.3 and 39.7 kg ha^{-1} d^{-1} in BT and OT, respectively, $P<0.05$). The grassland H' was not influenced by the tree canopies, showing on average high levels (3.1±0.2), while the average plant species richness per sampling area was slightly higher in OT than in BT (44 vs 41 respectively, $P<0.05$). However, some 50% of the species in OT were different from those found in BT. The SOC content was always higher in BT than in OT along the soil profile with a sharper decrease with depth in BT (Figure 1). The periodic tillage and seeding that recurrently occur only in OT may have contributed to a more uniform SOC content along the soil profile.

Table 1. Pasture dry matter yield (DM), legume and grasses %, pasture utilization rate (UR) and pastoral value (PV), below (BT) and outside (OT) the tree canopy in winter and spring (averages from 2013 to 2015).[1]

Position	Winter				Spring				PV
	DM (Mg ha^{-1})	Legumes (%)	Grasses (%)	UR	DM (Mg ha^{-1})	Legumes (%)	Grasses (%)	UR	
OT	0.8	38	28	0.8	2.4	13	58	0.4	40.7
BT	0.7	3	39	0.4	0.9	2	62	0.2	32.9
P	ns	0.04	ns	0.01	0.03	0.01	ns	0.07	0.01

[1] ns = not significant ($P>0.05$).

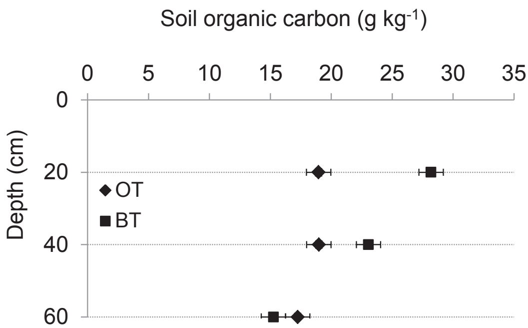

Figure 1. Soil organic carbon content (g kg^{-1}) below (BT) and outside (OT) the tree canopy in the wooded grasslands of the Berchidda-Monti observatory. Bars represents the standard error.

Conclusions

In the studied wooded grasslands, OT and BT canopy areas show different patterns of botanical composition and species richness that overall contribute to enhance the ESS. The higher availability of biomass, higher legume abundance and pastoral value in OT lead to higher pasture utilization rate than in BT. The soil in BT showed higher SOC than in OT, thus contributing to C-sink spots. Outside the tree canopy, agronomic practices such as tillage may have contributed to a more uniform SOC distribution along the soil profile. Further studies are worth to understand the complex relationships between biodiversity, productivity and the ESS of wooded grasslands, aiming to identify trade-offs between productive management and environmental goals.

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Nardus stricta L. and *Festuca rubra* L. meadow under management with organic inputs

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Abstract

Permanent grasslands dominated by *Nardus stricta* L. and *Festuca rubra* L. are an important source of fodder in the Dorna Region of Romania and are mostly used for cattle grazing. Improving soil fertility through the application of manure causes changes in floristic composition and proportion of species, fostering valuable species, thus contributing to the increase of production and the improvement of forage quality. The research was carried out in Şaru-Dornei, from 2007 to 2014, on an area located at an altitude of 940 m, with a slope of 10°. We followed the effects of fertilization with 20-50 Mg ha^{-1} manure, applied annually or every two years, on production, biodiversity and vegetation structure in a meadow dominated by *Nardus stricta* and *Festuca rubra*. Manure application had a positive effect on sward structure, reducing the cover of the dominant species *Nardus stricta*, in favour of valuable grasses (*Festuca rubra, Arrhenatherum elatius* and *Trisetum flavescens)* and legumes (*Lotus corniculatus, Trifolium repens* and *Trifolium pratense)*.

Keywords: manure, yield, efficiency, biodiversity, quality

Introduction

Sustainable management of grassland ecosystems requires knowledge of floristic composition, soil and climate conditions, these elements being connected each other (Janssens *et al*, 1998). Tracking the effects of improvement measures, especially of organic and mineral fertilization, and of management techniques, are therefore of particular importance (Hejcman *et al*, 2007). *Nardus stricta* L. and *Festuca rubra* L. grasslands from Romania's Carpathians represent the main source of forage for animals in local households. Fertilization with manure influences biodiversity, as well as the productivity and quality of these grasslands (Rotar *et al*, 2003; Vîntu *et al*, 2011), the main influenced parameters being crude protein content (CP), cell walls and digestibility of the forage.

Materials and methods

The research was conducted during 2007-2014 in the area of Saru Dornei in Dorna Basin, an area with an average annual temperature of 5.1 °C and a mean rainfall amount of 942 mm year^{-1}. A meadow dominated by *Nardus stricta* and *Festuca rubra* situated at an altitude of 940 m and with a slope of 10° was selected. Five treatments of organic fertilization were studied, monitoring the effects of increasing doses of well-fermented manure (Table 1). The experiment was arranged in completely randomised blocks, with four replications and a harvested plot size of 4×5 m. The chemical composition of 1000 kg of manure was 5.19 kg N, 2.83 kg P and 6.72 kg K. The cover of all vascular plant species was visually estimated in each plot using a metric frame methodology. Quality analyses, performed in a specialized laboratory, took into consideration the fodder from the first cut of 2014. Statistical analysis was performed by the SPSS statistic software using ANOVA and Least Significant Difference analysis (LSD).

Results and discussion

In the study period, the grassland productivity was influenced by the specific weather conditions of each year, but mostly by the amount of manure used and the mode of application. The average yields obtained in fertilized treatments were 100-133% higher than in the control and the highest efficiency (107 kg ha^{-1} DM per Mg ha^{-1} manure) was registered with V_4 (30 Mg ha^{-1} manure every second year,

Table 1. Fertilization levels (manure applied – Mg ha^{-1}).

Treatment	Year							
	2007	2008	2009	2010	2011	2012	2013	2014
V_1 – Unfertilized (control)	0	0	0	0	0	0	0	0
V_2 – 20 Mg ha^{-1} manure annually	20	20	20	20	20	20	20	20
V_3 – 30 Mg ha^{-1} manure annually	30	30	30	30	30	30	30	30
V_4 – 30 Mg ha^{-1} manure every 2nd year	30	0	30	0	30	0	30	0
V_5 – 40 + 0 + 50 + 0 Mg ha^{-1} manure	40	0	50	0	40	0	50	0

Table 2). As regards forage quality, CP and P content increased significantly and the content of cell walls, cellulose and lignin in particular (ADF) was lower by over 10%, leading to a higher forage value (Table 3). Increased production and a better fodder quality resulted probably from changes in vegetation structure due manure fertilization. In the study period, the percentage of grasses decreased from 78 to 48.7-52% (Table 4), together with the cover of the dominant species *Nardus stricta*, while that of more valuable grasses (*Agrostis capillaris*, *Arrhenatherum elatius* and *Trisetum flavescens*) was augmented. The share of legumes (*Lotus corniculatus*, *Trifolium pratense* and *Trifolium repens*) increased on average from 3.6 to 12.3-17.7%, improving forage quality. Total forbs cover increased from 18.0 to 32.4-36.9%. Manure application stimulated fair-to-good forage quality species such as *Achillea millefolium*, *Alchemilla vulgaris* and *Plantago lanceolata*.

Table 2. Dry matter (DM) production and fertilization applied (Mg ha^{-1}).[1]

Treatment	Year							Average	Efficiency (kg·ha^{-1} DM per Mg ha^{-1} manure)
	2008	2009	2010	2011	2012	2013	2014		
V_1 (control)	2.801	1.102	1.133	1.428	1.224	0.816	2.218	1.527	-
V_2	4.624	1.726	3.621**	4.222**	2.734**	1.534**	2.727*	3.097**	75
V_3	5.631*	2.404**	3.247*	5.006**	3.184**	2.326**	3.138**	3.528**	67
V_4	6.341**	1.527	2.939*	4.079**	2.835**	1.828**	2.522	3.126**	107
V_5	4.802	1.824*	4.317**	4.334**	3.616**	2.409**	3.116**	3.542**	89
LSD 0.05	2.234	0.631	1.539	1.528	0.627	0.331	0.527	1.092	
LSD 0.01	3.302	1.125	2.225	2.237	0.923	0.428	0.739	1.534	

[1] LSD = least significant difference. * $P<0.05$; ** $P<0.01$.

Table 3. Dry matter (DM) quality parameters.[1,2]

Treatment	CP (g 100 g^{-1} DM)	NDF (g 100 g^{-1} DM)	ADF (g 100 g^{-1} DM)	ADL (g 100 g^{-1} DM)	CA (g 100 g^{-1} DM)	CF (g 100 g^{-1} DM)	P (g 100 g^{-1} DM)
V_1 (control)	9.33	59.51	54.00	10.61	8.52	1.73	0.17
V_2	10.32**	58.09*	43.00**	9.82*	8.11	1.94	0.24**
V_3	11.61**	54.09**	42.62**	10.44	8.42	2.14**	0.26**
V_4	10.64**	53.91**	44.00**	9.92*	8.64	1.92	0.25**
V_5	10.31**	53.52**	43.00**	9.51**	8.92	1.91	0.28**
LSD 0.05	0.42	1.33	1.12	0.73	0.62	0.23	0.02
LSD 0.01	0.61	2.04	1.74	0.92	0.83	0.31	0.03

[1] CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; CA = crude ash; CF = crude fat; P = phosphorus.

[2] LSD = least significant difference. * $P<0.05$; ** $P<0.01$.

Table 4. Influence of organic fertilization on biodiversity (7 years average).

Species	Specific cover (%)				
	V_1	V_2	V_3	V_4	V_5
Agrostis capillaris L.	2.4	3.1	6.1	5.4	7.7
Anthoxanthum odoratum L.	3.1	2.6	3.6	3.6	3.4
Arrhenatherum elatius (L.) P. Beauv.	0.9	4.1	5.6	2.1	3.3
Festuca rubra L.	6.7	6.6	7.4	6.6	6.7
Nardus stricta L.	61.9	17.6	13.4	21.3	18.9
Trisetum flavescens (L.) P. Beauv.	1.6	9.0	7.7	4.0	7.3
Other grasses	1.9	4.7	6.0	5.3	4.9
Total grasses	78.4	47.7	49.9	48.3	52.1
Lotus corniculatus L.	0.7	1.4	5.7	3.4	3.9
Trifolium pratense L.	1.4	6.3	5.7	6.3	5.4
Trifolium repens L.	1.4	7.7	6.3	5.3	3.0
Total legumes	3.6	15.4	17.7	15.0	12.3
Achillea millefolium L.	2.9	4.6	6.0	8.1	5.9
Alchemilla vulgaris L.	1.9	4.3	5.7	5.6	4.0
Plantago lanceolata L.	1.9	5.4	2.7	3.1	3.0
Taraxacum officinale (L.) Weber	1.7	3.9	2.9	4.4	3.3
Veronica chamaedrys L.	1.1	6.7	5.6	5.3	8.9
Other forbs	8.6	12.0	9.6	10.1	10.6
Total forbs	18.0	36.9	32.4	36.7	35.6
Number of species	13	27	29	30	31

Conclusions

Organic fertilization positively influenced DM production in all treatments compared with the unfertilized control, increasing yield from +100 to +133%. Also, it improved the forage quality, resulting in higher crude protein and phosphorus content, and lower fibre content. Organic fertilization changed the structure and composition of the vegetation, as the dominant species, *Nardus stricta*, showed a substantial loss of specific cover in favour of species with a good forage value.

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STEPLA+ project: a tool based on information and communication technologies to monitor animal behaviour in grazing systems

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Abstract

Animal welfare is increasingly important for livestock, and likewise the use of pasture land in sustainable farming systems. Recent engineering advances, miniature technologies and the decreasing cost of electronic technologies has allowed the development of 'sensing solutions' that automatically collect and transfer data. Thus, STEPLA+ has been developing a tool that helps farmers to locate and monitor the activities of their herds especially in extensive farming systems. The tool was calibrated and validated in two periods through the elaboration of ethograms in Holstein cows in extensive production. The methodology used in ethograms was scan sampling by instantaneous observation looking at different behaviours: foraging, eating, rumination, drinking, grooming, resting, locomotion, agonistic, affiliative and sexual behaviours. The direct observations of the dairy cows during the regular milking (twice per day) together with GPS recorders and the three-axis accelerometer activity provide opportunities to increase data collection and improve the accuracy of this tool for extensive systems. Preliminary results from ethograms and the tool, showed that there is a correlation between the time spent grazing and temperature during August. The data of accelerometer in the 3-axes provided by the collars in October allowed differentiating the grazing activity.

Keywords: three-axis accelerometer, GPS, behaviour, ethograms, grazing systems

Introduction

Currently, the interest in developing electronic devices with higher sensitivity and larger data storage capacity for studying animal activity is increasing. In extensive systems, where livestock spend long periods ranging freely, these devices facilitate behaviour studies. However, the devices available on the market have batteries with short life. In this endeavour, it may be useful to try STEPLA+ tool, which provides GPS and three-axis accelerometer activity recorders for long periods. The information about individual and social behaviour could subsequently be used as indicators of health, welfare and reproductive status (O'Callaghan *et al.*, 2003; Roelofs *et al.*, 2005). As well as the study of specific activities such as grazing behaviour, it may improve the understanding of how the animals use the grass offered (Lockyer and Champion, 2001). Vázquez Diosdado *et al.* (2015) used a 3-axis accelerometer data to monitor cow behaviour when lying, standing and feeding. In addition, acceleration data were used in self-learning classification models in order to predict oestrus status in dairy cows (Yin *et al.*, 2013). The aim of this study was to test the STEPLA+ tool potential for the animal behaviour analysis. The influence of environmental conditions on animal behaviour was studied through the elaboration of ethograms in Holstein cows managed extensively.

Material and methods

The study was performed in two periods: August 2016 with seven Holstein cows (two multiparous and five primiparous), and October 2016 with five Holstein cows (two multiparous and three primiparous). Every animal carried STEPLA+ collars at their necks during the study. The collar contained a 3-axis high precision accelerometer, a GPS module, a microcontroller with embedded firmware and a long life battery. The hardware was designed to capture information each 11 min. Classification of different activities and postures of an animal requires an analysis system that decodes these data. A behaviour catalogue was drawn including ten conducts: grazing, research for forage, rumination, drinking, grooming, resting, locomotion, agonistic, affiliative and sexual behaviours. Animals were maintained throughout the experiment in pasture plots of 1 ha for 16 h day^{-1}. A topographic sketch of the grazing plots was made. The behaviour of the animals was recorded by ethograms performed between morning and afternoon milking by a multifocal scan sampling methodology. This method consists in recording instantaneous behavioural observation from each cow in a group, each two min. To validate this methodology a training session was hold for 3 h day^{-1} during 10 days. The ethogram recording time was from 10 to 20 min in August and total analysis time was 2,488 minutes during grazing periods for 11 days. The ethogram recording time was 30 min in October and total analysis time was 3,042 min during grazing periods for 11 days. In October, the database provided by the collars (one record each 11 min) with the observations recorded in ethograms (one observation each 2 min) was matched, and the ethogram observations that did not correspond with a record from collar at the same time were deleted. Therefore, the success rate of data were 403 out of 1,521 total records from the collars. Weather database information was included in each ethogram. The capacity of prediction of the behaviour with the data collars was evaluated using the Random forest method with R (R Core Team, 2016).

Results and discussion

The percentage of observed behaviours represents the time during which cows spent performing a behaviour during the ethogram data collection. In August (Table 1), the resting behaviour was the most common (34.0%), followed by rumination, grooming and grazing (20.7, 15.1 and 12.9%, respectively). In this period, the temperature registered varied from 21 to 27 °C and a decreased lineal correlation between grazing behaviour and the temperature was observed (R^2=0.80, Figure 1). In October the grazing behaviour was the most common (40.2%) followed by resting and rumination (24.0 and 20.6%, respectively).

Above 25 °C cows suffer heat stress (NRC, 1981) and this fact could have influenced dry matter intake and, in general, grazing behaviour as well. In contrast to August, in October the typical expression of grazing behaviour was evidenced within a thermoneutral zone that varies from 0 to 24 °C. In October the temperature registered ranged from 12 to 18 °C and the most common behaviour was grazing, as other authors also report (Kilgour, 2012).

The data values of the accelerometer in the 3 axes provided by collars in October allowed differentiating of the grazing activity from the other registered behaviours with a 70% success. With the acceleration data in the 3 axes collected in October trial, predicting resting and rumination activities was not possible.

Conclusions

Although differences between complex behaviours could not be distinguished, we evidenced the importance of developing a tool to collect big data, make appropriate predictions and incorporate weather factors into future models.

Table 1. Percentage of time spent for each activity during ethogram data collection from seven dairy cows in August trail and five dairy cows in the October trial.

Behaviour(%)	Grazing	Rumination	Resting	Grooming	Others	Observations	Total minutes
August	12.86	20.66	34.00	15.11	17.37	1,244	2,488
October	40.17	20.58	24.00	5.59	9.66	1,521	3,042

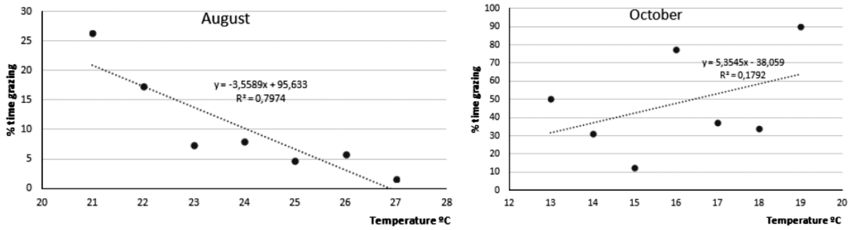

Figure 1. Percentage of time spent grazing during ethogram recording in August and October trials.

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Diffusion of heavy metals in plant species occurring in natural and improved grasslands of Sardinia

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Abstract

Although heavy metal (HM) contamination of crops is a matter of concern, such contamination has been little investigated in natural areas. Within the frame of a project funded by Regione Sardegna, we checked the spread of heavy metals in vegetation of natural and improved pasturelands selected according to their proximity to potential sources of pollution. We found a wide range of cadmium (Cd) concentrations, and generally a high Cd concentration in vegetation and soils of pasturelands close to military, industrial and mining areas, compared with control areas. The detected HM concentrations are discussed in terms of their impact on the food chain and compared them to values available in literature in order to ascertain critical values for sheep breeding.

Keywords: cadmium, lead, pasturelands, soils

Introduction

In the Sardinian census of polluted sites, 364 are listed, of which 157 are previous or current mining areas, 45 industrial areas, 59 disposal activities (checked or uncontrolled) of urban solid waste or needing reclamation, 98 are for storage or accidental loss of hydrocarbons, plus other emergencies (Action Plan Regional environmental-PAAR 2009-2013). The project CRP-24901-L.R. 7/2007, accomplished with regional funding, has provided data about the diffusion of heavy metals in pasturelands close to some of those polluted sites.

Materials and methods

Soils and plant species were sampled in six sheep-grazed areas considered at environmental risk due to their proximity to industrial, mining or military areas and in four control areas. Leaf samples from herbaceous, shrub and tree species in each of the ten areas were randomly sampled, taking into account leaf blade orientation and the predominant winds at each location. Topsoil was sampled at 0-10 cm depth inside 50×50 cm squares placed nearby sampled plants. Subsampling was done in the laboratory to obtain representative samples for analysis. Heavy metals were determined using a Shimadzu 6800 spectrophotometer (AAS) with flame (Flame Atomic Absorption Spectroscopy, FAAS), with graphite furnace (Graphite Furnace Atomic Absorption Spectroscopy, GFAAS) and hydride generator (HVG). Before analysis, soil and plant samples were previously acid digested by means of a microwave digestor MARS XPRESS/CEM according to manufacturer suggestions. We detected Cd, Co, Cr, Ni, Pb in plants and soils through GF-AAS, Hg and As through HGV, while through FAAS analysis we detected Cu, Fe, Zn, Pb and Cd. In this paper we discuss some data about cadmium, the most widespread element. The analysis of particulates deposited on leaf surfaces was done by means of an environmental electron microscope (ESEM) Zeiss LS10, using the detector of backscattered electrons (BSD-4 Quad Solid State Detector for Compositional Contrast), and the microanalysis scatter X-rays energy (EDS) INCAx-act (Oxford Instruments). Given the aims of the study, designed to identify any atmospheric deposition of elements on leaf surfaces, samples were not washed before analysis.

Results and discussion

With regard to the plant species, the scanning electron microscope combined with microanalysis allowed the detection of the presence of rare earth group elements (REE or lanthanide) (Figure 1) in some leaf samples. Although further investigations are desirable to determine the ability of HM to pass the cell wall, by analysing electronic microscope photos we observed that some of the particles deposited on leaf surfaces had a diameter smaller than the diameter of stomata (Figure 2), which may become a possible way to enter plants. In military areas, cerium, neodymium and lanthanum were often associated in the same particle together with other elements such as arsenic, aluminium, yttrium, cobalt, nickel and bismuth. With regard to the analytical determinations carried out on plants in AAS, a high Cd concentration was detected in the plant species sampled in areas adjacent to the mine Campo Pisano at Iglesias (Table 1), the Porto Torres petrochemical plant, the power plant Fiumesanto and the Ottana industrial site. Also high Pb concentrations were detected (up to 209 mg kg^{-1}) on *Verbascum* in Iglesias, much higher than the limits allowed by UE 1005/2015 Regulation (0.05-0.30 mg kg^{-1} Pb according to different edible plant categories).

Considering the soil samples examined during the research, it is worth mentioning the aspect related to the ingestion of soil by sheep grazing as a source of metal transfer from soil to the animals. In Iglesias we detected high concentrations in soil of Cd, Hg and Pb. These were several orders of magnitude higher than the permitted concentrations; for cadmium (Table 2) the average values were 38.2±43 mg kg^{-1}; much higher than the 2 mg kg^{-1} soil limit of Italian Decree Law n. 152/2006. Studies by Abrahams and Steigmaier (2003), Abrahams and Thornton (1994), Healy (1973,1967), indicate that, while factors related to soil chemical and physical composition and plant biology may limit the concentration of metals in plant species of the contaminated pastures, the soil ingested by animals can directly transfer potentially dangerous elements to grazing animals. Thornton and Abrahams (1983) conducted specific studies in England in areas contaminated by industrial and mining activities, and they concluded that cattle grazing can ingest unintentionally by 1-18% of ingested dry matter in the form of soil, while sheep can get up

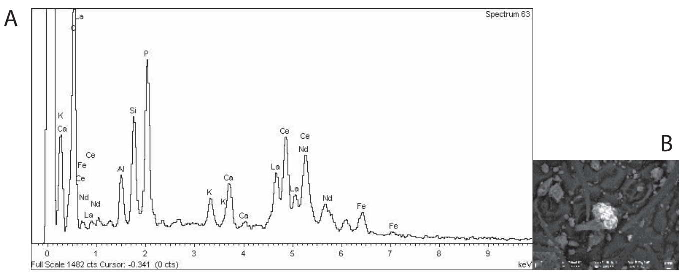

Figure 1. (A) Particle composition and (B) particle image on a *Malva* leaf sample from a military area examined by ESEM.

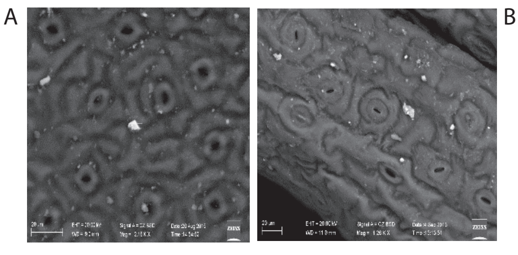

Figure 2. ESEM images of stomata and particles on leaves of (A) wild citrus and (B) wild fennel.

Table 1. Cadmium concentration in grass-legume mixtures in the control area. Cadmium concentration in some plants from the mine area.[1]

Sample		Grass/legume mg kg^{-1}	Grass/legume mg kg^{-1}	Grass/legume mg kg^{-1}	Grass/legume mg kg^{-1}	Grass/legume mg kg^{-1}
Control area	1	0.007	0.007	0.003	0.006	0,003
	2	0.016	0.031	0.019	0,015	0,02
	3	0.023	0.029	0.028	0,024	0,029
	Mean±SD	0.015±0.08	0.022±0.013	0.017±0.013	0.014±0.01	0.017±0.01
		lentisk mg kg^{-1}	wild fennel mg kg^{-1}	wild carrot mg kg^{-1}	Verbascum mg kg^{-1}	spurge mg kg^{-1}
Mine area	1	2.42	2.85	2.82	14.69	1.23
	2	2.86	2.05	2.74	13.06	1.42
	3	2.24	3.01	2.97	14.90	0.98
	Mean±SD	2.50±0.31	2.63±0.51	2.84±0.11	14.21±1.0	1.21±0.22

[1] SD = standard deviation.

Table 2. Cadmium concentration in five soils of the study areas.[1]

Sample	Mine area (SW) mg kg^{-1}	Industrial (SW) mg kg^{-1}	Industrial (NW) mg kg^{-1}	Control area (N) mg kg^{-1}
1	8.42	1.04	0.26	0.077
2	11.05	0.9	0.34	0.072
3	75.72	1.72	0.24	0.066
4	1.66	1.08	0.29	0.063
5	94.04	0.94	0.36	0.069
Mean±SD	38.18±43.25	1.14±0.33	0.30±0.05	0.069±0.005

[1] SD = standard deviation.

to 30%. The levels of ingested soil may vary seasonally and according to the type of management. The same authors showed that 9 to 80% of lead and from 34-90% of the arsenic ingested by cattle grazing on contaminated soils is due to ingested soil.

Conclusions

A study performed under the same project showed that southern Sardinian flocks exposed to environmental pollution had higher chromosome fragility and they were under a higher oxidative damage (Genualdo *et al.*, 2015). Such study was based on cytogenetic (sister chromatid exchange test) and physiological analysis (plasma concentration of non-enzymatic antioxidants and activities of enzymatic antioxidants) on sheep flocks reared in the same areas as those reported here. The plant and soil analysis we performed in such areas, confirm the environmental hazards due to higher HM concentrations. The presence of rare earth group elements, whose impact on human and ecosystem health needs to be deepened by toxicology and environmental chemistry research, could be another hazard. According to Pagano (2016), a more general outcome of several rare earth group elements toxicity studies consists of redox imbalances induced in cell systems, animals, and plants. Following the findings of this project, further investigations are advisable on pastureland species close to mining, industrial and military areas.

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Maintaining farming in wetlands: what are the challenges for breeders?

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Abstract

In Pays de la Loire (France), marshes and alluvial valleys are an integral part of the agricultural landscape: 140,000 ha are recognized as Natura 2000 areas, of which about 66,000 ha of agricultural land are operated by more than 2,500 dairy or cattle breeding farms. Since the second half of the 20th century, there has been reduced interest in wetlands for livestock farming, partly due to better forage yields on non-flooded agricultural lands, with the use of chemical inputs. A major challenge is therefore to maintain the attractiveness of these areas for new generations of farmers. However, little is known about the way these farmers manage their lands – mainly devoted to grasslands – with the flooding risk. The sociological survey we conducted, addressed to 46 breeders, reveals that they have specific know-how related to the large diversity of the meadows flora composition and yields. It also shows that natural risks are not perceived as a major problem. On the other hand, administrative difficulties and/or the confrontation between extensive farming, which relies on a significant use of natural grasslands, and more intensive systems, could be a real threat for the maintenance of cattle farming in these areas.

Keywords: grasslands, extensive farming systems, qualitative survey

Introduction

The French Environmental Code defines wetlands as 'lands, whether exploited or not, usually flooded or waterlogged (soft, salty or brackish) permanently or temporarily. The vegetation is dominated by hygrophilous plants for at least part of the year' (Environment Act, 2016). Wetlands have therefore a wide variety of profiles, ranging from marshes to alluvial valleys, and estuaries. Wetlands provide many physical, geochemical, hydrological, and climatic functions while allowing wealth creation through livestock (especially milk and meat) (Huyghe, 2009). In addition, wetlands are often part of the Natura 2000 network, because the rarity or fragility of their wild animal or plant species, and of the habitats that shelter them. As water is omnipresent, or always likely to flood the land, wetlands are considered as less favoured areas for agriculture. Since the second half of the 20th century, there has been reduced interest in wetlands for livestock farming, partly due to better forage yields on non-flooded agricultural lands, with the use of chemical inputs. Therefore, there is a strong challenge to renew the attractiveness of wetlands for new generations of breeders. However, little is known about the way farmers manage their lands – mainly permanent grasslands – with the flooding risk, and how they perceive their activity. Within the framework of a research programme aiming to bring to farmers technical and economic data to improve the extensive agricultural systems they use, a sociological study was conducted to indicate the specificity of the breeder's work when an important part of the farm is likely to be flooded. Floods can occur at different times of the year, and remain for short or long periods, and at high or less-high flood levels. This can have an impact on the flora of natural grasslands, but also on the possible access to the plots located in these wetlands (or even to the farm buildings). Flooding thus adds uncertainty to the farmer's work. In this paper, we report the specificity of knowledge that wetland farmers have, the way they perceive their job and how they imagine the possibility to transmit their knowledge.

Materials and methods

A qualitative approach was chosen to collect the comments of the livestock breeders. Initially, the Chamber of Agriculture, the French public extension service for agriculture, defined the sites to be surveyed in Pays de la Loire. In this region, 11% of the regional surface is covered by wetlands, which makes it second in the national ranking for this type of environment. There are 140,000 ha recognized as Natura 2000 sites, of which about 66,000 ha of agricultural land are operated by more than 2,500 dairy or cattle breeding farms. The Chamber chose to work on 11 Natura 2000 sites. A call for applications was launched in the regional press to create a network of 50 reference farms interested in participating in the project. The farms had to have at least 60% of their Usable Agricultural Area (UAA) in wetlands, be oriented towards dairy or cattle breeding, and to agree to communicate their technical and economic results. Semi-structured interviews were conducted in November 2015, by a group of six ESA students, as a part of their animal breeding specialization. They used an interview guide focusing on the general characteristics of the farm, the link of the breeding activity to wetlands, farmers' motivations to have their farm in such an area, the professional links and dialogue networks that exist among breeders, and finally how they think about the transmission of their knowledge. The complete transcription of the 46 interviews made a content analysis possible to achieve.

Results and discussion

Breeders have a detailed knowledge of the land they use: their comments confirm the large heterogeneity between wetland zones, but also within a zone. They report differences in altitude, soil bearing and composition, salinity, quality and floristic composition. Such differences affect yield, forage quality and how animals take advantage of the grasslands. Therefore, their use of wetland zones is also very diverse. Some meadows are only able to provide litter, while others allow the finishing of cattle with grass. Farmers located in alluvial valleys sometimes raise the beneficial contribution of the silt brought by floods. In all cases, farmers point out the forage security they can rely on, due to the ability of wet grasslands to produce forage all year long, even under summer drought conditions. Moreover, flooding risk often causes farmers (here two-thirds) to adopt extensive livestock systems, i.e. with more than 80% of grasslands in their main forage area. Valorisation of grasslands is associated with very low fertilizer inputs, these being brought more often in organic form. This enables breeders to comply with the standards set by the organic farming specifications and to get better prices for their products. In our sample, 14 out of 46 farmers are certified 'organic farming' which is well above the 5.7% observed in the region (ORAB, 2012). Also, nine out of the 46 prefer short distribution channels, which allow them to enhance their farming methods and to have a direct feedback on the quality of the products. In addition, they can benefit from European agri-environmental payments to preserve biodiversity. Here, 40 out of 46 farmers engaged a significant part of their grasslands in territorial agri-environmental contracts, and half of them even contracted 100% of their wet grasslands. Thus, unexpectedly, farming in wetlands can bring some technical and economic security. However, wetland farmers encounter a number of risks. They systematically stress the flooding risk, which requires a constant monitoring of water levels in order to adapt their work and to avoid the loss of animals when grazing. Benchmarks in the landscape related to the rise of water allow them to measure flows, and to estimate how long they have to remove animals from flooded grasslands. For some of them, this monitoring and the need to rebuild damaged fences after flooding, are seen as an additional workload. For those located in alluvial valleys, floodings can erode the banks and remodel the land on the long term. Many farmers also deplore the destructive effect of coypu (*Myocastor coypus*); this species digs holes that weaken the banks, and it may carry serious diseases including leptospirosis. As in the case of shepherds in mountain areas (Blanc, 2009), breeders acquire a local knowledge to cope with technical risks. Nevertheless, in the breeders' words, these risks are lower than the economic risk that is caused by the State's delay in making payments by more than one year. The administrative system is deeply criticized, especially since it goes with picky and badly explained controls. Finally, our survey also showed that wetland farmers have to confront the negative idea that other farmers have about wetlands. They report situations where the confrontation between them and intensive dairy or cattle breeding farmers located on cropped lands is hard to stand, because they are not seen as real professionals: 'Because we did not produce much, because we only made natural grassland, [...] we are considered to be small, small breeders who tinker.' In the end, natural hazards are not the ones that are most evident in what farmers say: we can assume that breeders are more easily able to cope with 'natural' uncertainties (floods, meteorological data, sanitary pressure, etc.) than with the economic, social, and administrative constraints to which their profession is increasingly confronted. Natural and technical hazards are seen as part of their work and their specificity in a wetland, not as major constraints. As one farmer explained: 'to be a wetland farmer, you need to be adventurous.' Despite its economic and environmental advantages, the extensive model developed by most of the farms surveyed still is depreciated, which can restrain the arrival of new farmers.

As they are fine connoisseurs of the environment in which they operate, breeders sometimes regard themselves as experts compared with the advisers or technicians with little training about farming in wetland conditions. One farmer explained: 'finally, we are experts, but with no recognition'. Therefore, when addressing the question of setting up new farmers in these areas, they stress the need to combine theoretical and practical knowledge. If they remain open to new scientific knowledge, they also explain the importance of the practical advice provided to them by colleagues or family to deal with the floods during informal exchanges. Their knowledge is a local and practical knowledge, learned from, and adapted for, their territory. Thus they promote the idea of developing wetland farmers' networks to shares experience between peers coming from similar zones.

Conclusions

Despite the natural diversity of the wetlands where the farmers of this study work, we conclude that all breeders of these zones acquire and use a specific local knowledge. Unexpectedly, the major difficulties perceived by farmers to valorise wet grasslands are not technical, but related to the administrative management of agri-environmental contracts and to the depreciation of extensive livestock farming by other farmers. Whereas their extensive husbandry is in line with society's demands, we can underline the existence of discordances within the agricultural profession in respect to agroecology transition.

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Nutrient availability and productivity gradients in permanent grasslands of the central Balkans

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Abstract

Permanent grasslands cover more than half of the agricultural land in Bosnia and Herzegovina and 88% in Montenegro, thereby playing an important role in forage production and ecosystem services. Extensively managed natural grasslands are predominant, and in general their productivity is extremely low. *Agrostietum capillaris* is one of such grassland communities, which is currently threatened by rapid changes in agricultural practices, primarily land abandonment. We surveyed pH, organic matter content and plant-available macronutrients in soils from these grasslands (five sites in Montenegro and two in Bosnia and Herzegovina) in order to evaluate the current production prospect of abandoned meadows and pastures, and see in which way soil properties affect their species diversity. Near the surface (0-10 cm) the soils were strongly acidic, well supplied with potassium, showing some organic matter fluctuation, but highly variable in phosphorus content. We also surveyed botanical composition and evaluated productivity for the year 2016. The results of two main interplaying mechanisms are examined: species pool and mass spatial effects. In our study, the species pool affected plant diversity more strongly than the mass spatial effect did.

Keywords: *Agrostietum capillaris*, crude protein, grassland, soil nutrients, DM yield

Introduction

Meadows and pastures are significant sources of feed for animals, and due to the global trend for safer food production, their importance is on the rise (Tomić *et al.*, 2005). Biodiversity protection has also become one of the main concerns since modern farming, forestry and meadow culture focus on high production, without concern for the quality of products or environmental health (Vintu *et al.*, 2011). Plant diversity patterns have mainly been explained by local determinants, such as soil properties, management and land use history. However, it was noticed that plant community structure and diversity may also be affected by nearby habitats via effects on the regional species pool and dispersal limitations (Janišova *et al.* 2014). Nutrient availability in permanent grasslands has a strong influence on plant species biodiversity, plant cover, and species' dominance in the vegetation canopy. The general characteristics of grasslands in the central Balkans are poor production and unsatisfactory quality (Simić *et al.*, 2016). Forbs make up over half of the plant production on areas that are not maintained by mowing and fertilizing. Natural grasslands and pastures in Bosnia and Montenegro take up more than half of agricultural land. The majority of natural grasslands are located in hill-mountain regions. The association *Agrostietum capillaris* prospers on low-nutrient acidic soils of this region. Many highly degraded areas are no longer able to support adequate biomass production. In this paper, we assessed the relationship between nutrient availability and production parameters of this community, and explored its link to biodiversity and abundance of three plant groups significant for fodder quantity and quality (grasses, legumes and forbs). We addressed the following questions: (1) does nutrient availability of these grasslands correspond to the productivity gradients (species number, dry matter yield and forage quality)? (2) To what extent can the overall features of a particular habitat be used as an indicator of the productivity gradient in *Agrostietum capillaris* communities?

Materials and methods

The survey was conducted on representative *Agrostietum capillaris* meadows found in diverse ecological conditions from seven sites in two countries (Figure 1). Three study sites were situated on the N- to NW and NE-facing slope and four on the S- to SE facing slope. The nutrient status of topsoil samples (0-10 cm) collected in summer at each study site was determined by standard methods: pH values in deionised water and 1M KCl (1:2.5 w v^{-1}); total organic C by dichromate redox titration method; total N by semi-micro Kjeldahl method; and available P and K by the AL-method (Pansu and Gautheyrou, 2006). Identified plant species were classified by their quality into three categories: quality grasses, quality legumes and forbs (harmful, useless or conditionally useful plant species from other plant families) (Tomić *et al.*, 2005), and the abundances of these yield-contributing species were noted. The study fields were mowed once, at the time of inflorescence formation of the dominant grasses (late June – early July). Forage dry matter yield (DM) was determined by weighing dry samples harvested from 5 m^2 area, and crude protein (CP) content was later determined over total nitrogen content in the samples.

Results and discussion

In all study sites soil pH was acidic, between 4 and 5 (Table 1). Both organic matter content and total N were high at all the locations, which was expected for meadow soils. Common for grassland-covered acidic soils of the Balkans, all the sites were very poor in readily-available phosphorus, except site no. 2, where apparently man-made inputs (i.e. fertilization) occurred. In grassland communities, the plant available P in topsoil is a good indicator of the intensity of grassland management as well as a measure of sustainable grassland management (Bohner, 2005), but in this study P content correlated weakly with DM yield or CP content. Species-rich grasslands were restricted to soils with low content of available P in the topsoil. Potassium was in adequate supply, as the geological formations have abundant K-holding minerals. In contrast to other nutrients, a relatively narrow range of K (212-455 mg kg^{-1}) was determined. The number of grass species found varied between three and nine. The proportion of legumes in the vegetation canopy was very low and none were found at location no. 2. However, a notable contribution of forbs to the canopy was seen. In alpine environments (locations 3 and 6) a significant production of legumes was observed, improving the quality of fodder and the crude protein content. High numbers of forbs suggest the importance of this association for biodiversity, but this also underlines poor quality and low production for livestock farming. In particular, high diversity was reported at location no. 7, where the influence of the Mediterranean climate on the harsh alpine weather was noticeable. The pattern of species number variation in surveyed meadows confirms the statement that surrounding habitats are sources of supplementary species the ecological optima of which are found in other vegetation types (Janišova *et al.*, 2014). In this way, both natural and non-natural habitats may constitute sources of additional plant species, thereby contributing to the local grassland diversity. Dry matter yield was low (2.22-3.89 Mg ha^{-1}), and the protein content was poor (55-93.5 g kg^{-1}), primarily due to the significant number of forbs and limited legume contribution. It is reasonable to suggest that proper management along with reasonable utilization and manure fertilization would lead to an improvement of production in these grasslands.

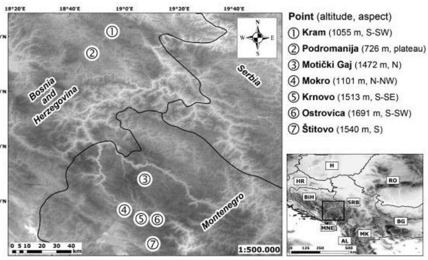

Figure 1. Ass. *Agrostietum capillaris* study locations in two countries.

Table 1. Chemical properties of the examined soils, share of quality plant categories, yield and quality in ass. *Agrostietum capillaris.*[1]

Location	pH		Nutrients					Plant categories				Yield and quality	
	H_2O	KCl	TOC	OM	N	P	K	G	L	F	T	DM	CP
1. Kram	5.33	4.42	58.9	101.5	4.08	32.2	349	7	5	10	22	3.23	55.0
2. Podromanija	5.11	3.98	39.5	68.0	3.24	237	227	3	-	4	7	3.10	62.7
3. Motički Gaj	5.82	5.11	72.7	125.3	1.46	19.0	455	9	4	14	27	3.89	90.5
4. Mokro	5.40	4.16	49.2	84.8	3.13	10.1	333	5	4	7	16	3.57	77.7
5. Krnovo	5.52	4.52	52.7	90.8	4.45	10.1	212	3	2	10	15	2.30	59.4
6. Ostrovica	5.03	4.18	97.4	167.9	8.18	31.3	219	4	3	4	11	3.69	93.5
7. Štitovo	5.26	4.16	104.8	180.6	8.44	13.7	277	4	4	20	28	2.22	57.8

[1] TOC = Total organic C (g kg^{-1}); OM = organic matter (g kg^{-1}); N = total N content (g kg^{-1}); P = AL-P_2O_5 (mg kg^{-1}); K = AL-K_2O (mg kg^{-1}); G = grasses; L = legumes; F = forbs; T = total number of species; DM = dry matter yield (Mg ha^{-1}); CP = crude protein content (g kg^{-1}).

Conclusions

We assessed the species richness and the productivity of extensive meadows of *A. capillaris* in relation to the soil nutrient status. In general, the examined soils were acidic and deficient in phosphorus, but rich in organic matter and well supplied with potassium. Botanical composition of the meadows varied, mainly due to a high number of site-specific forbs. The above-stated findings lead to poor production potential of this association for livestock production, nonetheless offering sustainable means of soil and biodiversity protection in the area.

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Influence of fertilization and cutting frequency on the characteristics of semi-natural grasslands in Protected Landscape Area of Žďárské vrchy Hills

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Abstract

Grasslands in central Europe are communities mainly replacing forests supplying several ecosystem services, including forage. The fulfilment of multiple functions depends on management, which should aim at enhancing biodiversity as well as forage quality and high yields at the same time. The aim of this paper was to evaluate the influence of fertilization (90 N, 180 N, P, K) and cutting frequency (2 and 3 cuts) on the diversity index and grassland forage quality. High levels of nitrogen were shown to be beneficial ($P<0.05$) for both the grassland quality and production. Fertilization had an adverse effect on biological diversity, which decreased ($P<0.05$) with a high level of fertilizers. Application of phosphorus and potassium increased ($P<0.05$) grassland forage quality, while maintaining species diversity.

Keywords: meso-hygrophytic grassland, fertilization, species diversity, grassland quality

Introduction

Grasslands are the most abundant type of vegetation cover of our planet after forests. A multi-functional harmonious landscape formed by grasslands provides economic benefits as well as ecological and social functions such as protection of biodiversity, soils and air (Kampmann *et al.*, 2011). As heterogeneous associations, grasslands feature considerable species diversity, which is reflected in their forage quality. Floristic composition is decisive for yield and forage quality, and is affected by site, fertilization and management practices. On the other hand, inadequate management methods such as excessive fertilization may result in the degradation of grasslands in terms of their botanical composition (Chapman, 2001). The simplest evaluation of grassland from a floristic point of view is needed for use in practice, to set the quality and forage value. The most inexpensive method is a method of estimation based on botanical composition, which enables us to set the quality of forage accurately, without demanding chemical analyses (Novak, 2004). The aim of this work is to evaluate the influence of NPK fertilization on the species diversity and grassland quality.

Material and methods

An experiment was established on the semi-natural meso-hygrophytic grasslands in the Protected Landscape Area of Žďárské vrchy Hills in 1993. The present paper summarizes the results achieved from 1993 to 2013. Mean annual temperature and precipitation (1951-2000) were 5.8 °C and 758.4 mm, respectively. Soil was an acidic Luvic Stagnosol on the gneiss diluvium. It was loamy sand to loam. The contents of available nutrients assessed by the Mehlich III method (Mehlich, 1984) were 13.4 mg kg^{-1} P, 51.4 mg kg^{-1} K, 94.3 mg kg^{-1} Mg, 1,253 mg kg^{-1} Ca, pH value was 4.2.

The experiment was laid out by using the design of split compartments in four repetitions with plots of 15 m^2 (1.5×10 m). Two factors were studied: (1) fertilization with four treatments: no fertilization (N0), PK fertilization (PK), N90+PK fertilization (N90) and N180+PK (N180); and (2) cutting frequency; 3 and 2 cuts per year. Nitrogen was supplied in the form of ammonium nitrate with limestone (LAV 27%, N 27%) at a total amount of 90 kg ha^{-1} and 180 kg ha^{-1} applied three times (30 or 60 kg ha^{-1} in spring, after 1st cut and after 2nd cut) or two applications (60 kg ha^{-1} or 120 kg ha^{-1} in spring and 30 kg ha^{-1} or 60 kg ha^{-1}after 1st cut). PK fertilizers were applied in spring: P in the form of Hyperkorn (26%) at 30 kg ha^{-1} and K as potassium salt (60%) at 60 kg ha^{-1}. The stands were harvested in early June, early August and early October (3-cut regime) or in mid-June and early September (2-cut regime). Grass was harvested by a mower (model MF-70) with a cutting bar of 1.2 m on an area of 12 m^2 at a height of 0.07 m. Characteristics assessed in the experiment included: the ratio of plant functional groups (i.e. grasses, legumes and forbs) at the 1st cut, Simpson's diversity index ($D = 1/ \Sigma Pi^2$; Pi is the rate of ith species), and evaluation of the forage quality ($E_{GQ} = \Sigma(Pi.FV)/8$; where Pi is the rate of ith species (%) in the harvested forage and FV is feeding value from -4 (highly poisonous) to 8 (highly valuable) of the given species) (Novak, 2004). In order to establish the rate of individual species or plant functional groups in the harvested forage, samples of aboveground biomass were taken from permanently staked plots (0.5 m^2), divided into individual species and dried at 60°C. Subsequently, they were weighed in a dry state and the proportions of individual species determined and expressed as percentages of total DM weight. Dry matter yields (103 °C) were the sum of all the cuts. Statistical analysis was conducted using the Statistica 10.0 CZ programme. The effect of fertilization was assessed by ANOVA and Tukey post-hoc test.

Results and discussion

Grasses (*Poaceae*) are the dominant component in the evaluated sward (Table 1). The proportion of grasses increased at the expense of herbs (P<0.05) by NPK fertilization, which increased especially *Alopecurus pratensis* L. on meso-hygrophytic site. Tall grass *A. pratensi*s L. dominates on moist sites (Gotze *et al.*, 2010). Use of fertilizers leads to a decline in the proportion of herbs (Hejcman *et al.*, 2007). On the other hand, a reduced competition of grasses at lower doses of N leads to an increase in the proportion of legumes (Obergruber, 1991), such as *Trifolium repens* L., which is very palatable species with high feed value (Novak, 2004). Two cuts increased tall grass to the detriment of *Trifolium repens* L., and it also reduced *Ranunculus acris* L. a poisonous plant. A higher rate of grasses and a lower rate of herbs led to a higher (P<0.05) forage quality (Table 2) and a decrease of diversity index (P<0.05). However, P and K fertilization improved grassland quality by increasing (P<0.05) the proportion of legumes and by maintaining species diversity.

Table 1. Effect of fertilization (1993-2013, n=21 years) on the rate (%) on the plant functional groups under 2- and 3-cut regimes.[1]

Dominant species/groups	3-cut regime				2-cut regime			
	N0	PK	N90	N180	N0	PK	N90	N180
Phleum pratense L.	2.4^{ab}	2.1^{a}	5.0^{b}	3.7^{ab}	1.9^{a}	3.6^{ab}	5.9^{b}	1.5^{a}
Festuca rubra L.	10.8^{a}	4.6^{b}	5.5^{b}	4.4^{b}	6.0^{a}	2.1^{b}	1.8^{b}	1.2^{b}
Alopecurus pratensis L.	7.4^{a}	15.8^{b}	28.3^{c}	31.9^{c}	7.5^{a}	26.5^{b}	50.2^{c}	48.3^{c}
All grasses	52.0^{a}	42.7^{b}	61.8^{c}	71.6^{d}	44.9^{a}	50.5^{b}	74.0^{c}	78.0^{c}
Trifolium repens L.	2.4^{a}	9.1^{b}	2.3^{a}	0.4^{a}	1.0^{a}	6.0^{b}	0.4^{a}	0.1^{a}
All legumes	4.3^{a}	14.6^{b}	3.1^{a}	0.4^{c}	2.6^{a}	9.1^{b}	0.5^{c}	0.0^{c}
Ranunculus acris L.	10.4^{a}	6.8^{b}	4.0^{c}	1.5^{d}	4.8^{a}	6.9^{a}	2.6^{b}	1.1^{b}
Bistorta major S.F. Gray	6.2^{a}	15.3^{ab}	16.2^{b}	9.2^{ab}	13.6^{ab}	1.5^{a}	8.8^{ab}	14.6^{b}
All forbs	43.7^{a}	42.7^{ab}	35.0^{c}	28.0^{d}	52.5^{a}	40.3^{b}	25.5^{c}	22.0^{c}

[1] In the same rows average values with different superscripts letters are significantly different at P<0.05.

Table 2. Simpson´s diversity index (D), grassland quality (E_{GQ}) and dry matter yield (t ha^{-1} year).[1]

Fertility	3-cuts			2-cuts		
	D	E_{GQ}	Dry matter yield	D	E_{GQ}	Dry matter yield
N0	7.3^a	30.4^a	3.9^a	6.0^a	37.8^a	3.9^a
PK	7.3^a	48.3^b	5.9^b	6.0^a	54.3^b	5.8^b
N90	5.2^b	57.0^b	6.9^c	3.6^b	63.5^b	6.6^c
N180	4.7^b	50.4^b	8.1^d	2.9^b	65.8^b	7.5^d

[1] In the same rows average values with different superscripts letters are significantly different at P<0.05.

Conclusions

The application of NPK fertilizer led, on one hand, to a reduction in species diversity, and on the other hand to a higher grassland forage quality. Applications of P and K fertilizers (without N) increased the rate of legumes, and thus the quality of grassland herbage, while also preserving species diversity.

Acknowledgements

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Cultivar choice in Italian ryegrass (*Lolium multiflorum* Lam.)

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Abstract

At the beginning of the sowing season, farmers have to choose the species and cultivars to grow. When meeting local farmers, a lack of knowledge on the use of the different forage species and cultivars has emerged. Market distributors import seed on the basis of the lowest international price. This focus on price makes finding the best-adapted varieties to our environmental conditions difficult. In this context, eleven cultivars of Italian ryegrass available on the local market were tested. The evaluation was carried out in an experimental farm located in southern Sardinia. Compared to similar tests performed over ten years ago, the range of varieties was remarkably different. Results show 6.33 t ha^{-1} of dry matter yield annual average, without significant differences among varieties. Conversely, some differences in winter growth and flowering date, ranging from the last week of April to the first week of May, were found. The choice of the variety should not be led by annual potential production only, but by seasonal production and class of earliness.

Keywords: *Lolium multiflorum* Lam., yield, earliness, variety

Introduction

At the beginning of the sowing season, farmers have to choose the species and cultivars to grow (Franca *et al.*, 1995; Tabaglio *et al.*, 2006, 2007). In several meetings with local farmers, a lack of knowledge on the use of the different forage species and cultivars has emerged. Indeed, market distributors import seed on the basis of the lowest international price. Hence, the focus on prices makes finding seeds belonging to the best-adapted varieties for our environment difficult. Compared to similar trial tests performed over ten years ago, the range of varieties is nowadays remarkably different. In this context, eleven cultivars of Italian ryegrass (*Lolium multiflorum* Lam.), all available on the local market, were tested.

Materials and methods

The trial was carried out in 'S. Michele' farm, an experimental station located in southern Sardinia (39°10'N, 9°06'E, 150 m a.s.l.) on a *Typic Calcixerept* soil (USDA, 2010). The average rainfall is 500 mm, irregularly distributed from October to May. Winter temperatures seldom fall as low as 0 °C; average maximum temperature is 32 °C in July.

Eleven variety of Italian ryegrass (*Lolium multiflorum* Lam.) listed in Table 1 were sown for three consecutive years, from 2011 to 2013 in mid-November, at a seed rate of 30 kg ha^{-1} in plots of 16 m^2 each. The experimental design was a randomized block design with three replicates. All plots were harvested at a cutting height of 5 cm according to the crop development (about 25 cm height in winter, and at flowering in spring). A mowing-weigher Hege 212 was used for harvesting. Plots were fertilized with 40 units of nitrogen after each winter utilization period. Dry matter (DM) percentage was calculated after oven-drying 500 g sub-samples in a forced draught oven at 65 °C for 72 h. In order to determine the class of earliness, weekly observations on the phenological phases were carried out from April to May. A factorial design was used to test total, winter and spring DM yield. (Snedecor and Cochran, 1956; Payne *et al.*, 2010).

Table 1. Varieties and their characteristics.

Variety	Seed company	Ploidy	Earlinesss
Jivet	Pasture Genetics	tetraploid	early
Lazo	Gentos S.A.	tetraploid	early
Jeanne	Psture Genetics	tetraploid	late
Atomic	Upper Murray Seeds	tetraploid	late
Pollanum	Dlf Trifolium AS	tetraploid	intermediate-late
Libonus	Deutsche Saatveredelung	tetraploid	early
Asso	Società Italiana Sementi	diploid	early
Teanna	Continental Semeces	tetraploid	intermediate
Nival	Deutsche Saatveredelung	tetraploid	late
Gepetto	Società Italiana Sementi	diploid	intermediate
Tiento	Gentos S.A.	tetraploid	intermediate

Results and discussion

The amount of rainfall during the growing seasons (October – May) was 387, 423 and 467 mm, for 2011-2012, 2012-2013 and 2013-2014, respectively, with remarkable seasonal differences. The DM yield was 6.68 t ha^{-1}, 5.50 t ha^{-1} and 6.87 t ha^{-1} in the three years, respectively.

The coefficient of variation (CV) of the total DM yield for the three years was 14.55%. The interaction between varieties and years was not significant at $P \leq 0.05$. Annual mean of DM yield was 6.34 t ha^{-1}, ranging from 5.93 t ha^{-1} for Nival to 6.94 t ha^{-1} for Teanna, without significant differences among varieties (F=0.17) (Figure 1). In contrast, significant differences at $P \leq 0.05$ in winter and spring were found. Asso proved to be the highest yielding variety in winter, whereas Gepetto, Teanna and Lazo were the highest-yielding varieties in spring (Figure 1).

Regarding the class of earliness (Figure 2), the behaviour of the different varieties was consistent across years. Atomic, Lazo, Gepetto, Pollanum showed the earliest cycle. In contrast, Libonus and Jeanne showed the latest cycle. In a few cases (Jivet and Libonus), the earliness class stated by the seed company did not reflect the response of the variety.

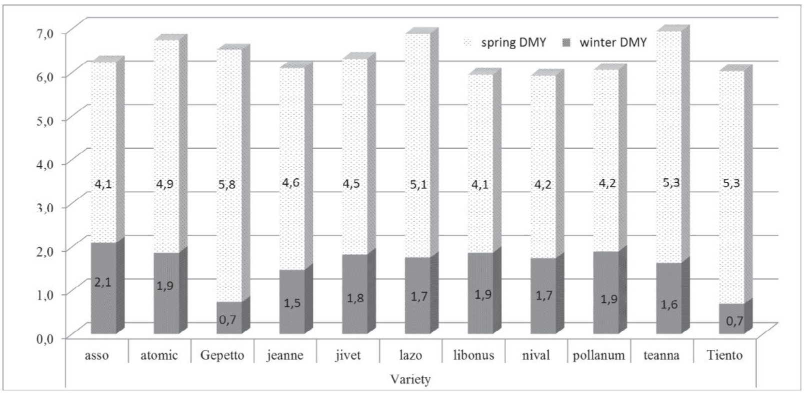

Figure 1. Dry matter yield (DMY) (t ha^{-1}) in winter and spring. Winter DMY least significant difference (LSD)=0.44 ($P \leq 0.05$); spring DMY LSD=1.32 ($P \leq 0.05$).

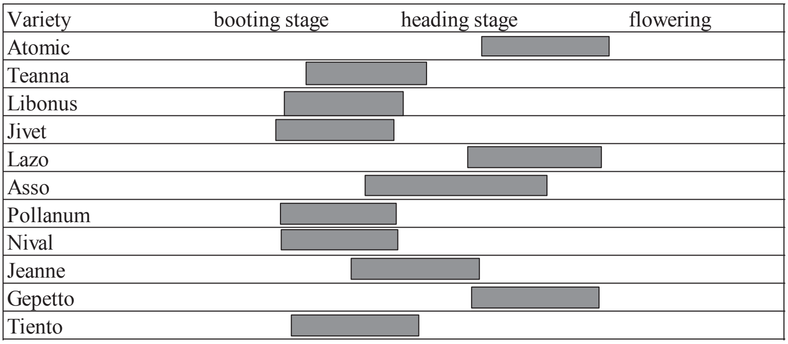

Figure 2. Phenological stages (7-9 May of each year).

Conclusions

In our Mediterranean environments, the tested varieties of Italian ryegrass showed reliable yield stability and could provide a good grazing crop in winter depending on their seasonal production. As a result, their choice should not be led by their annual yield potential only. Instead, seasonal production and class of earliness should play an important role. Hence, farmers should have the possibility to set up a forage chain able to ensure the feed supply by allowing a better distribution during winter and spring as well as a reserve of hay for the summer using different varieties characterized by different class of earliness.

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Persistence and establishment of red clover plants in extensive managed grassland in Norway

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Abstract

Red clover (*Trifolium pratense* L.) is normally a short-lived perennial and the number of plants in the field declines rapidly. In organic farming, the amount of clover in the field is decisive for the N_2 fixation and yield, and the protein content and quality of the forage produced. In Nordland County (66.27°N), there is a farm with some red clover plants in a >15-year-old grassland sward. In the presented study, we examined the grassland botanical composition and attempted to determine the age of red clover plants. Our hypotheses were that (1) extensive grassland management promotes self-seeding of red clover, which in turn, (2) would maintain a desired content of red clover over time. In addition, we tested two harvesting regimes of the first cut for seed maturation and seed quality at two locations in Norway. Red clover plants in old swards showed very high age and a branched root system. Very few seedlings were found in old swards, suggesting that self-seeding was insignificant. Experiments with leaving the grassland after the first cut for seed production of clover failed due to poor seed maturation. Surface seeding of red clover in pure-grass plots gave good results, especially with early spring seeding.

Keywords: longevity, self-seeding, surface-seeding, *Trifolium pratense*

Introduction

Red clover (*Trifolium pratense* L.) is normally a short-lived perennial and the number of plants in the field normally declines rapidly after seeding. Lunnan (2004) found a decline from 60-90 plants m^{-2} in the autumn of the year after sowing to 30-50 plants m^{-2} in the fourth production year, with a corresponding decline in clover proportion of the stand and in N_2 fixation. In Japan, Sakanoue (2002) reported that a red clover stand at an altitude of 1,200 m had persisted for 20 years since its establishment, under regular cutting. The ability of legumes to regenerate from the soil seed bank has been also observed in Australia and USA (Carr *et al.*, 2005). Some of the red clover seeds do not germinate immediately after falling to the ground. A large proportion of hard seeds germinate in the following spring leading to sufficient amount of clover in the subsequent year (Sakanoue, 2002). In the present study, we examined grassland botanical content and attempted to recognise the age of red clover plants in grassland that was almost 20 years old. Our hypotheses were that extensive grassland management promotes self-seeding of red clover, and that this, in turn, would maintain a desired content of red clover over time. We also tested two harvesting regimes of the first cut for seed maturation and seed quality at two locations in Norway.

Materials and methods

Farm study

This study was carried out at the Handnesøya in Nordland County (66.27°N). The farm has been managed organically for 20 years. The area of grassland allows for extensive management, with one cut during the growing season. The growing conditions are typical for areas with coastal climate. Closeness to Polar Circle results in 24 h of light during summer and compensates for to low air temperatures during the growing season. Winters are unstable with shallow snow cover and several freezing and thawing events. We chose to map 3 year old and 20 year old swards in early spring 2011. We attempted to assess clover age and its content in the sward. Age of red clover was evaluated randomly by digging up red clover plants and by assessing their root system. Plants were assessed to be young if the plants had white taproots and roots that were little branched, and assessed them to be old if the plants had brown, dark brown and a branched root system without taproot. The roots of more than 30 red clover plants were dug up and part of them was carefully washed under tap water. Botanical composition of the sward and the content of red clover were determined by the dry-weight rank method (t'Mannetje and Haydock 1963).

Field studies

In June 2012, red clover cvs. Bjursele (2x), Betty (4x) and Lea (2x) were sown in mixture with timothy (*Phleum pratense* L.) and meadow fescue (*Festuca pratensis* L.) at seed rates of 6 kg ha^{-1} red clover + 7 kg ha^{-1} timothy + 3 kg ha^{-1} meadow fescue at two sites in Norway [Løken (530 m a.s.l., 61.12° N) and Tjøtta (10 m a.s.l., 65.49°N)]. The cuts were carried out at two different points in time: at early heading of timothy and ten days later. In order to allow seed ripening about one-third of the plot containing cv. Bjursele was not harvested under the second cut. The forage yield was recorded under both cuts. In late autumn, non-harvested red clover plants were cut and placed on the surface of pure grass plots (1 m^2 each), which had also been established in June 2012. The establishment of red clover after different cutting regimes was recorded one year after and compared with the following surface seeding treatments: either 0, 2 and 10 kg ha^{-1} red clover surface seeded in late autumn or in early spring in pure grass plots. In addition, red clover seeds of cv. Bjursele after different cutting regimes were collected in late autumn and tested for germination. Seeds were placed on wet filter paper in Petri dishes for one week at 18 °C. Thereafter the number of germinated seeds was counted and germination capacity determined. All treatments described above had three replicates. Statistical analysis of ANOVA was performed to find out the effect of treatment.

Results and discussion

In the farm study, the assessment of botanical composition showed that timothy and meadow fescue dominated in both the 3-year-old and 20-year-old swards (Table 1). The content of red clover in the old sward was low, on average 4%. In the 3-year-old sward red clover content was significantly higher than in the 20-year-old sward and was on average 27%. Red clover plants from the young sward had a taproot, major part of roots were white and little branched (Figure 1A). In contrast, the plants from the old sward had brown, dark brown and a branched root system (Figure 1B). Those plants had no taproot and observations suggested that plants relied on lateral roots. Thus, red clover plants in the old swards showed very high age. This finding suggests that an extensive harvesting system with one cut during the growing season followed by a long regrowth period may preserve red clover plants rather well. However, we were not able to find out the exact age of the plants based on their morphological observations, but the root system suggested that red clover plants were old. The proportion of young red clover plants was low (Figure 1C). Very few seedlings of red clover were found in the 20-year-old sward, suggesting

Table 1. Botanical composition in 3 year old and 20 year old grassland swards at Handnesøya farm, Nordland County.

Name of species	Species proportion in grassland, %	
	3-years old	20-years old
Festuca pratensis	32.3	27.4
Phleum pratense	24.8	24.5
Poa pratensis	-	19.1
Agrostis capillaris	-	12.9
Trifolium pratense	27.3	3.8
Trifolium repens	7.3	2.1
Taraxacum officinale	4.5	3.2
Other species	3.9	7.0

Figure 1. A taproot of 3 year old red clover plant (A), a branched root system of a more than 3 years old red clover plant (B), and red clover seedling found in 20 year old grassland.

Table 2. Germination rate (%) of red clover seeds collected late autumn after early and medium harvest time in the first cut at Tjøtta and Løken.

	Early 1st cut	Medium 1st cut
Tjøtta 2012	43	12
Tjøtta 2013	9	13
Løken 2012	0	0
Løken 2013	25	17

that self-seeding was insignificant. In the field experiment, there were no difference in yield between red clover varieties, locations and years. On average for both locations, uncut red clover after the first harvest resulted in 3.25 tons DM ha^{-1} loss of forage yield. About 90% of red clover plants flowered at both sites and in both experimental years. However, cold and rainy weather in the second part of summer limited seed development and maturing, particularly in the first experimental year at Løken when there were no mature seeds at all (Table 2). Red clover seeds collected at Tjøtta showed better germination capacity at the first production year than at the second production year. Surface seeding in established pure grass stand gave better results than placement of cut red clover donor plants. The surface seeding in early spring showed better results of red clover establishment than the surface seeding in late autumn, regardless seed rate used. Thus, winter damage might be repaired by spring reseeding in young swards.

Conclusions

In northern climatic conditions, the growing season is too short for red clover seed production after the first cut. Thus, self-seeding of red clover would be rare in extensive managed grasslands. Assessment of red clover plants in old grassland showed that red clover plants might have a very long lifespan if only one cut is applied during the growing season. Spring surface sowing of red clover might work well in young grass swards.

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High nature value farmland management: innovative approaches to sustainability of HNV grassland systems in Ireland

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Abstract

The High Nature Value (HNV) farmland concept has developed from the recognition that low intensity farming is not only beneficial for biodiversity but also provides a large proportion of European environmental public goods such as clean water, climate change resilience and vibrant rural communities. Over the last 10 years a range of initiatives have taken place in Ireland to encourage and support the sustainable management of HNV farmland including grasslands and peatlands. We present a snapshot of the various initiatives from the development of results based agri-environment schemes to support provided under Ireland's Rural Development Programmes (RDP) and the development of regional, national and European innovation partnerships. We evaluate these various innovations, outlining progress made and highlighting issues remaining to be addressed to ensure a sustainable future for HNV grassland and peatland areas. We present examples of innovation partnerships that work together to produce locally adapted, results-oriented solutions for HNV grasslands and peatlands that could also be applied in other countries.

Keywords: HNV innovations, locally-adapted, farmland conservation

Introduction

Ireland is an island located off the northwest coast of Europe. The Utilisable Agricultural Area (UAA) of Ireland is approximately 4.5 million ha (65% of the land area). The UAA is dominated by pasture, which comprises a range of pasture intensities (including rough grazing/commonage), hay and grass silage. Many of these areas are considered to be High Nature Value farmland (HNVf). HNVf occurs in every country in Europe (Oppermann *et al.*, 2012) and has an important role in delivering biodiversity and other public goods such as clean air, clean water, climate regulation and aesthetic landscapes (Cooper *et al.*, 2009). Around 25% of the UAA of Ireland is HNV farmland (Matin *et al.,* 2016). HNVf areas in Ireland are diverse and include upland areas dominated by semi-natural vegetation such as blanket bog, wet heath and acid grassland along the west coast and upland regions in the east such as the Comeragh Mountains, Co. Waterford and the Wicklow Uplands, Co. Wicklow. Common land is a major component of farming systems in these regions. Common land is land held in common ownership on which two or more farmers have grazing rights (Lyall, 2000). Upland semi-natural vegetation communities extend to sea level in the west of Ireland due to the climatic conditions. Other areas are dominated by calcareous grassland such as island and coastal farms and the Burren, Co. Clare, or wet grasslands, e.g. farms in Counties Leitrim, Roscommon and Cavan where the prevailing soil type has limited potential for agricultural intensification. Land abandonment, afforestation and agricultural intensification are all threats to HNV farmland (DAFM, 2016; Keenleyside *et al.*, 2014; Terres *et al.,* 2015).

Innovative approaches to sustainability of HNV grassland systems in Ireland

In Ireland, a number of innovations have taken place to try to combat these threats and support HNV farmland (Figure 1). A key one was in 2004 when the BurrenLIFE project received EU LIFE Nature funding examining ways to manage and support HNV grasslands. This project resulted in positive conservation management outcomes through grazing and other farm management solutions (Dunford *et al,* 2010) as well as positive farmer engagement. It went on to be the flagship HNV farmland

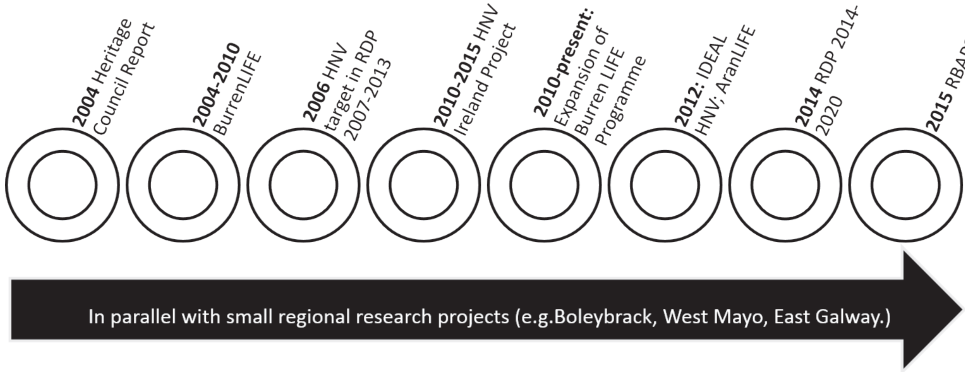

Figure 1. Timeline of HNV farmland support initiatives and projects.

demonstration project in Ireland and Europe. In 2010, the Burren Farming for Conservation Programme was rolled out to 160 farmers and further expanded in 2016. Despite this success, the process has not yet been replicated in areas outside of the Burren. At the same time as the BurrenLIFE programme was kicking off, a Heritage Council report called for HNV farmland to be defined, delimited and targeted for support (EFNCP *et al.,* 2004). In 2006, HNV farmland was incorporated as a specific target in Irelands 2007-2013 Rural Development Plan (RDP) under Axis 2. Another Irish innovation came about in 2010 when the Heritage Council provided funding to the European Forum for Nature Conservation and Pastoralism (ENCP) and IT Sligo to employ a HNV Ireland Officer. This role works on raising the profile of HNVf in Ireland, reporting on the needs of these HNVf areas and supporting HNV farming communities.

A number of farming groups were founded and supported in this time (e.g. Blackstairs, Nephin and Islands Farming Groups). These groups formed independently and their objectives were to support HNV farming in the local areas. In parallel, from 2013 to 2016, the IDEAL-HNV project was funded by the Department of Agriculture to identify the national distribution and extent of HNV farmland (http://high-nature-value-farmland.ie). The IDEAL-HNV project developed a national distribution map and described the national HNV types (which has yet to be done elsewhere in Europe). Organisations such as EFNCP, IT Sligo and the HNV Ireland Officer made submissions to ensure that HNVf was a clear RDP (2014-2020) priority. As a result of all this combined background work, the latest RDP included provision for the establishment of European Innovation Projects (EIPs) which, uniquely within Europe, focussed on HNV farmland. The competitive call for these closed in January 2017. Several of the newly formed farming groups are now working together with the HNV Ireland Officer, academic institutes and development companies to put together locally adapted EIP applications following the Burren process. These developments mean that HNV farming communities are now engaging with the HNVf concept and see that the supports being proposed will go to their areas.

The future of HNV farmland

The inclusion of the EIPs in the latest RDP has really engaged farming groups with over 100 applications from across the country. These projects, when funded, will be in a position to feed into the H2020-funded HNV-LINK project (http://www.hnvlink.eu) which aims to identify and catalogue HNV innovations so that they can be applied elsewhere. A results-based agri-environment pilot scheme (RBAPS) funded by DG Environment began in 2015 testing the Burren model in other regions of Ireland and Spain. The more results-based programmes are tested and applied in novel settings, the more likely they will be more broadly adopted across Europe going forward.

Despite the aforementioned positive developments, problems still persist, for example working co-operatives to deliver actions on common land do not exist yet in Ireland and farming groups are wary of some government departments due to historical differences. Criteria for land eligibility and the requirements to meet conservation objectives on common lands set by different government departments are often in conflict or are not clear to the farmers on the ground. It is possible that the EIPs will finally address some of these issues. Building partnerships and networks are essential to build capacity in HNVf areas so that potential funding, institutional support and community knowledge can be harnessed to conserve important semi-natural habitats such as grasslands and peatlands. The supports exist in the RDP but socio-economically disadvantaged areas need to develop the capacity and support infrastructure to avail of these opportunities. These areas need to overcome social disadvantages (e.g. remoteness, rural service and infrastructure decline) and distrust to capitalise on these supports. As the BurrenLIFE experience has shown (Dunford, 2016), a partnership approach can work to overcome some of these issues and develop innovative solutions to grassland conservation. This process, applied in other areas, should be supported by research, as initiatives needs to be based on sound science informed by local knowledge. Knowledge transfer and capacity-building supports through the national EIPs can be a real catalyst, though there is a need for these to feed directly into the next RDP (not just feeding into policy through standard consultation process) perhaps through a government working group on HNV farmland; policy development, implementation and monitoring.

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Drought resistance of functionally different forage species is related to their nitrogen acquisition and deficiency

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Abstract

We investigated whether losses in biomass yield under drought are related to species' nitrogen (N) acquisition and drought-induced N deficiency. In a three-factorial experiment, monocultures of four species that differed in their functional trait regarding N acquisition were established in a field experiment (first factor). Species were the non-legumes *Lolium perenne* L. and *Cichorium intybus* L. (no N_2 fixation), and the legumes *Trifolium repens* L. and *Trifolium pratense* L. (N_2 fixation). A summer drought was simulated with complete rain exclusion for five weeks, and drought effects were compared to rainfed control conditions (second factor). In addition, application of mineral N fertiliser was manipulated so that plots received no N during drought or 60 kg N ha^{-1} (third factor). Under drought, plant-available soil N was strongly reduced (P<0.001, compared to the rainfed control). Legumes were consistently less N-limited than non-legumes (P<0.001) and, at the end of the drought period, N derived from the atmosphere in the legume *T. repens* was 72%. Legumes' biomass yield was less affected by drought (-19%) than non-legumes' yield (-38%). Moreover, N fertilisation mitigated the drought effect on yield of non-legumes from -38% (no N) to -22% (60 kg N ha^{-1}). In conclusion, this improved yield-resistance to drought was related to mitigation of drought-induced N limitation through symbiotic N_2 fixation (legumes) or N fertilisation (non-legumes). Thus, cropping legumes could improve yield resistance against drought events as projected under climate change.

Keywords: drought experiment, drought-induced N limitation, symbiotic dinitrogen fixation, N fertilisation, yield resistance

Introduction

Projected frequencies and severities of drought events are expected to threaten grassland-based livestock farming by impairing forage production (Olesen *et al.*, 2011). In addition to direct water stress, drought can severely constrain the availability of soil nitrogen (N), for example by restricting mineral N fluxes (Durand *et al.*, 2010) or by inhibiting N mineralisation (Borken and Matzner, 2009). Indeed, soil N uptake by forage species was found to be reduced due to drought (Hoekstra *et al.*, 2015). If, under drought, soil N becomes a primarily limiting growth factor, cropping forage legumes in grassland mixtures could promote drought resistance in biomass yield because legumes do not depend solely on soil N due to their benefit from symbiotic dinitrogen (N_2) fixation. Thus, under drought, legumes may increasingly rely on N_2 fixation with an increasing limitation of soil N availability. In comparison, N fertilisation in non-legumes could help to overcome drought-enhanced N limitation, although the growth response to N fertiliser might decline with decreasing soil moisture (Colman and Lazenby, 1975). Drought resistance under increasing water scarcity might therefore be determined by the degree to which legumes can maintain symbiotic N_2 fixation and non-legumes can take up soil N. In contrast, if water is the primary growth-limiting resource, no gain from enhanced N supply should be expected (Hofer *et al.*, 2017). This study aimed at determining whether water or N is the primary growth limiting resource under moderate to severe drought, and to test whether increased N supply through symbiotic N_2 fixation or by mineral N fertiliser can increase resistance to such drought events.

Materials and methods

An experiment was carried out in a three-factorial design. Monocultures of four widely used forage species were selected for investigation (first factor). Species differed in their functional trait regarding

N acquisition, and were the non-legumes *Lolium perenne* L. and *Cichorium intybus* L. (no N_2 fixation) and the legumes *Trifolium repens* L. and *Trifolium pratense* L. (N_2 fixation), which were sown into plots of 5×3 m. A summer drought event with complete rain exclusion was simulated for five weeks from June to July 2013 by placing rainout shelters on the plots of the drought treatment, and drought plots were compared to a rainfed control (second factor). The application of mineral N fertiliser was varied, consisting of plots that were N fertilised during drought with 60 kg ha^{-1}, and plots not fertilised during the drought period (third factor). There were two or three replicates per treatment combination.

Plant-available soil N was measured by Plant Root Simulator (PRS)™-probes (Western Innovations, Canada). The percentage of N derived from the atmosphere (Ndfa) in the legume *T. repens* was measured by isotope dilution technique. Dry matter yield was determined at the end of the drought period. For the analysis of variance, data of the two non-legume and the two legume species were pooled.

Results and discussion

At the end of the five-week drought period, decreasing soil moisture contents reached the threshold of plant-available soil water at -1.5 MPa, indicating moderate to severe drought stress. Plant-available soil N was strongly reduced under drought compared to rainfed control conditions (P<0.001; Table 1). The N nutrition index (NNI) of the non-legume species was around 0.5 and 0.4 under control and drought conditions, respectively, indicating distinct N limitation (Table 1). By contrast, the NNI of the legume species was around 1 and 0.8 (control and drought), was significantly larger than that for non-legumes (P<0.001), and indicated no (control) or only marginal N limitation (drought). Taken together, this revealed significant N limitation for growth of non-legumes under rainfed conditions, which was even enhanced under drought in agreement with a recent study using the same species (Hofer *et al.*, 2016). Under drought, N limitation can further be increased by inhibited physical transport of mineral N in the soil solution (Durand *et al.*, 2010), reduced litter decomposition (Sanaullah *et al.*, 2012), or by restricted N mineralisation (Borken and Matzner, 2009).

Biomass yield of the non-legume species was reduced due to drought by -38% (Table 1, no N); however, biomass yield of the legume species was diminished only by -19% (no N). Nitrogen fertilisation increased biomass yields of non-legumes, but not of legumes (Table 1). More importantly, yield of N fertilised non-legumes was reduced under drought only by -22%, meaning that drought-induced yield losses in N fertilised non-legumes were very similar as for not N fertilised legumes (-19%). Percent N derived from the atmosphere of the legume *T. repens* was 68% and 72% under rainfed control and drought conditions, respectively, and was thus not affected by water scarcity (Table 1). This demonstrates that forage legumes can sustain symbiotic N_2 fixation at field conditions under projected drought events (Hofer *et al.*, 2017; Seneviratne *et al.*, 2012), and the result can well explain the consistently higher NNIs and biomass yields

Table 1. Growth variables of two functional types of species (non-legumes, legumes) grown for five weeks under rainfed control (Ctr) and drought (Drt) conditions, at which there was no N fertilisation (No N) or N fertilisation of 60 kg ha^{-1} (With N).[1]

Functional type	Non-legumes				Legumes			
N fertilisation	No N		With N		No N		With N	
Drought treatment	Ctr	Drt	Ctr	Drt	Ctr	Drt	Ctr	Drt
Soil N (µg cm^{-2} 7 d^{-1})	0.8 (0.17)	0.5 (0.06)	0.7 (0.08)	0.7 (0.19)	3.1 (0.21)	0.7 (0.18)	4.0 (0.35)	1.1 (0.31)
NNI	0.5 (0.04)	0.4 (0.02)	0.4 (0.03)	0.4 (0.01)	1.0 (0.03)	0.7 (0.09)	0.9 (0.03)	0.8 (0.05)
Ndfa (%)[2]	-	-	-	-	67 (8.1)	72 (13.2)	68 (3.4)	-
Yield (t DM ha^{-1})	1.6 (0.18)	1.0 (0.24)	2.3 (0.37)	1.8 (0.17)	3.2 (0.56)	2.6 (0.55)	3.3 (0.55)	2.6 (0.56)

[1] Provided are the means (± SE) of plant-available soil N (soil N), the N nutrition index (NNI), the percentage of N derived from the atmosphere (Ndfa) of the legume *T. repens*, and biomass yield (Yield).

[2] Ndfa = N derived from the atmosphere. *Trifolium repens* only.

in legumes than in non-legumes (Table 1). Similarly, our data show that additional N fertilisation under drought can partly mitigate yield losses in non-legumes as long as water stress is not extreme. Hooper and Johnson (1999) state that co-limitation of water and N occurs above a certain amount of water supply, but that water limits plant growth primarily when precipitation is extremely low. Therefore, at our five-week drought period, it can be inferred that soil N deficiency impaired plant N status and biomass yield as much as, or more than direct water deficiency. Yet, under conditions of prolonged drought stress and extreme water scarcity, a shift from N- to water limitation can occur (Hofer *et al.*, 2017).

This study demonstrates that the legumes' sustained ability of symbiotic N_2 fixation under moderate to severe drought could overcome drought-induced limitations in soil N, causing yield of legumes to resist increasing drought stress to some degree. Non-legumes grown in mixtures with legumes might therefore profit from the increased N acquisition not only under ample water supply (Nyfeler *et al.*, 2011) but also under drought, leading to increased yields in drought-stressed grasslands containing legumes (Hofer *et al.*, 2016).

Conclusions

Under moderate to severe drought, soil N seems to be a primarily limiting growth factor and, thus, legumes might be used to improve drought resistance in multi-species forage grassland. Legumes might therefore represent a mitigation option under projected drought events.

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Methane emission in Jersey cows during spring transition from indoor feeding to grazing

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Abstract

In this study we evaluated the effects of gradual transitioning from indoor winter-feeding to outdoor grazing, 8 to 11 h daily, on methane emission and milk yield in an organic Jersey herd in Denmark. A total of 151 mixed-parity lactating Jersey cows with free access to automated milking stations (AMS) were monitored from 30 d prior to barn release until 23 d post release. During each visit to the AMS, milk yield was measured and logged together with date and time. Methane (CH_4) monitoring equipment was installed in the feed troughs of AMS. Methane and carbon dioxide (CO_2) were measured continuously in ppm using a non-invasive 'sniffer' method, and the CH_4/CO_2 ratio was used as emission trait. This trait was averaged over milking. Milk yield was 19.4 kg d^{-1} in the indoor period and increased to 20.2 kg during grazing. A small increase (4%) in the CH_4/CO_2 ratio at herd level was found when comparing indoor period with grazing period, while the variation from day to day increased in the grazing period compared with the indoor period. The correlation between indoor and grazing period was very high; however, slight re-ranking of animals was detected.

Keywords: methane emission, grazing, pasture, dairy cattle, Jersey

Introduction

The contribution of agriculture to anthropogenic GHG (greenhouse gas) emissions is significant. Among agricultural-methane emissions sources, the most important is enteric fermentation from ruminants (40%) (FAO, 2016). Therefore the abatement of methane emission from cattle is a hot topic and various mitigation strategies including feed additives, genetic selection and feeding strategies have been proposed. However, there is limited knowledge about the effect on emissions of grazing compared with indoor feeding. We hypothesize that grazing influences methane emissions due to nutritional and activity changes in the animal's daily routine. Besides knowledge about the absolute effect of transition from indoor feeding to grazing, we are also interested in the ranking of animals in the two periods, as well as estimating the transition period before a new constant level of emission was established.

Materials and methods

Methane and carbon dioxide concentrations were measured from 151 lactating cows in a Danish organic Jersey dairy cattle herd managed in one group for the entire lactation. Gas ppm concentration was logged continuously each second with non-dispersive infrared (NDIR) devices installed in feed troughs of automated milking stations (AMS). The experiment lasted for 53 days, between 7 April and 30 May 2016. Cows were let out into the pasture for the first time on 7 May 2016. Herd average dry matter intake (DMI) of roughage and concentrate mixed ration (PMR) and AMS-feed was measured every day in the whole period and pasture intake estimated daily assuming same daily total DMI as average of the indoor period (Figure 1). The proportion of DMI of concentrate offered in the (AMS) was identical before and after start of grazing, while average daily intake of total mixed ration (TMR) was reduced from 14.0 to 7.4 kg DM due to grazing. Access to the pasture was regulated by one-way gates with minimum 8 h and maximum from 11 hours daily in the first days after transition, increasing to 16 h in the major part of the period. Herbage height measured by plate meter was 10.0 to 12.4 cm. Botanical composition of the cultivated grassland was 78% grass, 7% white clover and 11% non-sown species; crude protein content

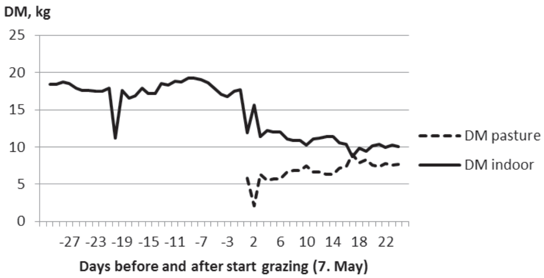

Figure 1. Dry matter (DM) intake indoor and at pasture in the transition period as average of lactation cow in a Jersey herd (kg DM cow^{-1} daily).

was 10.0 to 13.4% of DM and net energy 6.6 to 7.0 MJ kg^{-1} DM based on weekly cut herbage samples simulating grazing.

Ratio of CH_4 to CO_2 was calculated from average gas concentrations during each milking. Phenotypes were generated by a custom made program for wave-shaped data. Significance of all covariates was checked via univariate mixed model. Covariates assessed in the model were: days in milk, milk yield, parity, dry matter intake from the pasture (nested within grazing), 5th harmonics of Fourier series approach (Løvendahl & Bjerring, 2006) which denotes for fraction of a day, animal effect, grazing period effect (in-barn vs grazing), AMS. Next, the experiment was divided into two periods (in-barn and grazing) to check correlation between them and differences in repeatability. Modelling was done in DMU (Jensen and Madsen, 1992) software. A bivariate mixed model approach was used in the analysis. The primary trait was split into two: gas ratio during non-grazing and grazing period, respectively.

Results and discussion

All covariates specified in the univariate model turned out to be significant with period (indoor vs grazing) being highly ($P<0.001$) significant. Average yield per milking (6.70 kg in indoor period vs 8.46 kg during grazing) and average milking frequency (2.90 vs 2.39 milking cow^{-1} day^{-1}) was significantly different. Average amount of model-predicted emitted methane in comparison with carbon dioxide increased significantly during the grazing period compared to the indoor period (0.036 vs 0.040, $P<0.001$) and with an increased daily variation during grazing compared with indoor feeding (Figure 2). The repeatability within cow for methane dropped slightly from indoor to grazing (0.19 vs 0.18).

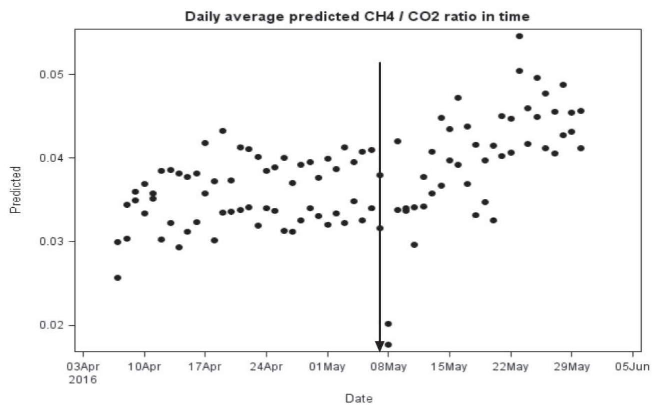

Figure 2. Model predicted values of daily average CH_4/CO_2 ratio from an organic Jersey dairy cattle herd. The black arrow indicates date when grazing period begun.

The animal rank correlation between the two periods was estimated at 0.85, similar to findings of Bell *et al.* (2014). This indicates that both periods agree well; however, slight re-ranking of animals takes place. The gas ratio started to stabilize at 2-3 weeks after first grazing day. This might be an effect of ruminal microflora adaptation to new dietary and maintenance conditions, as well as stabilization in amounts of grazed DMI.

Conclusions

Grazing compared with indoor feeding significantly influenced enteric methane emission from dairy cows. Methane ratio to carbon dioxide increased together with increasing DMI from pasture; however it tended to stabilize a few weeks after beginning of grazing. The correlation between in-barn and grazing periods was very high; however, some slight re-ranking of animals was detected. Therefore grazing should be considered as a significant factor during methane emission analysis and further research is needed to investigate this effect and its persistence.

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Indirect measurements of Mediterranean grassland vegetation characteristics using plenoptic camera and NIRS

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Abstract

The description of the grassland vegetation and its multiple functions such as forage production, biodiversity and nutritive values requires the collection of many types of in-field data. This collection is in general cumbersome and laborious (e.g. counting plants by species in sampled quadrats) and, when destructive, it does not allow monitoring the dynamics of vegetation during the growing season. As an alternative, indirect measurements can be done using optical tools. In this work, we tested the interest of combining plenoptic images and NIRS information to characterize different variables of Mediterranean grasslands. A plenoptic camera is a multifocal camera that produces clear images on all the deepness of the images. We tested this protocol on two grassland mixtures for estimating the functional diversity of the mixtures. NIRS and plenoptic images were taken at the same time. Images were analysed using Trainable Weka Segmentation ImageJ. The outputs of the images and NIRS analyses were afterwards correlated with the field measurements.

Keywords: plenoptic, NIRS, grassland, functional diversity

Introduction

Grassland and rangeland can provide many services to humanity. In many livestock systems of the world, they are the main source of animal feed. They can also contribute to climate regulation (carbon sequestration), nutrient recycling, biodiversity conservation, and landscape aesthetics. Some descriptors of the vegetation are generally good indicators of these different services. Biomass and chemical composition are related to the value for livestock nutrition. Some functional diversity variables, especially those calculated with leaf traits (specific leaf area and leaf dry matter content) are linked with carbon sequestration and nutrient recycling. Many variables can be used to describe grassland and rangeland vegetation. These variables require the collection of many types of field data (biomass, traits, species composition, etc.). Most of these measurements are destructive and quite laborious and time consuming. These measurements cannot be repeated on the same plot during a growing season. Indirect measurements can be performed using optical tools. The spectral specificity of vegetation, especially in the red and the near infrared, is used to assess the state of the vegetation in remote sensing, mainly to assess biomass, or in laboratory using NIRS tools to assess chemical composition. Photography is also used to assess the presence of weeds (Gebhardt and Kühbauch, 2007) or the ratio between legumes and grasses (Himstedt *et al.,* 2009). One limit on the use of photos is the focus problem. Some new technology such as the plenoptic (i.e. multi-focal) camera provide pictures without such problem. Very few studies tested the utility of such optical approaches on the functional diversity of vegetative cover and also combined photography and NIRS. The objective of this work was to test the possibility to use plenoptic camera and SPIR to evaluate grassland vegetation features.

Materials and methods

In the south of France, we tested two legume mixtures in four plots: the first one with *Trifolium alexandrinum, Medicago sativa, Vicia sativa* and *Avena sativa*; the second one with the same species but with *Trifolium michelianum* instead of *Vicia sativa.* Two plots (one for each mixtures) were cut and measured on 16 June 2016 and the other two on 29 June. In each plot 10 quadrats (0.5×0.5 m) were used to survey, on two individuals per species per quadrat: vegetative height (H), specific leaf area (SLA) and leaf dry matter content (LDMC). After cutting the quadrat (at 5 cm), the different species were sorted and the biomass per species was recorded. A photograph was taken vertically at 1 m using a Lytro® Illum camera. Forty spectres per sample were taken using an ASD NIRS on the green vegetation just after the cut. The sharp plenoptic image classification was realized with a machine-learning method based on a reduced number of descriptors and a TreeForest classifier. The accuracy of the results was evaluated onto a recomposed image where the part and the arrangement of each species was known: it ranged from 50 to 95% according to the cases. This resulting classification allowed the estimation of the visible species ratio for each studied plot and was used as predictors of the field variables. Moreover, from the images, the mean value and standard deviation were calculated for Red, Green and Blue bands of the image and also from the 'depth-map'. The depth-map is given by LYTRO desktop software and represents for each pixel the focus used to create a clear picture. It could be a proxy of the distance between the objects on the camera. We used these average and standard deviation of these four band as predictors. For the NIRS, for each wavelength, we calculated the average and standard deviation of the 40 spectres per quadrats. We performed a PCA on average values, on standard deviations and variation coefficients. We used the two axis of each PCA as predictors. For the field measurements, botanical composition was described by the fresh mass of each species in the quadrats and the total fresh biomass per quadrat. Functional diversity indexes: community weighted mean value (CWM), and functional diversity (FD) using the Rao index were calculated for each traits separately. These indexes were calculated using FD packages on Rcore Software (Laliberté *et al.* 2014). Spearman correlation was used to compare the different field variables and the variables from images or NIRS.

Results and discussion

Table 1 shows the correlation between different descriptors of vegetation and NIRS PCA. The three community weighted mean value (CWMH, CWMSLA and CWMLDMC) are linked with the second axis of the PCA made on the mean spectre of the sample with r coefficient of 0.66, 0.35 and -0.35, respectively. The specific leaf area and leaf dry matter content are two traits linked to the leaves economic spectrum and consequently to the biochemical composition of the leaves that were assessed using NIRS. These community-weighted mean values were also linked with the red, blue and green value of the pixels on the images (Table 2). The r coefficient between the mean red values of the images was of 0.53 with the CWMH, 0.56 with the LDMC and -0.52 with the CWM SLA. Total biomass and the biomass of *Vicia sativia* were also linked with NIRS outcomes with r coefficient of 0.40 and 0.43, respectively, with the first PCA axis calculated on mean spectre (Table 1). The weed biomass was linked with the depth map extracted (r=-0.32, Table 2). Indeed, the depth map was able to detect the presence of the tall

Table 1. Spearman correlation coefficient between NIRS variables and field measurements (in bold the significant effect).[1]

	Mean NIRS		SD NIRS		CV NIRS	
	PCA1	PCA2	PCA1	PCA2	PCA1	PCA2
CWM.H	0.06	**0.66**	**0.50**	-0.25	**0.41**	**-0.43**
CWM.LDMC	0.00	**0.35**	**0.45**	-0.28	**0.44**	-0.29
CWM SLA	-0.18	**-0.35**	**-0.41**	0.28	**-0.34**	**0.32**
FD.H	0.11	-0.23	0.03	0.16	0.02	0.19
FD.LDMC	**0.34**	-0.03	0.25	0.01	0.14	-0.01
FD.SLA	0.16	-0.18	0.19	0.09	0.13	0.16
Biomass	**0.40**	-0.17	-0.12	**0.35**	**-0.33**	0.23
TA biomass	-0.26	0.01	-0.04	-0.02	0.02	0.08
TM biomass	**-0.35**	**-0.53**	-0.05	-0.10	0.12	0.23
VC biomass	**0.43**	**0.32**	-0.13	**0.37**	**-0.35**	0.00
Weed biomass	**0.33**	-0.26	0.04	-0.09	-0.05	0.01

[1] H = vegetative height; SLA = specific leaf area; LDMC = leaf dry matter content; CW = community weighted; FD = field measurement.

Table 2. Spearman correlation coefficient between images variables and field measurements (in bold the significant effect).[1]

	Depth map		Red		Blue		Green	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CWM.H	0.22	0.26	**0.53**	**0.56**	0.20	**0.45**	0.04	**0.51**
CWM.LDMC	-0.17	0.18	**0.56**	**0.41**	**0.44**	0.34	**0.41**	**0.42**
CWM SLA	0.24	-0.24	**-0.52**	**-0.38**	**-0.42**	-0.27	**-0.36**	**-0.37**
FD.H	-0.27	-0.16	-0.25	-0.18	-0.11	-0.13	0.02	-0.17
FD.LDMC	**-0.39**	-0.01	0.04	-0.03	0.19	-0.10	0.22	-0.05
FD.SLA	-0.27	-0.10	-0.04	-0.04	0.12	-0.02	0.20	-0.03
Biomass	-0.30	0.26	**-0.50**	-0.15	-0.18	-0.03	-0.05	-0.20
TA biomass	0.11	**0.36**	-0.13	0.21	-0.14	0.28	-0.05	0.19
TM biomass	0.03	-0.23	-0.20	-0.06	-0.15	0.06	-0.01	-0.02
VC biomass	-0.17	0.11	-0.06	-0.17	0.10	-0.20	0.04	-0.20
Weed biomass	**-0.32**	0.12	-0.17	-0.02	-0.11	-0.08	-0.06	-0.08

[1] H = vegetative height; SLA = specific leaf area; LDMC = leaf dry matter content; CW = community weighted; FD = field measurement.

weed species. The percentage of images per species obtained from the segmentation were linked to their biomass for the *Trifolium michelianum* (r=0.77) and for the *Vicia sativa* (r=0.88) (Table 3).

Conclusions

These results showed some interest for NIRS and the analysis of images, especially plenoptic images, for assessing biomass and species composition but also for the functional diversity of grasslands.

Table 3: Spearman correlation coefficient between the percentages of species segmented in the image and field measurements (in bold the significant effect).[1]

	TA_image	TM_image	VC_image	Soil_Image
CWM.H	-0.24	-0.12	0.33	**0.46**
CWM.LDMC	-0.30	0.07	0.08	0.17
CWM.SLA	0.22	0.02	-0.20	-0.21
FD.H	0.04	0.16	-0.21	**-0.48**
FD.LDMC	-0.02	0.12	-0.10	-0.09
FD.SLA	-0.02	0.23	-0.27	**-0.36**
Biomass	**0.47**	**-0.48**	0.34	-0.10
TA biomass	0.24	-0.30	0.16	0.02
TM biomass	-0.34	**0.77**	**-0.86**	**-0.82**
VC biomass	**0.52**	**-0.86**	**0.88**	**0.60**
Weed biomass	-0.13	0.26	-0.26	**-0.35**

[1] H = vegetative height; SLA = specific leaf area; LDMC = leaf dry matter content; CWM = community weighted mean; FD = functional diversity.

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Water vole damage to grasslands is affected by farming practices and landscape

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Abstract

Human-wildlife conflicts occur throughout the world and involve a wide variety of wildlife species and a diversity of problems, including damage to agricultural crops. During winter 2014-15 several of the farmers in a small-scaled farming area at the coast of northern Norway experienced an explosive population growth of water vole (*Arvicola amphibius*), resulting in a dramatic invasion into farmland. This study investigated the impact of farming practices (mowing, grazing, ploughing, etc.) and landscape composition on the damage to permanent and temporary meadows from water vole diggings. Preliminary results show that meadows embedded in a landscape with pastures, road verges, open ditches, and abandoned grasslands had increased damage, while grazing and ploughing within the meadows reduced damage. For the farmers the damage caused serious economic loss. This incident is an example of a human-wildlife conflict that may cause abandonment of farming. Several of the farms are located next to the Vega Archipelago World Heritage site. Maintaining extensive farming in this region is thus of national and international interest. The results will be used to implement preventive measures to reduce damage from future outbreak of voles.

Keywords: human-wildlife conflict, water vole, *Arvicola amphibius*

Introduction

Semi-natural habitats are considered as the main source of biodiversity in agricultural landscapes, and still are predominant in many countries in areas less suitable for agriculture where they are sustained within small-scaled farming systems (Niedrist *et al.*, 2009). Small-scale farming is particularly vulnerable to social, political, environmental and climate changes, which in severe cases my lead to cessation of agriculture and abandonment of land-use. Abandonment of land-use is today the most important pressure on semi-natural habitats in Norway, as in the rest of Europe, and maintaining such farms is thus a major concern for many conservationists. One potential driver for abandonment of small farms is damage from wildlife. Human-wildlife conflicts occur throughout the world and involve a wide variety of wildlife species and a diversity of problems, including damage to agricultural crop and forest resources, impacts to livestock production, issues related to invasive and exotic species, and wildlife-human disease transmission (Madden, 2004; Messmer, 2009). Water vole (*Arvicola amphibius*) is a rodent that has caused substantial damage and severe economic losses for farmers in France and Switzerland (Berthier *et al.*, 2014; Giraudoux *et al.*, 1997; Halliez *et al.*, 2015). Water vole is a relatively large rodent (max. 200-300 g) that is adapted to a wide range of habitats from vegetated river banks and emergent vegetation (Strachan and Jefferies, 1993) to grasslands and agricultural land (Morilhat *et al.*, 2008). During the winter 2014/2015 several small farms at the coast of northern Norway experienced a major outbreak of water vole, which for many farmers caused severe damage to winterfeed, meadows, pastures, open ditches and transport infrastructures. A case study was set up to understand better why the extent of water vole damage varies between neighbouring farms and meadows in the area, by investigating how landscape structure, land-use and agricultural practice may affect damage. The results are expected to serve as a basis for future management and damage control.

Material and methods

The study area (13 km^2) is located on one island at the outer coast of Helgeland in northern Norway (65°54' N, 12°19' E). There are ten active farms and 151 ha of cultivated land in the area and average meadow size was 0.9 ha. A majority of the farmers rely on off-farm income, and farming is upheld only as long as it is less labour-intensive and reasonably profitable. The study area is located close to the Vega Archipelago World Heritage site, recognised for its unique landscape and land-use qualities and covering vast areas of species-rich semi-natural grasslands and coastal heathlands. To maintain agriculture and a sustainable land-use in this area, it is of great importance to develop measures to reduce the damage from future water vole outbreaks. Today, there is no form of economic compensation to farmers when crops or livestock are lost to protected species. There are only avian predators on the island, as the population of American mink (*Neovison vison*) recently has been diminished through conservation efforts. Pest control using rodenticide is not considered an option because of the conservation status of the area and the high ecological value of water vole as prey for vulnerable species such as Eagle owl (*Bubo bubo),* Short-eared owl *(Asio flammeus)* and migrating birds of prey. The variation of vole damage to meadows was analysed in a GIS environment; a suitable approach for handling spatial information at different scales. Within the study area 24 circular plots were established at the centre of selected meadows, each of 0.2 ha, with one plot in each meadow. In all plots, new water vole diggings were counted in November 2016 and recorded at high spatial precision GPS (<0.3 m) and used as response variables in a Poisson regression analyses. For each plot, soil type, land-use (recently ploughed, unploughed) and land-use after summer harvest was recorded, and used as local meadow explanatory variables. Landscape variables were recorded from surrounding vegetation cover within a 100 m buffer zone outside each plot and adjusted for unsuitable area. Vegetation cover was interpreted from colour aerial orthophotos and verified through field inventories. An initial full model was estimated using all variables. Significance was assessed by a stepwise backwards elimination from the full model. Variables returning high (>5.0) Variation inflation factor (VIF) were assumed correlated and removed from the model. The first final model suggested some over-dispersion, and the final model was scaled by a scale factor assessed from the ratio deviance/degrees of freedom (df). The statistical analyses were performed in Minitab Inc. 17.2.1.

Results

We found diggings from voles in 92% of the plots. From the Poisson regression analysis it is evident that six variables related to landscape characteristics and three variables related to meadow properties significantly affected the response (Table 1). Total variation in the data set described by the model was 52.7%. Landscape properties explained 28.1% of the variation, and local meadow properties 24.6%. Among the landscape properties the variables 'pasture' and 'road verge' returned the highest positive estimates, associated with increased damage. This implies that increasing the proportion of pasture with one areal unit results in an increase of water vole diggings in the plots by 1.8%. For a unit of road verge the result is 1.7% increase. Presence of seashore and forest in the surroundings are most likely to reduce damage to meadows. For the inside meadow variables, grazing during autumn significantly reduced damage compared with meadows that were harvested only once a year, as well as to those that were mown late in autumn. Meadows recently ploughed had reduced, but non-significant, damage compared with permanent meadows. Finally, we found that meadows on peat soil were less damaged than those on shore deposits. The results are preliminary and further research is needed. This needs to include increased number of plots and explanatory variables.

Discussion

The results show that water vole damage is associated with resource availability both inside the meadow and in the surroundings. This is consistent with results from Morilhat *et al.* (2007). The areas with a combination of permanent meadows and remaining harvest until late autumn, embedded in a landscape with intensive grazing and linear features such as road verges and open ditches, were the most affected by

Table 1. Effects of surrounding landscape and meadow properties on water vole diggings estimated with Poisson regression using natural log (Morilhat *et al.*, 2007) as link function.[1]

Variables	Coef	SE	*P*-value
Constant	1.707	0.250	0.000
Landscape properties (continuous)			
Seashore and salt marshes	-0.059	0.021	0.004
Land-use abandoned	0.006	0.001	0.013
Pasture	0.018	0.001	0.000
Forest	-0.021	0.001	0.000
Open ditch	0.007	0.001	0.000
Road verge	0.017	0.001	0.000
Local meadow properties (categorical)			
Recently ploughed	-0.217	0.044	0.122
Soil type peat (vs shore deposits)	-0.509	0.040	0.000
Second crop un-mowed (vs grazed)	1.208	0.058	0.000
Mowed (vs grazed)	0.978	0.055	0.000

[1] Coef = coefficient values; SE = standard error. R^2= 52.7% and AIC= 307.5.

voles in this study. This implies that meadows embedded in habitats poor in resources (such as intensive grazed pastures) or unsuitable (forests/spruce plantations) move the voles to dig for food inside the meadows. In contrast, increased resource availability outside the meadows reduced the damage in the meadows. This is especially evident with the high negative coefficient value found for un-grazed seashore habitats, which is highly preferred by the voles. Thus, competition for resources between herbivores seems to be an important driver for damage to the meadows. The importance of road verges is interpreted as the need for a dry and safe habitat for lairs, as roads are constructed on a foundation of rocks. Grazing the meadows through autumn may reduce vole damage to the meadows. However, from other parts of the study, plant species abundance and herbivore preferences are also important. Both sheep and voles avoid species such as *Filipendula ulmaria*, which are abundant in the area, while the roots of *Anthriscus sylvestris* are highly preferred by voles. This latter species is stimulated by nutrient input from agriculture and is abundant in meadows and field margins. Together with reduced pressure from predation by mink, this can explain some of the extreme population growth of water vole.

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Comparing diversity indices in rangelands in different areas of Sardinia (Italy)

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Abstract

An evaluation of diversity indices was carried out on 144 plots in Sardinia (Italy), located in natural or semi-natural rangelands, in three different sites (Goceano, Ogliastra and Marmilla-Sarcidano-Barigadu) across a geographic gradient. These rangelands are grazed by different animal species (sheep, goats, pigs, cattle, horses, donkeys) throughout the year. Vegetation surveys were conducted applying the Braun-Blanquet phytosociological method. Significant similarities and differences were found between diversity indices in the three sites examined. In this study, we propose a methodology for selecting a reduced set of diversity indices for Sardinian rangelands.

Keywords: species richness, biodiversity, diversity indices

Introduction

The effects of livestock grazing on biodiversity have been addressed by a number of studies, such as those of Papanastasis *et al.* (2002) and Camarda *et al.* (2016) for the Mediterranean region. However, despite these extensive conceptual frameworks, we still lack a general understanding of how grazing affects biodiversity in rangelands (Hanke *et al.*, 2014) and in protected areas. The management of livestock grazing must necessarily be based on a deep knowledge of natural resources, including their variability along the year, in order to preserve rangelands, which are important ecosystems for the maintenance of biodiversity (Sabatini *et al.*, 2001). Diversity is one of the most important traits in the study of plant communities and many methods are available for its measurement or assessment. The present research compared 12 commonly used diversity indices for the characterization of plant communities in three rangeland sites.

Materials and methods

The study was carried out on 144 plots located across a geographical and environmental gradient in three sites on the island of Sardinia in Italy (Goceano, Ogliastra and Marmilla-Sarcidano-Barigadu, coded as GOC, OGL and MSB, respectively), within large forest areas managed by the Sardinian Forest Agency (Fo.Re.S.T.A.S.) and grazed by different animal species (sheep, goats, pigs, cattle, horses, donkeys) throughout the year. Vegetation surveys were conducted applying the phytosociological method of Braun-Blanquet (1951). We considered twelve commonly used diversity indices, i.e. Species richness (SR), Dominance (D), Simpson (S) (Simpson, 1949), Shannon-Wiener (H) (Shannon and Wiener, 1949), Evenness (EVN) (Pielou, 1975), Brillouin (BR), Menhinick (ME), Margalef (MA) (Margalef 1968), Equitability (EQ), Fisher alpha (FA), Berger-Parker (BP), Chao (CH), as implemented in the software PAST (Hammer *et al.*, 2001). Plant identification followed Arrigoni (2006-2015).

Results and discussion

The floristic surveys detected a total number of 223 plant species in the 21 plots located at GOC, 320 species in the 63 plots at OGL and 155 species in the 60 plots at MBS. As expected for Sardinia, this number included a significant percentage of endemic taxa and a prevalence of pluriannual species, mostly hemicryptophytes and chamaephytes. The dominant species belonged to *Fabaceae*, *Asteraceae* and *Poaceae*, so that, e.g. in OGL *Vulpia myuros*, *Asphodelus microcarpus*, *Trifolium campestre*, *Poa bulbosa, Brachypodium retusum* and *Carlina corymbosa* occurred in nearly 80% of the investigated plots. In general, we detected significant differences between all the diversity indices calculated in the three sites. However, Dominance, Simpson and Berger-Parker indices were less significantly different after ANOVA. At the same time, the correlation analysis, after hierarchical clustering, highlighted the strong negative correlation between Dominance and Berger-Parker indices as well as among other indices (Figure 1 and Figure 2; Table 1).

Conclusions

The ANOVA and the correlation matrix highlighted the most significant similarities and dissimilarities among the 12 indices in the three sites. Interpretation and comparison of indices from different samples and plant communities is often difficult. Despite the strong relationships between the diversity measures,

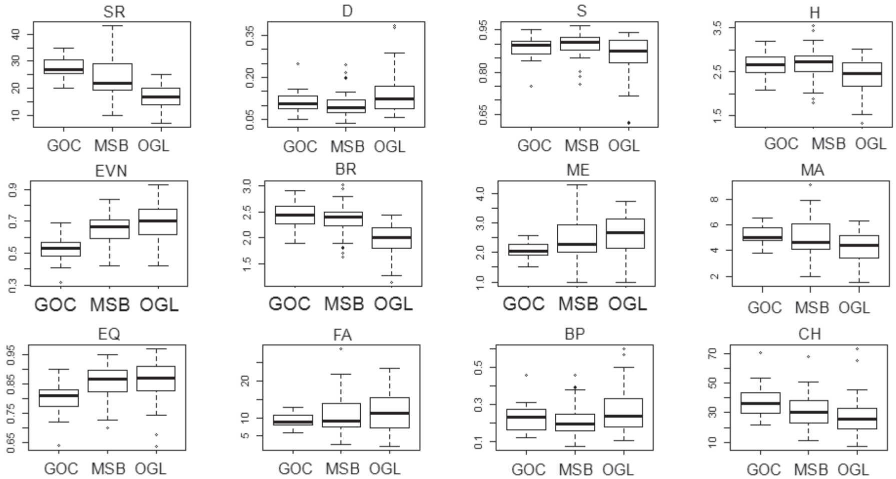

Figure 1. Boxplots for the 12 diversity indices calculated for 144 plots in the three sites (GOC, MSB, OGL). Codes are indicated in the materials and methods. The probability values for one-way ANOVA (Welch-like correction for non-homogeneity) are as follows: 7.9e-15, 0.01698, 0.01697, 0.0007166, 2.594e-09, 5.881e-11, 2.618e-06, 0.0002877, 0.0004224, 0.009816, 0.03917 and 0.005322.

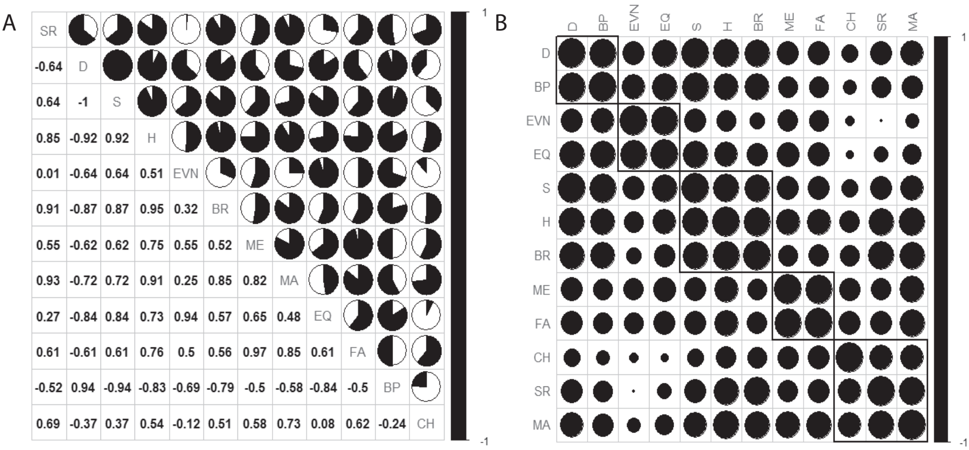

Figure 2. (A) Graphical and numerical display of the correlation matrix for the 12 diversity indices considered in the present study. Pie filling is proportional to the correlation coefficients, which are reported in the left lower part of the graph. (B) Correlation matrix after hierarchical clustering order. The rectangles around the chart of the correlation matrix are based on the results of hierarchical clustering.

Table 1. Significance values for the correlation matrix (Spearman).

	SR	D	S	H	EVN	BR	ME	MA	EQ	FA	BP	CH
SR	0	1.05e-20	1.08e-20	1.46e-40	0.896876	5.79e-58	6.12e-11	1.32e-58	0.0030299	8.26e-19	2.97e-10	2.05e-30
D	1.05e-20	0	0	8.51e-71	1.16e-21	5.50e-41	4.57e-19	5.27e-28	1.31e-38	2.75e-23	2.27e-60	4.19e-09
S	1.08e-20	0	0	9.33e-71	1.14e-21	6.05e-41	4.39e-19	5.21e-28	1.25e-38	2.62e-23	2.10e-60	4.17e-09
H	1.46e-40	8.51e-71	9.33e-71	0	2.55e-10	5.12e-62	1.56e-24	1.07e-55	2.18e-21	4.23e-33	2.95e-31	2.06e-17
eVN	0.89688	1.16e-21	1.14e-21	2.55e-10	0	0.00151	1.57e-12	0.003567	9.49e-85	3.16e-09	4.98e-25	0.7527
BR	5.79e-58	5.50e-41	6.05e-41	5.12e-62	0.001513	0	4.88e-09	1.82e-38	2.12e-09	2.41e-14	1.35e-23	2.34e-15
Me	6.12e-11	4.57e-19	4.39e-19	1.56e-24	1.57e-12	4.88e-09	0	1.71e-32	4.37e-19	7.50e-87	1.30e-09	1.35e-16
MA	1.32e-58	5.27e-28	5.21e-28	1.07e-55	0.003567	1.82e-38	1.71e-32	0	1.23e-08	2.41e-49	8.52e-13	1.31e-36
eQ	0.00303	1.31e-38	1.25e-38	2.18e-21	9.49e-85	2.12e-09	4.37e-19	1.23e-08	0	1.64e-16	2.90e-36	0.06412
FA	8.26e-19	2.75e-23	2.62e-23	4.23e-33	3.16e-09	2.41e-14	7.50e-87	2.41e-49	1.64e-16	0	1.72e-11	1.10e-23
BP	2.97e-10	2.27e-60	2.10e-60	2.95e-31	4.98e-25	1.35e-23	1.30e-09	8.52e-13	2.90e-36	1.72e-11	0	0.00019
CH	2.05e-30	4.19e-09	4.17e-09	2.06e-17	0.752698	2.34e-15	1.35e-16	1.31e-36	0.0641233	1.10e-23	0.00019	0

they are not interchangeable and there has been much debate over which is appropriate in various contexts (Morris *et al.*, 2014). However, in this study, we suggest a statistical methodology for reducing the set of diversity indices for Sardinian rangelands in the three study sites. On the basis of ANOVA and hierarchical clustering order, we propose the following set of indices: SR (Species Richness), EVN (Evenness), ME (Menhinick) and BR (Brillouin) although weather including or not the Shannon-Wiener index (H) should be tested with additional studies.

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Forage legumes and grasses. Which is better: pure stands or mixture?

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Abstract

Intercropping of grasses and legumes could represent a viable alternative to rotation due to the ready availability of nitrogen as well as their complementary development. The present data have allowed an accurate assessment of convenience for the cultivation in pure stands or mixture some legumes (berseem and squarrose clover, hairy vetch and burr medic) and grasses (ryegrass and oat), commonly used in the Mediterranean environments. In a 5-year trial, the average annual dry matter yield was 4.56 Mg ha^{-1}. Annual and seasonal yield were very variable, due to weather variability that determined a different timing of growing. The drought in the last year caused a reduction in the average production. Protein content analyses have allowed a further assessment of the protein production of pure stands and mixtures. Land Equivalent Ratio (LER) was calculated, for every season and year, both for forage and protein production.

Keywords: yield, vegetal protein, intercropping, rotation, legumes, grasses, sustainability

Introduction

The adoption of sustainable techniques, starting from rotation with legumes, could allow the preservation of soil fertility and mitigation of the impacts of intensive agriculture. Legumes are a natural source of vegetable proteins and play a central role in the sustainability of cereal and forage systems, especially in the nitrogen enrichment of soils (Giambalvo *et al.*, 2011). The validity of grass-legume intercropping compared with using a pure-crop rotation is debated (Mariotti *et al.,* 2006, Tuna *et al.*, 2007; Willey, 1979). In mixtures, the legume *rhizobia* activity and the different development of root and air systems could lead to a better use of nutrient and light resources. The present trial was carried out for five years over the period 2012-2016 in a semi-arid Mediterranean environment, with the aim of evaluating forage and protein production of several annual grasses and legumes, managed both in intercropping and rotation.

Materials and methods

The experiment was carried out in southern Sardinia (39°10' N, 9°06' E; 150 m a.s.l.) on a Typic Calcixerept soil (USDA, 2010). Long-term annual rainfall is 500 mm, irregularly distributed from October to May. Winter temperatures seldom reach as low as 0 °C and average maximum temperature is 32 °C in July. Several annual legumes and grasses (Table 1), for pastures in winter and for hay in spring, were grown in order to test stable intercropping (sown every year in the same plot) and pure-crop rotation. For all years, crops were sown in mid-November, in plots of 14 m^2 with three replicates.

In the first year, pure grasses were fertilized with 40 units of nitrogen after each utilization. For each crop, cuts were made at a height of 10 cm, whenever plants reached 20-30 cm in winter and at the flowering stage in spring. The whole-plot forage yield was evaluated by weighing the mechanically cut herbage.

Table 1. List of species in intercropping and pure stands.[1]

1. Squarrose clover – *Trifolium squarrosum* L. (25 kg ha^{-1})	4. Burr medic – *Medicago polymorpha* L. (25 kg ha^{-1})
2. Berseem clover – *Trifolium alexandrinum* L. (25 kg ha^{-1})	5. Ryegrass – *Lolium multiflorum* Lam. (25 kg ha^{-1})
3. Hairy vetch – *Vicia villosa* Roth (160 kg ha^{-1})	6. Oat – *Avena sativa* L. (160 kg ha^{-1})

[1] Seed rates in brackets refer to pure stands; the rate is halved in intercropping.

A sub-sample of 500 g per plot was then separated and oven-dried at 65 °C for three days to evaluate dry matter (DM) percentage and to analyse crude protein (CP) content. In mixture samples, the single species were separated previously. The productive efficiency of intercropping was determined using land equivalent ratio (LER) (Mead and Willey, 1980; Willey and Rao, 1980).

Results and discussion

In the five years of the experiment, total annual rainfall was 387, 423, 467, 408 and 213 mm, with remarkable seasonal differences. The average DM yields were 5.0, 5.6, 4.6, 4.4 and 3.1 Mg ha^{-1} in the five years, while the corresponding CP production was 0.87, 0.68, 0.68, 0.59, 0.52 Mg ha^{-1}; differences depending on seasonal and annual rainfall. Average annual DM yield (DMY) was 4.6 Mg ha^{-1}, concentrated mostly in spring (69%), ranging from 3.1 Mg ha^{-1} for burr medic in pure stands to 6.1 Mg ha^{-1} for medic-oat mixture (Table 2). Seasonal distribution showed high differences among the examined species: in spring, the hay yield ranged from 38% in burr medic to 88% in squarrose clover. Medic-ryegrass mixture had the most balanced seasonal production. The annual average protein production was 0.69 t ha^{-1}, from 0.43 t ha^{-1} (ryegrass) to 1.07 t ha^{-1} (hairy vetch) (Table 2), and proved to be more balanced than forage yield, with 57% of protein in hay in spring on average, ranging from 31% in burr medic to 84% in squarrose clover. As reported in Table 2, oat was the most productive of the grasses in all seasons, both in terms of DMY and protein. Similarly, it was the most competitive in the mixture, increasing the DMY, though not always with an increase in protein. Hairy vetch in pure crop showed the greatest stability throughout the years (from 3.30 to 5.39 Mg ha^{-1} y^{-1}) and also the highest annual protein production. Moreover, hairy vetch showed the highest competitiveness towards ryegrass after the winter season. Among legumes, burr medic showed the greatest suitability for grazing and for protein production in winter, both in pure crop and intercropping. Of the two clovers, squarrose was more productive in annual forage yield (but not of protein); berseem was more productive in winter for forage and protein.

As shown in Figure 1, LER values showed superiority of the mixtures (LER>1), but not in every season nor for every species. Annually and in spring, intercropping did not result in an advantage for squarrose-ryegrass and berseem-ryegrass. Moreover in winter, the mixture was favourable for burr medic-grasses

Table 2. Average of annual and seasonal forage and crude protein production (t ha^{-1}) of the five years of trial and, in brackets, the percentage of legume in the mixture.[1]

Mixtures and pure crops	Annual		Winter		Spring	
	DMY	Protein	DMY	Protein	DMY	Protein
V. villosa / L. multiflorum	4.8 B-D (67)	0.89 B (75)	1.2 D (81)	0.30 D-G (86)	3.7 B-E (62)	0.59 A (70)
V. villosa / A. sativa	5.4 A-C (47)	0.90 B (57)	1.4 CD (37)	0.30 D-G (47)	4.0 AB (51)	0.60 A (62)
T. squarrosum / L. multiflorum	3.8 E-H (48)	0.47 D-G (61)	0.6 E (19)	0.12 HI (24)	3.1 B-G (50)	0.35 CD (65)
T. squarrosum / A. sativa	5.8 A (39)	0.63 CD (51)	1.1 D (11)	0.20 GH (14)	4.7 A (45)	0.43 BC (62)
T. alexandrinum / L. multiflorum	3.7 F-H (37)	0.47 D-G (49)	1.1 D (44)	0.21 GH (51)	2.5 G-J (34)	0.26 DE (45)
T. alexandrinum / A. sativa	4.8 B-D (29)	0.55 C-G (35)	1.5 CD (20)	0.28 E-G (23)	3.3 B-G (36)	0.27 DE (47)
M. polymorpha / L. multiflorum	4.3 D-F (58)	0.85 B (70)	2.1 AB (63)	0.49 AB (74)	2.2 H-J (44)	0.36 CD (50)
M. polymorpha / A. sativa	6.1 A (30)	0.82 B (40)	2.2 A (20)	0.40 B-D (24)	3.9 A-C (31)	0.42 BC (39)
M. polymorpha	3.1 H	0.80 B	1.9 A-C	0.55 A	1.2 K	0.25 DE
T. alexandrinum	3.2 H	0.60 CD	1.2 D	0.27 E-G	1.9 JK	0.33 CD
T. squarrosum	4.2 D-G	0.63 CD	0.5 E	0.10 I	3.7 B-E	0.53 AB
V. villosa	4.5 C-E	1.07 A	1.5 B-D	0.45 BC	3.0 C-H	0.63 A
A. sativa	5.7 A	0.59 C-F	2.0 A	0.33 D-F	3.7 B-E	0.26 DE
L. multiflorum	4.4 D-F	0.43 FG	1.5 CD	0.22 F-H	2.9 E-H	0.22 DE

[1] Means followed by the same letters are not significantly different at P= 0.01 (Duncan test)

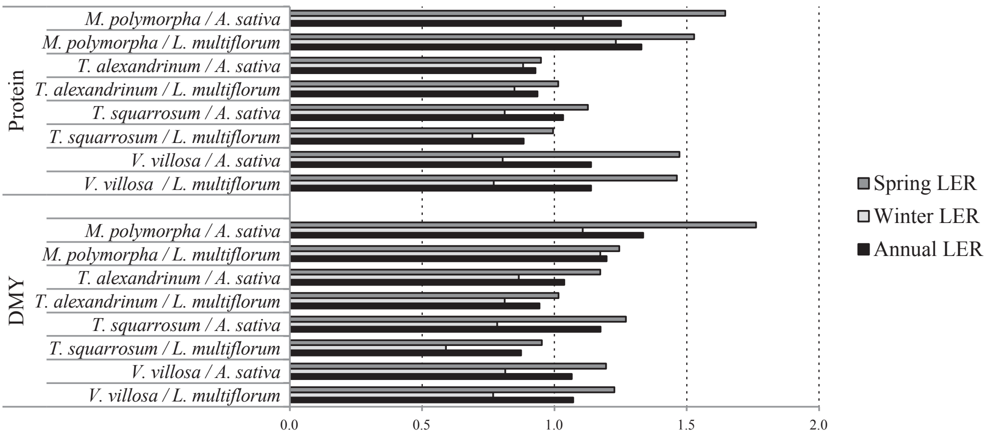

Figure 1. Annual and seasonal average of land equivalent ratio (LER) for forage and crude protein in the five years. DMY = dry matter yield.

only. Regarding protein production, the LER value of hairy vetch or burr medic in mixture strengthened the validity of their intercropping with grasses. In contrast, in clover mixture, LER showed no advantage for berseem-oat.

Conclusions

General conclusions on the superiority of mixtures in comparison with pure stands cannot be drawn. Each species showed a different behaviour in relation to the companion species in the mixture. Nevertheless, some results proved to be useful to enable farmers to decide whether to sow in pure stands or in mixtures. Hairy vetch in pure stand showed the greatest productive stability and the highest crude protein production as well as a good floristic balance in mixture. In addition, one can assess the advantage of mixture for burr medic and hairy vetch. As regards squarrose and berseem clovers, our results showed little or no advantage for mixtures with grasses, particularly for their protein production.

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Utilization efficiency of nitrogen and phosphorus and their response on dry matter accumulation in different forage mixtures

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Abstract

Crop response to applied nutrients is a global phenomenon. Studies on the efficiency of applied nutrients such as nitrogen and phosphorus and their response on dry matter yield (DMY) in subterranean clover (*Trifolium subterraneum* L.; cv. Clare), perennial ryegrass (*Lolium perenne* L.; cv. Meretti) and crested wheatgrass (*Agropyron cristatum* L.; local population), either grown alone or in binary mixtures (clover:grass 1:1) were carried out through a field experiment at the Institute of Forage Crops, Pleven, Bulgaria. Our observations showed that ryegrass displayed the maximum agronomic nitrogen-use efficiency. The lower dry mass accumulation associated with the lower nitrogen-use efficiency of crested wheatgrass was compensated by growing this crop in combination with subterranean clover. Agronomic efficiency of applied nitrogen was highest for subterranean clover-ryegrass mixtures. The amount of nitrogen accumulated in the DMY by mixtures was found to be higher than in pure stands. In pure stands subterranean clover fixed 31.4 kg N t^{-1} dry matter (DM), which significantly increased by an additional 2.4 kg N t^{-1} DM when mixed with ryegrass. These observations suggested that both nitrogen and phosphorus were used more effectively under mixed cropping of subterranean clover-ryegrass mixtures than in pre stands.

Keywords: nitrogen use efficiency, phosphorus use efficiency, subterranean clover, wheatgrass, perennial ryegrass

Introduction

Consequent upon climate change, increasing frequency and duration of droughts strongly requires adaptation of forage crops and their diversification under changed agro-pedological conditions. In grass-legume mixtures, where legumes fix symbiotic nitrogen (N) and grasses have high N utilization levels, the total N requirement may be reduced significantly through improving the N-use use efficiency (Ledgard and Steele, 1992). Thus, mixed cropping of grasses and legumes has an inbuilt advantage and may provide an adaption to certain environmental issues (Luscher *et al.*, 2014; Porqueddu *et al.*, 2003). The purpose of this study was to evaluate the utilization efficiency of nitrogen and phosphorus and their accumulation in the dry matter yield of crested wheatgrass and perennial ryegrass, in pure stands and in mixtures with subterranean clover.

Materials and methods

A four-year field trial was carried out at the Institute of Forage Crops, Pleven, Bulgaria, using different forage crops, viz., subterranean clover (*Trifolium subterraneum* L. cv., Clare), wheatgrass (*Agropyron cristatum* L., local population) and perennial ryegrass (*Lolium perenne* L., cv. Meretti) either in pure stands or in binary grass-legume mixtures (ratio of grass:legume=1:1) were evaluated. A completely randomized block design was performed (4 replications, plot size=10 m^2). Phosphorus (P) as triple superphosphate (30 kg ha^{-1}) and potassoum (K) as potassium chloride (15 kg ha^{-1}) were applied at stocking stage. Nitrogen was applied as ammonium nitrate at sowing in autumn. Swards were cut at plant height of about 20 cm. Dry matter yield was determined after drying a 200 g sample of biomass to constant weight at 60 °C. Nitrogen-use efficiency (NUE) and P-use efficiency (PUE) were determined according to Bowen and Zapata, (1991). The N and P that accumulated in the DM were calculated by multiplying dry matter yield (DMY) by tissue N and P concentrations. Sustainable yield index (SYI) was used for the assessment of N (NSYI) and P (PSYI) accumulation in DMY (Singh *et al.*, 1990). The amount of fixed nitrogen (N fix) was calculated for subterranean clover according to Carlsson and Huss-Danell (2003),

Results and discussion

The N usage varied among the different crops. Subterranean clover absorbs mineral nitrogen from roots and, by nitrate reductase, assimilates it as organic nitrogen, in addition to symbiotic N_{fix}. The NUE of subterranean clover was 76% (Table 1). The accumulation of nitrogen in grasses is the consequences of nitrate-N assimilation through the roots due to the elevated activity of nitrate reductase. However, the efficiency of N use in perennial ryegrass was high. Vazquez de Aldana *et al.* (1997) reported similar observations. Grasses vary in their efficiency of N utilization (Inostroza *et al.*, 2015). Perennial ryegrass has an excellent ability to absorb N (Wilkins *et al.*, 1997). The accumulation of nitrogen in DM of wheatgrass was the lowest. A lower efficiency of N use (8 points) was observed from crested wheatgrass. It is assumed that this is due to the comparatively smaller leaf area of the crop coupled with weaker photosynthesis (White, 1983).

The low NUE of crested wheatgrass was further compensated when combining this grass with subterranean clover. In subterranean clover-ryegrass mixtures, N was efficiently utilized due to mutualistic competition between the two components for available nitrogen. Thus, the NUE of subclover-ryegrass mixture was 11.2% higher than NUE of subterranean clover grown alone and 8.5% higher than the NUE of ryegrass pure stand. Inostroza *et al.* (2015) consider that the possibility of grasses to absorb the fixed N from the legumes is of variable magnitude, depending on the activity of roots. Subterranean clover accumulated maximum N in the DM in pure stands. The amount of nitrogen accumulated in the DM of both mixtures was similar. Nitrogen accumulated in DM of mixed crops was significantly higher than that of pure stands.

The PUE varied within a narrow range, in the pure stands as well as in mixtures. In mixtures with perennial ryegrass, PUE was 11.2% higher, compared with the PUE of the subterranean clover pure stand. The amount of P accumulated in the DM in the mixture of subterranean clover-ryegrass was 13.6% higher than pure ryegrass and 16.4% higher than subterranean clover.

The sustainable yield index (SYI) of different crops was observed in the following increasing order: perennial ryegrass, crested wheatgrass, subterranean clover. Sustainable yield index in crop mixtures was higher than in pure stands. In our study, the amount of N fix in subterranean clover was 31.4 kg N t^{-1} DM.

Table1. Nitrogen (NUE) and phosphorus (PUE) use efficiency, sustainable yield indices (NSYI and PSYI), accumulation in dry matter (DM) yield and amount of fixed nitrogen (Nfix) for pure stands and legume-grass mixtures.[1]

Treatments	NUE (%)	N in DM (kg ha^{-1})	NSYI	PUE (%)	P in DM (kg ha^{-1})	PSYI	N fix (kg N t^{-1} DM)
Subclover	76.1	12.6	0.2	20.3	1.7	0.2	31.4
Wheatgrass	70.2	8.9	0.23	18.7	1.5	0.2	
Ryegrass	78.0	11.6	0.28	20.8	1.7	0.2	
Subter+wheatgrass	77.5	13.3	0.2	20.7	1.7	0.2	32.6
Subter+ryegrass	84.6	14.0	0.2	22.6	1.9	0.2	33.9
CV	26.4	3.9	0.0	1.9	0.0	0.0	1.5
LSD 5%	3.6	0.6	0.1	0.9	0.1	0.1	0.3

[1] CV = coefficient of variation; LSD = least significant difference.

Lucas *et al.* (2010) found approximately 30 kg N t^{-1} of dry biomass. According to Sebastia *et al.* (2004), legumes effectively regulated the process of N fixation in mixtures with grasses and the amount of fixed N varied depending on the competition among the mixture components. The mixture of subterranean clover-ryegrass fixed 1.2 kg N t^{-1} DM more than the pure stand.

Conclusions

The role of mixtures based on legumes for sustainable agriculture was shown. The legume component in the intercropping pasture system with grasses contributes to more effective usage of resources. Mixed cropping of subterranean clover-ryegrass was more effective over pure stands with regard to the use of both nitrogen and phosphorus.

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Influence of management on *Festuca valesiaca* grasslands from Romania's forest steppe

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Abstract

The natural grassland area of Romania, comprising over 4.8 million ha, of which 3.3 million ha are pastures and 1.5 million ha hayfields, represents 33% of the total national farming area, and thus a huge potential of natural resources for the economic development of the country. This potential is weakly capitalized, due to the state of degradation of most grasslands, with low yields and reduced quality of fodder, owing to improper floristic composition. In this context, our study aims to identify the best rates of organic fertilizers that may improve the productivity and quality of *Festuca valesiaca* Schleich. ex Gaudin ecosystems, under conditions of environmental conservation. This paper presents the results obtained during the period 2010-2015, regarding dry matter production, crude protein, acid detergent fibre and neutral detergent fibre content and vegetative cover evolution.

Keywords: permanent grassland, fertilization, productivity, quality, *Festuca valesiaca*

Introduction

In Romania, permanent grassland in the forest steppe covers over 250,000 ha, a large part of which is dominated by *Festuca valesiaca*, used for grazing and mowing. Owing to climate conditions, soil characteristics and their manner of management, these grasslands have a low productivity and a reduced quality. The objectives of our study aimed at an increase in productivity and at the improvement of vegetative cover structure and the quality of fodder from *Festuca valesiaca* grasslands fertilized with organic fertilizers. Fodder productivity and quality are influenced by soil fertility, agronomic practices, climate conditions, grassland floristic composition, and phenological stage at harvest (Andueza *et al.*, 2010; Hopkins *et al.*, 1999; Poetsch *et al.*, 2005; Pozdicek *et al.*, 2008; Vintu *et al.*, 2011).

Materials and methods

At Iasi Research Station, Research Centre Ezareni, on a *Festuca valesiaca* grassland, located at an altitude of 95 m, on a soil with 32-37 mg kg^{-1} mobile phosphorus and 231-315 mg kg^{-1} mobile potassium, pH of 6.5-6.9 in a layer of 0-20 cm, we conducted a monofactorial expriment, in three replicates, with the size of experimental plots of 3×4 m, in which we monitored the influence of composted cattle manure, applied annually or every two or three years, in doses of 10-40 Mg ha^{-1}. The management scheme variants were: V_1, unfertilized control; V_2, 10 Mg ha^{-1} cattle manure annually; V_3, 20 Mg ha^{-1} cattle manure applied every 2 years; V_4, 30 Mg ha^{-1} cattle manure applied every 3 years; and V_5, 40 Mg ha^{-1} cattle manure applied every 3 years. The manure contained 4.45 g kg^{-1} N-total, 2.12 g kg^{-1} P_2O_5 and 6.95 g kg^{-1} K_2O, and was applied during autumn at the end of vegetative period (October). The multi-annual mean temperature was 9.6 °C, and mean rainfall was 518 mm y^{-1}. In the area, *Festuca valesiaca* grasslands are used mainly for sheep and cattle grazing or for mowing. Harvesting was done as hay, at the earing phenological stage of dominant grasses, and determinations were carried out on samples taken at the first vegetation cycle, the data representing the average for years 2010-2015. The Kjeldahl method was used to determine crude protein; Van Soest method for acid detergent fibre (ADF) and neutral detergent fibre (NDF), while relative feed value (RFV) was determined through the relation proposed by Undersander and Moore (2002). The study of vegetation was conducted by the Braun-Blanquet method (1964) and the analysis of biodiversity with the help of the PC-ORD software, for the interpretation of floristic data (McCune and Grace, 2002) using MRPP (Multi Response Permutation Procedure) and multidimensional scaling NMS Autopilot. Data were analysed using analysis of variance (ANOVA) and least significant difference (LSD) test (P<0.05-0.001).

Results and discussion

Fertilizing *Festuca valesiaca* grasslands with organic fertilizers leads to an improvement in productivity and quality. Manure applied at 10-40 Mg ha^{-1} contributed, together with other climatic factors, to a significant production increase, ranging between 54 and 67%, in comparison with the non-fertilized control, for all the studied treatments. On average, for six years, 2.30 Mg DM ha^{-1} and 3.55-3.83 Mg DM ha^{-1} were obtained for the control and fertilized treatments, respectively (Table 1).

Fertilization with organic fertilizers at 10-30 Mg ha^{-1} of manure resulted in increased CP content from 78 g kg^{-1} DM (control) to 100-104 g kg^{-1} DM (30 and 10 Mg ha^{-1} manure, respectively) and increased the CP quantity, from 179 kg ha^{-1}(control), to 355-398 kg ha^{-1} (V_4 and V_2, respectively). Alongside the important CP increase, the manure doses applied resulted in a reduction of the NDF and ADF content in the fodder (Table 1), as a result of changes in the floristic composition, a prolonged duration of the vegetative phases and an alteration of the ratio between stems and leaves in favour of the leaves, in comparison with the control treatment, which led to the increase of RFV, from 65 to 87-93 units, influencing in a positive manner the fodder digestibility and palatability. Organic fertilization also resulted in important changes in vegetation structure. In Figure 1, fertilized treatments (V2÷V4) are grouped in the graphic representation, but without overlaps, indicating a different effect of different fertilization treatments, while the unfertilized control is well separated, emphasizing the changes in the vegetative cover.

Conclusions

The fertilization of *Festuca valesiaca* grasslands with 10-40 Mg ha^{-1} manure resulted in an increase of DM production of 54-67%, and also changes in fodder chemical composition, which improved substantially its quality, by increasing the CP content and reducing NDF, thereby increasing fodder digestibility and palatability. The application of 10-40 Mg ha^{-1} manure resulted in significant variations in the floristic composition, namely a reduction of *Festuca valesiaca* species and an increase in *Fabaceae* presence (*Lotus corniculatus, Trifolium pratense* and *Trifolium repens*) and in good forage quality grasses. These grasslands may be improved by manure fertilization and reasonable utilization, applying a suitable management (10 Mg ha^{-1} manure annually, 20 Mg ha^{-1} manure every 2 years and 40 Mg ha^{-1} manure every 3 years).

Table 1. Influence of organic fertilization on grassland productivity and fodder quality (average 2010-2015).[1]

Variant[2]	Production		Quality			
	DM (Mg ha^{-1})	CP (kg ha^{-1})	CP (g kg^{-1} DM)	NDF (g kg^{-1} DM)	ADF (g kg^{-1} DM)	RFV
V_1	2.30^{C}	179^{C}	78^{C}	752^{C}	468^{C}	65^{C}
V_2	3.83***	398***	104***	608^{000}	420^{00}	87***
V_3	3.78***	367***	97***	570^{000}	419^{00}	93***
V_4	3.55**	355***	100***	568^{000}	422^{00}	93***
V_5	3.70***	340***	92**	591^{000}	424^{00}	88***
* LSD 0.05	0.62	79	9	44	23	9.2
** LSD 0.01	0.90	115	12	65	33	13.5
*** LSD 0.001	1.35	172	19	97	50	20.2

[1] DM = dry matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; RFV = relative feed value.

[2] V_1 = unfertilized (control); V_2 = 10 Mg ha^{-1}cattle manure annually; V_3 = 20 Mg ha^{-1}cattle manure every 2 years; V_4 = 30 Mg ha^{-1}cattle manure every 3 years; V_5 = 40 Mg ha^{-1}cattle manure every 3 years.

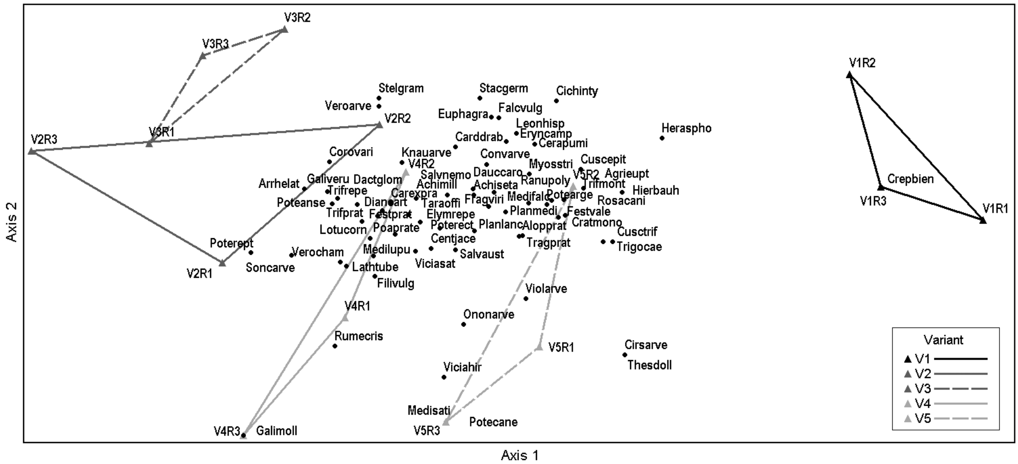

Figure 1. Influence of fertilization on floristic composition order. R_1, R_2 and R_3 are replicates.

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Rangeland Rummy: a tool to trigger discussions between pastoral farmers about their grazing system and co-construct adaptive strategies to climatic hazards

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Abstract

Sylvo-pastoral systems in Mediterranean areas are increasingly recognised for their natural, social and cultural value. However, they strive for economic and ecological sustainability, while constantly coping with climatic hazards. Rangeland Rummy is a serious game aimed at helping farmers to design sustainable feeding strategies for sylvo-pastoral systems. It is to be played by a group of farmers accompanied by a technician. A game board, sticks and cards are used to represent the feeding system, in which performance is evaluated by a simple simulator. Various animals, vegetation types and grazing practices can be simulated. The local climatic conditions and climatic hazards can be taken into account with basic calibrations before or during the game. Thus, farmers can play in a context close to their own farm. The latest version of Rangeland Rummy was tested and validated during summer 2016 with three groups of farmers. The users described it as a serious and technical game. It was deemed most useful for players sharing similar production systems and either starting in the activity, envisaging changes in the grazing system or having to face climatic hazards. Rangeland Rummy is also being used for educational purposes in agricultural faculties.

Keywords: rangeland, management, serious game, climate change

Introduction

Silvo-pastoralism is a widespread land-use system in Mediterranean areas, involving livestock husbandry in rangelands or in wooded pastures. Silvo-pastoral systems are highly diverse in terms of vegetation types, animal species and associated products, and management practices. However, they share common features (Jouven *et al.*, 2010): (1) securing economic sustainability by minimising inputs and maximising forage intake at pasture; (2) providing ecosystem services through an ecologically sustainable use of natural vegetation; and (3) coping with climatic hazards, which increase the variability and unpredictability of forage availability over seasons and years. In order to meet such challenges, farmers need to design management strategies taking the best advantage of the biological diversity of rangelands and animals and including securities in case of climatic hazards. Farmers' management strategy can be evaluated based on the results obtained each year, then modified if deemed inefficient in relation to the issues presented above. In order to speed up this process, we designed and tested a serious game called 'Rangeland Rummy' capitalising on both the available scientific knowledge and on the local ecological knowledge of the farmers playing the game.

Materials and methods

The basic concept of Rangeland Rummy is to put together a small group of farmers, playing together at designing on a physical board, with cards and sticks, a sustainable pasture-based feeding system. Next to them, a technician enters the chosen setup in a simple simulator, which provides the group with the predicted performance of the system (feeding management in line, or not, with animal requirements, forage self-sufficiency, reliance on grazed forage and, on rangeland, the sustainability of rangeland utilisation). One or more strategies can be tested, thus allowing *ex-ante* optimisation and learning about pastoral farm management. This concept was first applied to a game intended for grassland-based systems (Forage Rummy: http://www.scoop.it/t/rami-fourrager). In 2013, the concept was transferred to sylvo-pastoral systems (Farrié *et al.*, 2015), which implied changing the underlying conceptual model and including a qualitative approach to the grazing system. New vegetation types (rangelands and woodlands) and new animals (sheep) were introduced. Forage availability (in grazing days) was represented as a function of grazing management and season (instead of grass growth rates). This first version was tested in 2013 with groups of farmers and was used in 2013, 2014 and 2015 with groups of students. On these occasions, a number of limitations were identified. In 2016 the tool was further refined, in order to: (1) focus on grazing management, (2) increase the diversity of rangelands documented (>400 sticks based on available pastoral references), (3) describe the local vegetation dynamics and the changes in animal body reserves, (4) provide indicators of sustainability of rangeland utilisation, and (5) introduce climatic hazards in terms of timing and amount of pastoral resources available.

Results and discussion

The physical component of the Rangeland Rummy is presented in Figure 1. A set of 'vegetation × utilisation' sticks is prepared by the technician before the game, by applying a calibration for seasonal dynamics (and if needed climatic hazards) to a selection of vegetation types chosen from the database available in the simulator. In order to do this, it is important that the players anticipate the type of

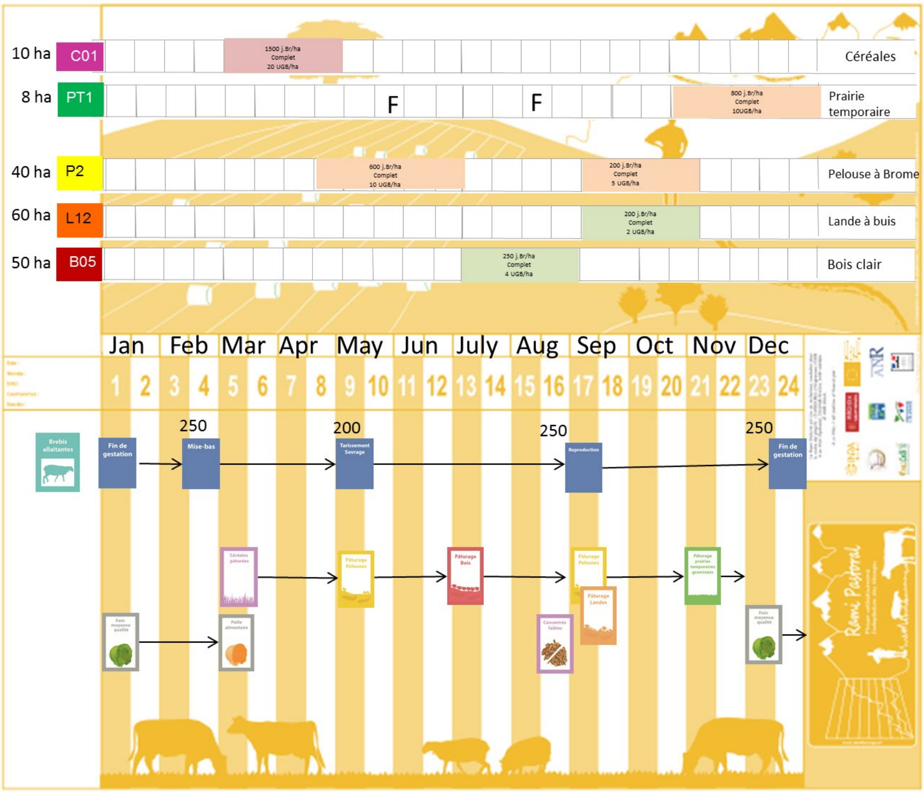

Figure 1. Example of layout for the game board. In the upper part, the sticks represent the chosen utilisations of the available types of vegetation (grazed crops: lilac, grassland: green, open rangeland: yellow, shrubland: orange, woodland: red) and the surface area devoted to each. In the lower part, the plain blue cards enable players to trace the various physiological stages of the animal group (here only one group represented) and the number of animals present through time. Below, coloured cards represent the types of vegetation grazed during the year, and/or the conserved forage and concentrate distributed by the farmer.

farm they want to represent. The game lasts two to three hours. First, the areas available per vegetation type and the composition of the animal groups in terms of number of individuals and physiological stage are set. Up to three different animal groups can be identified, with for each group one or two types of animals and a specific feeding sequence. Then, 'vegetation × utilisation' sticks are chosen among the available set in order to feed the animal groups at pasture during the grazing season. Based on the knowledge of the players, an area is attributed to each stick and conserved feed can be added. The simulator provides a comparison between the requirements of each animal group and the amount and quality of the predicted intake with the chosen feeding management. The users are also informed if a change in body condition is likely. At farm scale, the simulator provides indicators of self-sufficiency for forage, concentrate consumption and reliance on grazed forage at farm scale plus the contribution of each vegetation type to the feeding system. For each 'vegetation × utilisation' it also calculates the actual utilisation of the available biomass and informs the user in case of under-utilisation throughout the year. Based on simulation results, farmers can discuss and modify the feeding strategy.

Rangeland Rummy was used in the summer 2016 by three groups of 3 to 4 'pastoral' farmers interested in discovering the game and in discussing their grazing and feeding practices with peers. The farmers within a group came all from the same area, and sometimes knew each other. GROUP1 included farmers breeding sheep for meat and starting in the activity, interested in finding a sustainable sizing for their system; GROUP2 included farmers breeding goats for milk, with different levels of experience, interested in testing the inclusion of new pastures (in terms of vegetation or surface area) in their feeding system; GROUP3 consisted in farmers breeding either sheep or cattle for meat, which were part of a group already involved in training sessions and workshops about grazing management, and which interest lied in evaluating the potential of the game. These tests confirmed that the Rami Pastoral triggered discussions among farmers about their grazing and feeding practices at all stages of the game. The players found the game very sharp on technical aspects. Based on the outcomes of the game sessions (and on previous tests), we determined the following criteria for optimal use: (1) mix unexperienced farmers with experienced ones; (2) represent a system close to that of one of the players, but in a simplified version, (3) set clearly an objective for the session, known to all participants.

Conclusions and perspectives

Rangeland Rummy is a serious game which helps to trigger and focus discussions among 'pastoral' farmers about their grazing and feeding system. The game is useful for participants envisaging changes in their system, either because it is not stabilised yet, or because of climatic hazards or specific opportunities. The latest version of Rangeland Rummy will be available very soon in the French version for extension services and farmers' associations, and to be used for educational purposes in agricultural schools and faculties.

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Theme 3.
Alternative and multiple-uses of grassland resources

Mediterranean grassland species from traditional to multiple uses

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Abstract

We focused our attention on grassland species suitable for multiple uses, as source of bioactive compounds for healthcare of domestic animals, phytoremediation, biomass production for bioenergy and for wild fire prevention. Plant secondary metabolites such as polyphenols exhibit a wide range of biological effects, many of which are associated with their free radical scavenging and antioxidant activity. We looked at available data on natural antioxidants derived from grassland plants for the identification of species to develop new natural products for phytotherapy and as feed additives to improve animal production. We also made a screening of available data about the phytoremediation potential of grassland species taking into account also eco-toxicological aspects related to the utilization of biomass harvested from contaminated sites. Furthermore, we discuss examples regarding the novel role of grasslands to supply bioenergy and bio-based products such as biofuels, biochemicals and biomaterials from bio-refinery. Energy generation from biomass of extensive, high-diversity grasslands has been claimed in areas of central Europe, whereas autochthonous Mediterranean perennial grasses are currently being investigated for bioenergy. In Europe, about 65,000 fires occur yearly, burning half a million hectares of vegetated areas. We analysed results of available experience of grazing and wood-pasture management as tools to reduce available fuel, and to protect species and landscape biodiversity.

Keywords: bioenergy, fire prevention, grasslands, grazing, phytoremediation, secondary metabolites

Introduction

The herbaceous layer of grazed lands is usually represented by grasses but several other plant species can also cover large areas. We focused our attention on grassland species suitable for emerging and multiple uses, such as isolation of bioactive compounds for healthcare of domestic animals, environmental remediation, biomass production for bioenergy and wild fire prevention.

Plant secondary metabolites, due to their biological activities, have been used for centuries in traditional medicine. Nowadays, ethno-pharmacology and ethnobotany studies are widely used to discover new active compounds and to preserve traditional knowledge as a source for the development of new therapeutics and nutraceuticals from traditional herbal remedies; grassland species are a precious reservoir of secondary metabolites for such purposes (Bullitta *et al.*, 2007a; Camara *et al.*, 2016; Heinrich *et al.*, 2016; Piluzza *et al.*, 2015; Piluzza and Bullitta, 2011).

Local soil contamination in 2011 was estimated at 2.5 million potentially contaminated sites in European countries. Contaminated soil continues to be commonly managed using 'traditional' techniques, e.g. excavation and off-site disposal, which accounts for about one-third of management practices (EEA, 2014), although the use of green plants as a remediation tool in environmental cleanup is currently considered a useful tool, provided that the many complex interactions among soil, contaminants, microbes and plants are elucidated, and that each intervention of phytoremediation is considered a site-specific solution.

The generation of energy from biomass of extensive, high-diversity grasslands has been claimed as an alternative to their abandonment. Moreover, according to Prochnow *et al.* (2009a), in Europe 13-22%

of the permanent grassland area could be surplus to requirements by 2020, while green refineries using whole green crops can generate materials as well as energy or fuels, thereby meeting the requirements for producing value-added products from widespread raw materials. It is important to consider the performance of conventional bioenergy species when grown under Mediterranean conditions, because unfortunately, in Mediterranean environments, the sustainable cultivation of lignocellulosic non-food crops relies on a limited assortment of dedicated species and varieties.

Some issues deriving from the use of grassland species for their bioactive compounds, phytoremediation potential and suitability as raw materials for bio-refinery are here discussed.

Wildfires are a relevant problem, particularly in rural areas, all over the World and they represent an ever present and increasing threat because of climate change. Fire can affect a wide array of vegetation characteristics including nutritive value and production of herbage, species composition, plant density and size, root development, and seed survival and germination. Soil characteristics that may be affected include temperature, moisture, pH, nutrients and erosion (Volesky *et al.*, 2006). Therefore, the paper also discusses appropriate wildfire prevention in the context of protecting biodiversity and landscapes.

Ethnobotany and bioactive compounds

The Mediterranean basin area has been inhabited for millennia and humans have contributed to increase the diversity of landscapes and habitats, and to make of it one of the 25 global biodiversity hot spots (Di Novella *et al.,* 2013). During the development of civilizations, humans used plants for their basic needs: food, clothing, shelter, hunting and nursing (Di Novella *et al.,* 2013). However, many socio-economic changes took place towards the end of the last century, moving away from the sustainable use of natural resources and there was a parallel increase in the erosion of traditional plant knowledge. The loss of knowledge on traditional plant uses has also been heightened by a decrease in native plant species diversity due to various human activities, such as an alteration of natural ecosystems, indiscriminate harvesting of wild plants, irrational grazing and forest fires (Tuttolomondo *et al.*, 2014).

Several studies on ethno-pharmacology and ethnobotanical and ethno-veterinary uses have been carried out in Italy (Bullitta *et al.*, 2007a; Di Novella *et al.*, 2013; Piluzza *et al.*, 2015; Tuttolomondo *et al.*, 2014), Spain (Benítez *et al.*, 2012), Serbia (Jarić *et al.*, 2007), Turkey (Polat *et al.*, 2015), Tunisia and Italy (Viegi and Ghedira, 2014); Mediterranean areas (Pieroni *et al.*, 2006), Poland (Łuczaj, 2010), Romania (Kołodziejska-Degórska 2012), Czech Republic (Simkova and Polesny, 2015) and Hungary (Dénes *et al.*, 2012).

The recovery of traditional plant knowledge linked to the medicinal use of plants is, therefore, one of the most urgent and immediate issues needing attention. International research has confirmed that the recovery of popular traditions can contribute not only to the identification of little-known plant species but also to the discovery of new, biologically active molecules for the treatment of diseases (Tuttolomondo *et al.,* 2014). The identification of 'new' plant species of interest for both conventional and non-conventional medicine represents an important opportunity to use the wide plant biodiversity of sustainable agro-ecosystems (Tuttolomodo *et al.,* 2014). Ethno-pharmacology, as the scientific study of indigenous drugs and their biological activities, and ethnobotany, which focuses on the study of the relationship among people and plants as a cultural value, are nowadays widely used as useful tools to discover new active compounds and to preserve traditional knowledge (Eissa *et al.,* 2014; Heinrich *et al.*, 2006). In fact, the information obtained from biological effects of traditional herbal remedies becomes a valuable contribution to the discovery of new active compounds and allows the development of new therapeutic lines (Eissa *et al.,* 2014). Plant secondary metabolites, due to their large biological activities, have been used for centuries in traditional medicine. They are usually classified according to their biosynthetic pathways (Min *et al.*, 2003). Three large molecule families are generally considered: phenolics, terpenes and steroids, and alkaloids. A good example of a widespread metabolite family is given by phenolics; because these molecules are involved in lignin synthesis, they are common to all higher plants (Bourgaud *et al.,* 2001). Phenolics comprise a class of bioactive compounds commonly found in the plant kingdom, and they include several groups of different substances, among them tannins, flavonoids and phenolic acids, and are one of the most important classes of compounds for their biological activities, especially for their antioxidant properties (Conforti *et al.*, 2008; Piluzza and Bullitta, 2011; Sulas *et al.*, 2016) and related implications in animal nutrition (Piluzza *et al.*, 2013). In northern Poland, Balcerek *et al.* (2009) studied the antioxidant capacity and total phenolic compounds in extracts of selected species: *Miscanthus sinensis* Anderss., *Dichanthium caucasicum* (Trin.) S.K. Jain & Deshp, *Phragmites australis* (Cav.) Trin. ex Steud. The average amount of polyphenols and antioxidant capacity in these selected species was low, but the extraction was performed with water. According to Boeing *et al.* (2014) the extraction of phenolic compounds is significantly affected by solvent combinations, and organic solvent-water mixtures are more efficient in extracting antioxidant compounds.

Hărmănescu (2011a) investigated the effects of fertilization on polyphenol content of forages harvested in spring from hilly permanent grassland in Romania. The highest polyphenol concentration was identified in unfertilized forages and lower concentrations were evidenced when mineral nitrogen was supplied. The increased nutrient bioavailability from fertilization could reduce the plant stress and, presumably, decrease the quantitative biosynthesis of polyphenols and/or could enhance the presence of species with smaller quantity of such compounds (Hărmănescu, 2011b). Another study on polyphenolic composition of a Romanian permanent pasture under influence of organic fertilization showed different results between the experiments carried out in 2010 and 2011. Fertilization with manure increases the total polyphenol and tannin content compared with the unfertilized control in the first year only (Tarcau *et al.,* 2013). Therefore, fertilization affects the floristic composition of grassland, influencing directly the polyphenol content of forages (Hărmănescu, 2011a). Additional studies regarding the influence of single plant species and phenological stage could be useful.

In France, Copani *et al.* (2015) investigated the potential positive effects of the addition of legumes that contain bioactive compounds in combination with grass for silage and found an improved rumen fermentation, and undegraded or poorly degraded proteins in silages. Synergistic effects have also been observed during *in-vitro* fermentation of sainfoin (*Onobrychis viciifolia* Scop.) and cocksfoot (*Dactylis glomerata* L.) (Niderkorn *et al.*, 2012).

Tannins are an extremely complex group of polyphenolic compounds conventionally classified into hydrolysable and condensed tannins (CT), the latter being more widely distributed in nature (Min *et al.*, 2013). CT, also named proanthocyanidins (PA) because this term is more correlated with their chemical structure, present several implications for the nutritive value of temperate forages fed to ruminants and have been studied by various authors (Häring *et al.*, 2008; Min *et al.*, 2003). Tannins are poorly understood and their relationship with animal nutrition involves a number of different research areas such as the interactions between PA and bacterial cells and the interactions between PA and forage proteins (Piluzza *et al.*, 2013).

Condensed tannins are oligomers and polymers of flavanol and have been found in the leaves and stems of several forage legumes, such as birdsfoot trefoil (*Lotus corniculatus* L.), sainfoin, sulla (*Hedysarum coronarium* L.) sericea lespedeza (*Lespedeza cuneata* G. Don) and also in the flowers of *Trifolium* species, sainfoin and sulla (Mueller-Harvey, 2006; Piluzza *et al.*, 2013; Waghorn, 2008). Total concentrations and compositions depend on genotype (Häring *et al.*, 2007; Stringano *et al.*, 2012), season (Theodoridou *et al.*, 2011), plant organ (Häring *et al.*, 2007; Piluzza and Bullitta 2010; Re *et al.*, 2014a) and growth stage of species (Guglielmelli *et al.*, 2011; Häring *et al.*, 2007; Piluzza and Bullitta, 2010; Re *et al.*, 2014a; Theodoridou *et al.*, 2011). A study investigated thirty-seven sainfoin (*Onobrychis viciifolia* Scop.) accessions from the EU 'Healthy-Hay' germplasm collection for proanthocyanidin content and composition. Cluster analysis revealed that European accessions clustered into two main groups: Western Europe and Eastern Europe/Asia. In addition, accessions from USA, Canada and Armenia tended to cluster together (Stringano *et al.*, 2012). Re *et al.* (2014a) showed that sainfoin, field-grown under rain-fed Mediterranean conditions, showed beneficial CT concentration that can be considered suitable for contributing to the improvements of animal performance and health.

Hatew *et al.* (2016) investigated the structure variation of CT extracts, obtained from four sainfoin accessions, on the *in vitro* ruminal methane production and fermentation characteristics; they found that tannin polymer size is an important factor affecting *in vitro* CH_4 production, which may be linked to the CT interaction with dietary substrate or microbial cells. Plant secondary metabolites offered multiple opportunities that require elucidation of concentration and chemical composition and *in vivo* studies to better establish the functionality of the Mediterranean grassland species.

Polyphenols and condensed tannins offer several opportunities to farmers for managing the health of their herds and flocks (Wang *et al.*, 2012). For instance, bloat is a serious digestive disorder, which causes painful suffering or death to animals and also financial losses to farmers. It generally occurs when plants degrade too fast in the rumen; this produces a stable proteinaceous foam that traps fermentation gases, which can no longer be eructed by the animal (Wang *et al.*, 2012). However, plants containing condensed tannins, such as sainfoin, birdsfoot trefoil, crownvetch (*Coronilla varia* L.) and cicer milkvetch (*Astragalus cicer* L.), either as sole feeds or in mixtures with potentially bloat-forming forages, never cause bloat (Mueller-Harvey, 2006).

Phytoremediation potential of wild and domesticated species

The use of green plants as a remediation tool in environmental cleanup has offered some potential, as plants can uptake and bioaccumulate (phytoextraction) as well as immobilize (phytoimmobilisation) certain trace elements enhanced by their rhizospheric processes (Adriano *et al.*, 2004). Phytoextraction-related traits for a plant are the capacity to assimilate and translocate metals and to produce high biomass, traits that are usually difficult to find together in the same plant. According to Moreno-Jiménez *et al.* (2011), Mench *et al.* (2009) and Kidd *et al.* (2009), the ability to survive under the prevailing environmental conditions, depletion of contaminant availability in the soil, native character and a low soil-to-shoot transfer of the contaminants are the most important plant traits for the application of phytostabilisation. Such techniques involve the establishment of a plant cover on the surface of the contaminated sites with the aim of reducing the mobility of contaminants, thereby reducing off-site contamination (Bolan *et al.*, 2011). According to Poschenrieder *et al.* (2001), many of the metal-rich soils in the Mediterranean region occur in small, heavily disturbed areas and do not show a typical metallophyte flora, and such sites usually are colonized by plants that also occur outside the metal-rich habitats. Such pseudometallophytes have attracted less attention than the obligate metallophytes, despite their potential for phytoremediation, due to their adaptation to a wide range of adverse soil conditions, higher biomass production and their ability to be good competitors in soils with moderate metal toxicity. According to Bothe (2011), in Central Europe most heavy-metal soils carry four to six typical metallophytes: (*Viola lutea* ssp. *calaminaria* (Ging.) Nauenb., *Minuartia (Alsine) verna* (L.) Hiern, *Silene vulgaris* (Moench) Garcke, *Armeria maritima* Willd., *A. maritima* ssp. *halleri* (Wallr.) Rothm., *Thlaspi alpestre* (L.) syn. *T. caerulescens* (J. Presl. & C. Presl.), *Thlaspi praecox* Wulfen).

Heavy-metal tolerance has been developed by plants of totally unrelated taxonomic affinities; it is frequent in Brassicaceae, and also seen in Caryophyllaceae, Plumbaginaceae, Violaceae, Asteraceae, Poaceae, and others (Bothe, 2011). With the exception of iron, all heavy metals above a concentration of 0.1% in the soil become toxic to plants and therefore change the community structure of plants in a polluted habitat. However, each plant species has a specific threshold value for each heavy metal. An important issue is the role of rhizosphere and associated microbes in heavy-metal polluted sites. Root exudates influence the structure and function of microbial populations that in turn mediate the various biochemical transformations in the root zone including redox reactions and chemical speciation. Rhizosphere processes play in this way an important role in transformation, mobility and uptake of trace elements (Adriano *et al.*, 2004).

In the Sulcis Iglesiente area (SW Sardinia, Italy), the development of metalliferous mining activities dates back to 3000 BC. Consequently, serious conditions of pollution and environmental hazard are due to diffusion of heavy metal contaminants in soil and water. A negative landscape impact is also due to large areas being devoid of topsoil and vegetation, because of the formation of vast spoil heaps of mining waste. Those mining areas are included in the Sardinian geo-mining, historic and environmental park recognized by Unesco in 1998. Autochthonous populations of pasture legume species were studied by Safronova *et al.* (2012) for tolerance to Zn, Cd and Pb and for the effect of associative and nodule bacteria on growth and nodulation frequency of legumes. The trial was performed on Campo Pisano (Sulcis Iglesiente area) mine waste. A combined inoculation with plant growth-promoting rhizobacteria (PGPR) and nodule bacteria of the legume species *Lotus edulis* L. resulted in synergistic and additive effects on growth, mineral nutrition, heavy metal accumulation and adaptation of such species to unfavourable soil conditions. *Lotus ornithopodioides* L. showed a high translocation capability for Cd and Pb from roots to shoots; the translocation factor (TF) value for Pb was above 1, suggesting that this natural population has a trait considered typical of hyperaccumulators (Safronova *et al.*, 2012). The heavy metal tolerant *Variovorax paradoxus* strain 5C-2 having 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity was isolated in the roots of the metal-accumulating plant Indian mustard (*Brassica juncea* L. (Czern.) grown on mine waste from Campo Pisano (Sulcis Iglesiente area) in Sardinia. This strain is suitable as an inoculant to improve the growth of the metal accumulating plant *B. juncea* in the presence of toxic Cd concentration and for the development of plant-inoculant systems useful for phytoremediation of polluted soils (Belimov *et al.*, 2005). Natural populations of *Hedysarum coronarium* L., *Chrysanthemum coronarium* L., *Lolium rigidum* Gaud., *Lotus ornithopodioides* L. and *Scorpiurus muricatus* L. were suitable to revegetate mine spoils in the Sulcis Iglesiente area (SW Sardinia) due to their tolerance to heavy metals. In particular, *C. coronarium* displayed significantly higher Pb and Zn accumulation than the other species, together with appreciable dry matter (DM) yield, that would make it useful for stabilizing such contaminated areas (Bullitta *et al.*, 2007b).

Among the Mediterranean shrub species tested by Moreno-Jiménez *et al.* (2011), *Retama sphaerocarpa* (L.) Boiss. was the best candidate for phytostabilisation of arsenic-polluted soils.

According to Zornoza *et al.* (2016), the Mediterranean species *Lygeum spartum* L., *Atriplex halimus* L., *Helichrysum stoechas* (L.) Moench., *Dittrichia viscosa* (L.) Greuter, *Piptatherum miliaceum* L. Coss., and *Limonium cossonianum* Kuntze were introduced in acidic mine soils. *P. miliaceum* was the best candidate for phytostabilisation, owing to its lower translocation and bioaccumulation factors, higher biomass, and higher colonization of the area. *Atriplex halimus* seemed a potential candidate for phytoextraction rather than for phytostabilisation of soil Cd, with high translocation and bioaccumulation factors, high biomass, and fast growth.

Heckenroth *et al.* (2016) developed an effective and non-destructive method for the selection of native Mediterranean plants with phytostabilisation potential based on their spontaneous recovery capacities. They indicate the species *Coronilla juncea* L. and *Globularia alypum* L. for phytostabilisation purposes in soils with metal and metalloid mixed contamination. Another environmental issue is the emission of fly ashes enriched with heavy metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn), a worldwide problem deriving from the coal-based thermal power plants. Several species have been studied for their phytoremediation potential on fly ash landfills, among which are *Chenopodium album* L., *Cynodon dactylon* (L.) Pers., *Typha latifolia* L., the latter considered more promising (Pandey, 2012).

An issue related to phytoextraction that has not been extensively addressed is the disposal of contaminated plant material after phytoextraction. Sas-Nowosielska *et al.* (2004) and Abilash and Yunus (2011) consider disposal of crops after phytoextraction an unsolved problem. A suggested use of biomass after phytoextraction is the disposal at an energy plant for energy recovery, provided that appropriate filters for the reduction of emissions of heavy metals into the atmosphere are available. Also suggested are the disposal for waste fermentation and production of biogas, or as sources of raw materials for industry. In the absence of suitable re-use of contaminated material, an alternative is landfill, after pre-treatment such as composting, compaction or pyrolisis.

According to Lasat (2000), progress in phytoremediation has been precluded by limited knowledge of basic plant remedial mechanisms and, in addition, by the poor understanding of the effect of agronomic practices on these mechanisms. Another limitation lies within the biological nature, as potential for phytoremediation depends upon the interaction among soil, contaminants, microbes and plants. This complex interaction, affected by a variety of factors suggests site-specific phytoremediation practices. More recently, Bothe (2011) stated that phytoremediation appears to be an attractive field that is in its infancy due to its complexity, and up to the present, remediation of soils polluted by heavy metals is governed mainly by chemists, and biologically based applications await future directives.

Biomass for energy and bio-based products from grasslands

In Europe, the research on energy crops has been mainly focused on conventional species such as miscanthus (*Miscanthus* spp.), switchgrass (*Panicum virgatum* L.), reed canary grass *(Phalaris arundinacea* L), giant reed (*Arundo donax* L.) and woody species. However, in some areas of central Europe, changes in livestock production systems have led to land-use abandonment, especially of semi-natural grassland (Rösch *et al.*, 2009). The generation of energy from biomass of extensive, high-diversity grasslands has been claimed as an alternative to their abandonment (Prochnow *et al.*, 2013). Anaerobic digestion is one possible method for converting grassland biomass into energy and a new technology for the conversion of biomass to energy was developed (Wachendorf *et al.*, 2009). This procedure separates the ensiled biomass into a liquid phase for biogas production and into a solid fraction to be used as fuel, taking into account the negative impact of high fibre concentrations on anaerobic digestion and the detrimental influence of high mineral and N concentrations on combustion (Prochnow *et al.*, 2009a,b). Moreover, changes in the herbage chemical constituents that are relevant for combustion and methane production were also investigated across different combinations of plant functional groups (Khalsa *et al.*, 2014; Melts *et al.*, 2014).

Green bio-refineries are multiproduct systems, which utilize green biomass as an abundant and versatile raw material to convert biomass feedstock into a range of fractions and products with near zero CO_2 emissions (Mandl, 2010; Sharma *et al.*, 2011). Green refinery can use whole green crops (i.e. different kinds of grass, clover, lucerne) to generate chemicals, materials, as well as energy or fuels. Hence, value added products can be produced sustainably from a simple and widespread raw material, which is becoming a surplus resource. Instead of using fresh grass, the Austrian green bio-refinery system is based on grass silage to ensure a decentralized and seasonally independent feedstock system (Ecker *et al.*, 2012). Lactic acid and the mixture of free amino acids, both generated during the ensiling process of grass, are key compounds, whose utilization fields comprise fodder, food beverage and cosmetic applications.

Moreover, the plant also produces biogas and organic fertilizer (Mandl, 2010). Pilot scale processing trials in Austria, Denmark, Switzerland and Germany have produced lactic acid, amino acids, leaf juice protein concentrate, fibre products and biogas by setting up integrated refineries (Mandl, 2010). Therefore, the multifunctional utilization of silage/hay/forage from grassland offers an opportunity to produce chemicals from biomass in an ecological and sustainable way, and can represent relevant opportunities with economic potential.

Novel plant species have been recognized in Europe as high-yielding and promising bioenergy species. In fact, the tall perennials Virginia fanpetals (*Sida hermaphrodita* L. Rusby) and rosinweed or cup plant (*Silphium perfoliatum* L.), native to the Eastern USA are being evaluated mainly due to their high productivity, excellent material quality and presumably low invasive potentials (Franzaring *et al.*, 2015; Gansberger *et al.*, 2015). The green shoot mass of *S. perfoliatum* is harvested in late summer and brought to biogas plants in Germany. The dry stems of *S. hermaphrodita* are harvested on cold and dry winter days to be used as a solid fuel in biomass cogeneration plants in Poland. The interest in novel species, originally evaluated for forage aims, also testifies to the increasing trend in the sector of bioenergy.

Regarding the performance of conventional bioenergy species, the estimates of mischanthus yields for Southern European countries, are in the range 13.4-22.7 Mg ha^{-1}, when the crop is rainfed (Zub and Brancourt-Hulmel, 2010), but also indicating that higher yields can be obtained only with irrigation supply. As regard switchgrass, a study carried out in two dry Mediterranean sites of Italy and Greece showed that the best performing lowland varieties of switchgrass reached about 20 Mg ha^{-1} of DM when an additional 250-400 mm of water were applied (Alexopoulou *et al.*, 2008). Similar information was also reported by Cosentino *et al.* (2007), with evidence that conventional bioenergy species need irrigation to achieve high dry matter yields in Mediterranean areas.

Native Mediterranean perennial grasses, which are able to face summer drought under rainfed regime, and have low input requirements, adequate productivity and ability to grow on marginal lands, may represent a suitable feedstock for bioenergy production and bio-based products. Perennial grasses are more promising candidates, compared to annuals, for biomass production, especially in marginal land. Nonetheless, native germplasm of Mediterranean perennial grasses is still little investigated for forage purposes and almost unexplored in terms of bioenergy aims. However, relevant research is currently in progress to increase knowledge on autochthonous and still undomesticated Mediterranean perennial grasses from native flora that could also support multiple uses (fodder, bioenergy, etc.). There are interesting examples of species that might be easily introduced into the Mediterranean cropping systems.

Autochthonous populations of smilo grass (*Piptatherum miliaceum* (L.) Coss.) are being evaluated for maturity, biometric traits, biomass production and quality in Sardinia (Italy).

They differ for flowering date, biometric traits, yield and biomass quality traits (Sulas *et al.*, 2015), out-yielding the reference species (tall fescue). A significant correlation was found between dry matter yield and number of tillers per plant. Smilo grass has also a wide range of ethnobotanical utilizations across Mediterranean basin.

Saccharum spontaneum L. spp. *aegyptiacum* (Willd.) Hack. (African fodder cane) is a perennial, herbaceous, rhizomatous grass, widespread in South Mediterranean regions. It is under investigation for biomass yield, water use efficiency, and biomass quality under different soil water availability, as a lignocellulosic energy crop in semi-arid Mediterranean area (Cosentino *et al.*, 2015). This species possesses a range of agronomically desirable traits such as high biomass yield (22 to 27 Mg ha^{-1} under rainfed condition), active assimilation rates during drought-stress periods, efficient water use, and satisfactory biomass quality.

Phalaris aquatica L. is a perennial cool season herbaceous plant, native to the Mediterranean area, which has been used for forage breeding programmes in Greece due to its high biomass yield potential and drought tolerance (Pappas *et al.*, 2010). *Phalaris aquatica* is also an alternative perennial feedstock for second generation bioethanol production; moderate acid pretreatment followed by enzymatic hydrolysis proved to be an effective biochemical method to release sugar monomers (Karapatsia *et al.*, 2017). In the framework of the Optima project, other native perennial grasses namely *Ampelodesmos mauritanica* Th. Dur. & Schinz, *Hyparrhenia hirta* (L.) Stapf, *Cymbopogon hirtus* L. Janchen, *Lygeum spartum* L., and *Sorghum halepense* L. (Pers.) are currently under investigation for the same purposes

In addition to the above-mentioned native perennial grasses, other Mediterranean species may be considered. A recent paper deals with the potential of sulla as a feedstock for bioenergy and protein (Amato *et al.*, 2016). Authors evaluate the potential value for energy production of sulla, under rainfed conditions, in terms of theoretical ethanol and predicted biomethane potential yields (about 350 l Mg^{-1} and 280 L CH_4 kg^{-1} DM, respectively). They also pointed out that sulla requires very low-energy inputs and the use of traditional agricultural equipment and offers, in meantime, great agronomic and environmental benefits. On the other hand, cardoon (*Cynara cardunculus* L. *var. altilis* DC), which is an emerging species for bioenergy and bio-based products (Deligios *et al.*, 2017), well adapted to Mediterranean rainfed conditions, has also potential as a forage species.

Grassland wildfire: land management and land resilience

Out of 1,053,000 km^2 of grasslands in the EU, 19.3% are represented by wood-pastures and the largest extent of wood-pastures is found in Spain, France and Romania (Plieniger *et al.*, 2015). Grasslands are of great importance for the quality of life on earth. They provide various goods and services, such as food and fibre, carbon sequestration, genetic diversity, recreational areas and heritage values (Glasser and Hadar, 2014). Plieniger *et al.* (2015) estimate that in Europe in the 28 EU member states wood pastures cover approximately 203,000 km^2 (about 4.7% of total Europe); 109,000 km^2 being pastures with sparse trees, 85,000 km^2 pastures in open woodlands and 9,000 km^2 pastures with cultivated trees, mainly grazed olive groves and fruit trees. Pastures in open woodlands with sparse trees are important in Spain and Portugal, called dehesas and montado, where holm oak (*Quercus ilex* L.) and cork oak (*Quercus suber* L.) are present. Although rangelands and woodlands in Europe have an exceptional ecological value for their contribution to landscape-level biodiversity and for their role as repositories of genetic resources (Plieniger *et al.*, 2015), an average of about 65,000 fires occur every year, burning approximately half a million hectares of vegetated areas. Moreover, more than 85% of the area affected by fires is in Mediterranean countries (southern France, Greece, Italy, Portugal and Spain) (San-Miguel-Ayanz and Camia, 2010). Fires can become uncontrolled and cause large environmental and economic damage. Human activities are the main cause of forest fires and are responsible for over 97% of all European fires (Ganteaume *et al.*, 2013). Riva *et al.* (2016) describe the factors that influence the resilience of forests to fires. They focused on impacts of land management to forest resilience and several other factors that can increase resilience and improve the post-fire regeneration. Firstly, grazing and wood-gathering activities (that were part of forest use for centuries) reduce the amount of available fuel in forests, and have represented an important factor in the changing of fire regimes of many Mediterranean countries. Firebreaks are important tools and most common management practice in regional and national programmes to reduce spread and risk of fire. Riva *et al.* (2016) define them as linear features within the forest where the vegetation has been removed and which are placed in relation to the slope, location of road, wind direction, important not only against fire spread but also to provide access and increase safety for firemen, to protect roads and areas of special value.

In continental climate regions such as the southern slopes of the Alps, fire regimes differ from those of the Mediterranean area. The main fire season occurs in winter, fires are mainly orographically driven and very often human-ignited (Rizzolo, 2016). Although forest fire danger is often underestimated and less studied than in Mediterranean regions and other countries, as in Italy, France, Greece and Portugal, wildfires in the Alps can have severe impacts on mountain forests affecting their protection capacity against rock falls and avalanches and increasing flood runoff, mud and debris flows (Moody and Martin 2001; Robichaud *et al.*, 2007). Accordance to Arpaci *et al.* (2014), the Alpine environment is highly diverse in terms of climate, geology and topography, which lead to different levels of susceptibility to fires. Riva *et al.* (2016) reported post-fire regeneration from the seed bank as a forest revegetation mechanism. According to Sanna *et al.* (2016), seed bank ecology allows a substantial flexibility for potential changes in the plant community and can provide important information on the dynamics of vegetation and its persistence. Sanna *et al.* (2016) emphasize that an appropriate and site-specific management may represent a strategy for the conservation of resilient and biodiverse grasslands within Mediterranean oak-based silvopastoral systems. The same authors reported in Sardinia a potential number up to 28,264 seedlings m^{-2} of ready germinable seedbank, and up to 6,436 seedlings m^{-2} of permanent seedbank in grazed areas and found average grasses abundance; similarly reported by Koc *et al.* (2013) and Iannucci (2014). Grazing is probably the most ecologically sound technique for creating discontinuities in fuels, mainly at the shrubby layer and disrupting fuel ladders (Lovreglio *et al.*, 2014). Moreover, grazing can directly reduce the frequency and intensity of fire by removing fine fuel, and indirectly by causing a shift in plant community composition to less-productive and more-ephemeral species (Fuhlendorf *et al.*, 2008). In Europe the first experiment of controlled grazing for a brush clearing action dates to the 1980s in France, where the Forestry Services of Gard wanted to clear a space to serve as a fire-break. A local goat-breeder arranged to clear the area using his goat herd, at considerably lower price than a traditional land-clearing team. Further experiments were carried out in Languedoc-Roussillon region (1985-2005) on about 27,000 hectares. In Spain, farmers take part in wildfire prevention programs letting their livestock graze intensively in the fuel break areas defined by Forest Services, with an appropriate stocking rate; in exchange they receive money and/or in-kind remuneration (Ruiz-Mirazo and Robles, 2012). In Italy, Talamucci *et al.*, (1997), refer on the possibility of maintaining a flock grazing in a system based on native pasture, subterranean clover on strips thinned out and improved firebreaks lines, to balance seasonal distribution of forage and contributing to reduce fire hazards and flame diffusion speed. Grazing enhances biodiversity and positively affected ecological (Shannon index) and agronomic value (Huntsinger, 2016; Re *et al.*, 2014b) of the pasture vegetation. The same authors describe positive effects of moderate to heavy grazing on biodiversity. On the other hand, grazing effects on biodiversity can vary widely depending on conditions, such as invasion of certain plant species, grazing history, site productivity, plant palatability and plant regeneration requirements (Lunt, 2005).

Conclusions

Several of the grassland species we mentioned are not only of potential use, but actually do have multipurpose uses to be added to the ecosystem services and socio-economic benefits traditionally recognised as applying to grasslands. It is undoubtedly wise, therefore, to take into account the available ethno-botany and agronomic studies especially for Mediterranean species, traditionally known in the past and nowadays recovered for novel uses. According to the literature overview, the appropriate management of vegetation components that could act as fuel in case of wildfires is one of the main tools for controlling wildfires and to protect grassland and rangeland biodiversity. Given the plant biodiversity values of grassland habitats and species, the effective conservation and sustainable use of grassland biodiversity will also ensure the non-food supply of suitable species for the fulfilling of new emerging demands for bioenergy and bio-based products, for environmental remediation, for new natural therapeutic products.

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Ecological and social perspectives of European wooded grasslands

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Abstract

Wooded grasslands, also termed wood-pastures or silvopastures, are archetypes of multifunctional land-use systems in Europe and hold exceptional ecological, social, and cultural values. Yet, wooded grasslands have been through a sharp decline all over Europe, mainly due to processes of agricultural intensification and land abandonment. Recently, wooded grasslands have found increasing attention from conservation science and policy across Europe. This keynote paper synthesizes insights from recent efforts in the European AGFORWARD and other research projects to quantify and map the distribution, threats and changes as well as the ecological and socio-cultural values of a variety of wooded grassland systems in Europe. Substantial ecological values are revealed in terms of landscape level biodiversity, ecosystem dynamics, and genetic resources. Socio-cultural values are related to aesthetic values, cultural heritage, and rich traditional ecological knowledge. The paper highlights some of the agricultural and conservation policy challenges around wooded grasslands and concludes that research should be guided by a holistic vision that integrates information about ecology, societal values, and institutional arrangements.

Keywords: silvopastoral systems, wood-pastures, multifunctional land use, social-ecological systems, ecosystem services, land-use change

Introduction

Wooded grasslands – grassland systems in which livestock grazing co-occurs with scattered trees and shrubs – are archetypes of multifunctional land-use systems in Europe and hold exceptional ecological, social, and cultural values (Bergmeier *et al.*, 2010). They represent an important part of the European cultural and natural heritage, but are also mirrors of dramatic changes in the relationship between people and their natural environment. The existence of veteran trees in wooded grasslands whose age sometimes exceeds 500 years reflects long-term stability of land management practices. Wooded grasslands occur in all major European biogeographic regions, including dehesas and montados on the Iberian Peninsula, wood pastures of Eastern Europe, and orchard meadows in Central Europe (Figure 1).

This paper conceptualises wooded grasslands in Europe as social-ecological systems by emphasising the links between humans and their environment through time and space, which is subject to both ecological and social dynamics (Plieninger and Bieling, 2012). Despite the importance of social processes in shaping ecological dynamics, theory and practice of natural resource management often still treat social and ecological dynamics separately. This separation is an obstacle to sustainable management and to understanding rural landscapes in Europe, which are socially and ecologically integrated. The extraordinary biodiversity of European wooded grasslands has been shaped by a long-lasting and complex history of human uses, so that their social and ecological values are intimately connected (Hartel and Plieninger, 2014). A social-ecological perspective therefore is suitable to integrate perspectives from both the natural and social sciences, to understand how ecosystems have been shaped by humans in the past, and how they may be governed in the future.

This keynote paper synthesizes insights from recent efforts in the European AGFORWARD (www.agforward.eu) and related research projects (1) to quantify and map the distribution of wooded

Figure 1. Examples of Mediterranean oak dehesas (left), wood-pastures in Eastern Transylvania, Romania (right), and orchard meadows in Southern Germany (bottom).

grasslands, (2) to identify the threats and social and ecological drivers of wooded grassland losses, and (3) to assess the ecosystem services of wooded grassland systems in Europe. It mainly builds on information from Hartel *et al.* (2015), Plieninger *et al.* (2015), Fagerholm *et al.* (2016) and Torralba *et al.* (2016).

Distribution of wooded grasslands across the EU

Combining spatial information from the Pan-European LUCAS survey, we estimate that wooded grasslands cover a total of approximately 203,000 km^2 in the EU27 (4.7%, Figure 2), with roughly 109,000 km^2 being grasslands with sparse trees, 85,000 km^2 grasslands in open woodlands, and 9,000 km^2 grasslands with cultivated trees (mainly grazed olive groves and fruit trees). Out of 1,053,000 km^2 of grasslands in the EU, 19.3% are wooded grasslands. The largest extent of wooded grasslands is found in Spain, France, and Romania. Grasslands with sparse trees have their largest surface in the Mediterranean (Spain, France, Italy) and Eastern European countries (Romania, Bulgaria). Grasslands in open woodlands are particularly concentrated in Spain and Portugal, where they occur mainly as holm oak (*Quercus rotundifolia*) and cork oak (*Quercus suber*) dehesas and montados. Grasslands with

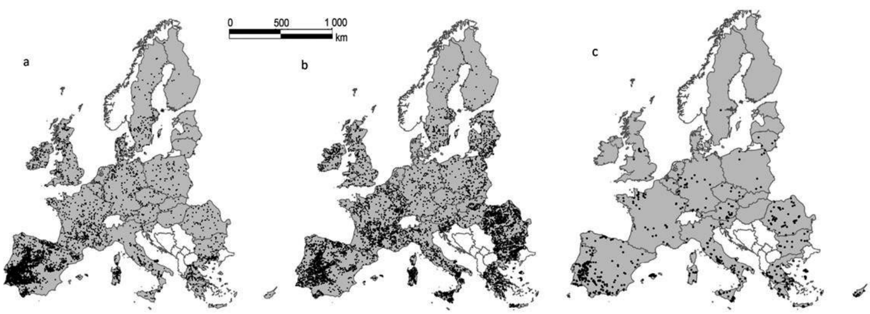

Figure 2. Distribution of wooded grassland systems across Europe. (a) Grasslands in open woodland, (b) Grasslands with sparse trees, (c) Grasslands with cultivated trees (Plieninger *et al.*, 2015).

cultivated trees are found across the Mediterranean, with the highest extent being found in Spain, Greece, Portugal, and Italy. Wood-pastures cover 10.8% of the Mediterranean biogeographical region, 5.6% of the Black Sea region, and 4.7% of the Alpine region (Plieninger *et al.*, 2015).

Threats to wooded grasslands

Despite growing recognition of their unique values, the future of wooded grasslands remains threatened, and the consequences of their reduced capacity to deliver important ecosystem services are poorly understood. Today, most wooded grassland systems are under intense pressure from socioeconomic and environmental changes. Direct causes contributing to their degradation include legal or illegal clearing of trees, a lack of tree regeneration, shrub encroachment (which, in Mediterranean Europe is often related to increased wildfire hazards) and abandonment of traditional grazing regimes, agricultural intensification, and urbanisation (Bergmeier *et al.,* 2010; Hartel *et al.*, 2015). Indirect causes, such as changes in the institutional arrangements or economic conditions, have further underpinned this decline. Generally, the financial revenues of wooded grassland systems are lower than those of other agricultural systems. Silvopastoral practices do not lend themselves to intensification, and most wooded grasslands occupy areas only marginally suitable for agriculture. Broadening and deepening farm activities like tourism and recreation is a more viable development alternative in many landscapes, but this, too, is only feasible in some situations. Moreover, these kinds of activities do not per se the maintenance of wooded grasslands. Maintaining wooded grasslands therefore increasingly depends on European or national income support schemes, and specifically on payments for biodiversity and ecosystem services (Bugalho *et al.*, 2011). Here, I point particularly to management changes, policy mismatches, tree decline, and lack of regeneration as major threats to wooded grasslands (Hartel *et al.*, 2015).

Management changes

Management changes can be grouped under the headings of land-use intensification, land abandonment, and the conversion into other land cover forms. Land-use intensification typically involves the increase of grazing livestock density alongside the removal of woody vegetation and the use of chemical fertilizers. The overall result is a grassland with low biodiversity value and potentially low resilience, especially towards climatic variations. The decrease of grazing intensity, or its abandonment, results in shrub expansion and reforestation of wooded grasslands. Land conversion may be to built-up areas, infrastructure such as roads, or conversion to arable land. Any woody vegetation is usually removed in the process. While the immediate driving force for these changes is local economics, often they are encouraged by national and regional policies.

Policy mismatches

Through much of the late 20th century agricultural and rural development policies supported the destruction of many wooded grasslands in Europe. Public infrastructure programmes converted thousands of hectares of Spanish and Portuguese cork oak and holm oak wooded grasslands into irrigated land, eucalyptus plantations, artificial water bodies, or industrial units. From the 1950s onwards, public agricultural policies in Southern Germany provided landowners with grants for clearing scattered fruit trees. Even today state forest services seek to abolish the grazing rights in the mountain forests of the Alps. Even when there are public incentives for wooded grassland conservation, for example through agri-environmental schemes, land managers may be reluctant to participate in them. An analysis of schemes for wooded grasslands and other farm woodlands in the German state of Saxony, identified high production costs and opportunity costs for land use, contractual uncertainties, land-tenure implications and variable societal preferences for ecosystem services of wooded grasslands as obstacles to scheme uptake (Schleyer and Plieninger, 2011). The multi-functional nature of wooded grasslands is extremely difficult to manage under institutional structures that are organised as single land-use sectors. Under the Common Agriculture Policy there are restrictions on agricultural support for wooded grasslands as some regulations consider them to be forests rather than pastures (Beaufoy, 2014) and forest policy falls within the competence of the member states, not under the European Union. Yet, although these sectors are concerned with wooded grasslands at various administrative levels, a lack of integration between them pose major challenges to the design of effective mechanisms to safeguard wooded grasslands.

Decline of old, hollowing or dying trees

Worldwide the value of the large number of old, hollow trees found in most wood pastures for their important role as keystone structures in ecosystems has only recently been recognized (Lindenmayer *et al.*, 2014). Their cultural value is also increasingly emphasized (Blicharska and Mikusinski, 2014). These trees are in sharp decline due to human-related factors such as cutting, reforestation, and uncontrolled pasture burning and land abandonment. High losses of these trees often go unnoticed, especially in the traditional rural landscapes of Europe. For example, in central Romania many ancient oaks collapsed due to severe, uncontrolled burning applied to wooded grasslands in 2012 (Hartel *et al.*, 2013).

Lack of regeneration

Intensive grazing, often linked with shrub removal is detrimental to tree saplings, causing lack of regeneration in wooded grasslands. This failure of tree regeneration has however also been noticed in traditionally managed wooded grasslands. It threatens the future continuity of the veteran tree populations.

Rates and social-ecological drivers of change

A systematic review of 14 studies quantifying spatial-temporal changes of the most significant European wooded grassland systems – the dehesas and montados of Iberia – shows a consistent decline of density and cover of scattered trees on pasturelands over the past five decades. Average loss rate was 0.33% of initial area per year. However, the synthesis illustrates great heterogeneity, with mean net change rates ranging between -2.07% per year (area loss) and 2.11% per year (area gain). Loss rates have increased from the late 1980s onwards (Figure 3). The review reveals that the primary causes for this decline result from the interplay of technological factors (e.g. mechanisation) that mainly impact the understory layer. These factors have particular impact on tree regeneration, also in combination with shrub encroachment and increasing livestock pressure. Wooded grassland landscapes could become severely degraded if all trends of their recent dynamics should be maintained (Costa *et al.*, 2014).

Ecosystem services of wooded grassland systems

In European wooded grasslands multiple ecosystem services have traditionally co-occurred: Wooded grasslands are multifunctional by definition. The variety of provisioning ecosystem services of wooded grasslands comprises food for humans and animals, firewood, charcoal, gums, resins, dyes, pharmaceuticals, cork, and aromatic plants. Moreover, wooded grasslands provide many less tangible

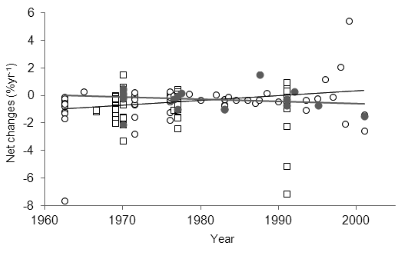

Figure 3. Trends for net change rates (% per year) of dehesa and montado landscapes in Iberia. Open circles: *Quercus suber* dehesas and montados, Grey circles: *Quercus rotundifolia* dehesas and montados, White squares: mixed dehesas and montados. Lines represent linear net change trends for cork oak (black line) and holm oak (grey line) wooded grasslands (Costa *et al.*, 2014).

regulating and cultural ecosystem services, such as soil protection, stabilization of soils, microclimate amelioration, reduction of water runoff in hilly areas, maintenance of landscape beauty, cultural heritage and recreation. Throughout Europe, wooded grasslands are known to be local-scale biodiversity hotspots, although they differ vastly in their land-use history (Bergmeier *et al.*, 2010).

Ecological valuation

To upscale existing case-study insights on the biophysical provision of ecosystem services from wooded grasslands, the European AGFORWARD project conducted a meta-analysis on the effects of wooded grasslands and wooded croplands (jointly termed agroforestry) on ecosystem service provision and on biodiversity levels. A total of 365 comparisons was extracted from 53 publications and selected for the meta-analysis. Based on these comparisons, we calculated response ratio, which is an unweighted index widely used for meta-analysis in ecology when primary studies differ in the indicators and methods used. To quantify the overall effect of agroforestry on ecosystem service provision and biodiversity, effect sizes were used as dependent variables to construct a random-effect model (effect sizes nested within studies) (see Torralba *et al.*, 2016 for further details). Results revealed an overall positive effect of wooded grassland and cropland systems (effect size = 0.454, $P<0.01$) over conventional agriculture and forestry. However, results were heterogeneous, with differences among the types of agroforestry practices and ecosystem services assessed. Erosion control, biodiversity, and soil fertility are enhanced by wooded grasslands (Figure 4). The effect on biomass production is negative. Comparisons between wooded grassland types and reference land-uses showed that wooded grassland systems increase ecosystem service provision and biodiversity, especially when compared with forestry land (Torralba *et al.*, 2016).

Socio-cultural valuation

To assess how people perceive ecosystem services, AGFORWARD, developed a Public Participation GIS (PPGIS) approach. PPGIS is a relatively new field of research that evaluates the spatial distribution of ecosystem services according to the perceptions and knowledge of stakeholders. As a complement to expert-driven ecosystem services mapping and modelling, PPGIS allows for the participation of various stakeholders in the creation of an ecosystem services map (e.g. community members, environmental professionals, NGO representatives, decision-makers) and integrates their perceptions, knowledge and values into ecosystem services assessments (Brown and Fagerholm, 2015). In our approach, we engaged the general public (2139 respondents in total) to identify and map a range of ecosystem services from 13 wooded grassland (and other agroforestry) landscapes in the EU (Figure 5). Outdoor activities were

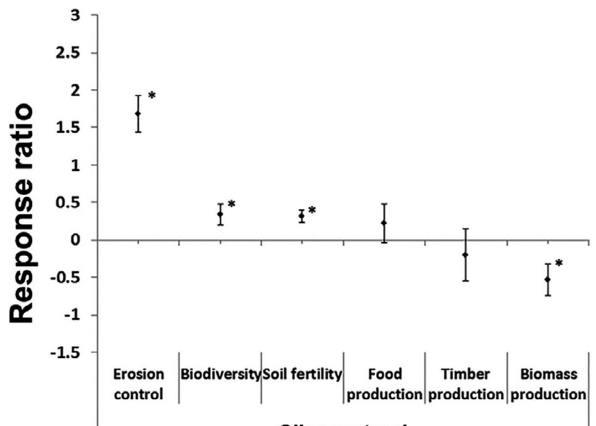

Figure 4. Mean effect size (response ratios) of wooded grasslands on different ecosystem services, compared to alternative land-use systems. * Effect sizes differed significantly from zero ($P<0.05$) (Torralba *et al.*, 2016).

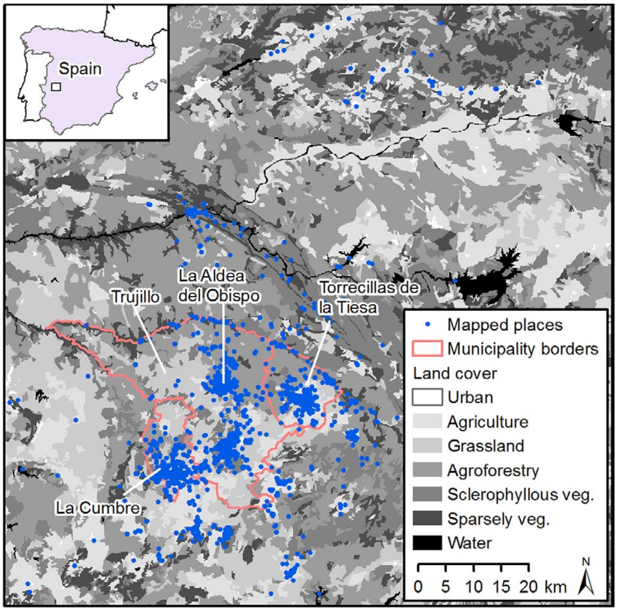

Figure 5. Example for the socio-cultural valuation of ecosystem services, as mapped through a Public Participation GIS approach in a Spanish wooded grassland site (Fagerholm *et al.*, 2016).

clearly the most mapped ecosystem services (attributed to 17.1% of all mapped places), followed by aesthetic values (13.1%) and sites for social interaction (12.9%). A mosaic of landscape types (i.e. the landscape) provided more ecosystem services (especially cultural and provisioning) to people compared to individual wooded grasslands. However, land tenure and public access significantly guided the spatial practices and values of the people beyond the preferred landscape types (Fagerholm *et al.*, 2016, Fagerholm, unpublished data).

Conclusions

Given that the social and ecological values of wooded grasslands are the result of a long-lasting and complex interaction between humans and nature, a broad, interdisciplinary research agenda is needed to provide solutions for the sustainable conservation of wooded grasslands. Therefore, research should be guided by a holistic vision which integrates information about ecology, societal values, and governance. Grassland and other ecological science could provide information on biodiversity, patterns in species distribution and abundance, and the ecological processes underlying these patterns, the keystone structures for biodiversity, and the status of and main threats to wooded grasslands. In many European countries there is little large-scale spatial and process-based monitoring of wooded grasslands. Data are lacking on surface area, species composition, animal density and herding seasonality, tree age structure and rejuvenation, tenure, and current and past land use. This is the evidence needed to develop policies to protect and maintain wooded grasslands. A second research dimension would identify the societal value of wooded grasslands, including their ecological and socio-cultural values. The knowledge generated by ecosystem service research can be a powerful tool in developing contextual policies for wooded grasslands, because it gives insights into the societal relevance of these systems under various bioclimatic, social-cultural and economic settings. A third research dimension would address the institutional arrangements which govern wooded grasslands. Wooded grasslands form heterogeneous landscapes with elements of woody vegetation and open areas, and a varied institutional framework to match. The future of wooded grasslands depends on the ability of these various institutional arrangements to form a common vision and to show the flexibility to implement such a vision. Research could support such visioning by facilitating an understanding of the nature of these potential institutional barriers and of the kinds of innovative changes that could be adopted in order to maintain the ecological and social-cultural values of wooded grasslands.

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Spatial and temporal influence of oilseed-rape and semi-natural habitats on wild bees in permanent grasslands

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Abstract

Wild bees need continuous food resources throughout the season as found in semi-natural habitats (SNH). Oilseed rape (OSR) fields provide a short but rewarding food resource used by some species. The aim of this study was to understand the effects of landscape area covered by OSR (%OSR) and SNH (%SNH) on species richness and abundance of three different sociality functional groups captured in permanent grasslands. We found that in SNH-rich landscape, %SNH and %OSR significantly impacted bee abundance but not species richness. Responses greatly depended on bee sociality: %SNH enhanced eusocial bee abundance in the late season, but reduced other social bee abundance suggesting a dilution effect. The %OSR had a global positive effect on eusocial species but a negative effect on other social bees in permanent grasslands, illustrating a competition between OSR and SNH for other social bees. Our results highlighted the relevance of temporal approaches.

Keywords: bees, sociality, semi-natural habitats, oilseed rape, temporal effects

Introduction

Wild bees (*Hymenoptera*: *Apoidea*: *Anthophila*) are major pollinators of wild plants and crops. One of the causes of wild bee decline is the loss of beneficial habitats such as semi-natural habitats (hereafter called SNH). SNH (including hedgerows, orchard meadows, permanent grasslands and woodlots), are essential for bees because they provide refuge and food resources during the entire season. However, the SNH surface area has been decreasing across Europe (Cousins, 2009) and therefore the potential effects on wild bee conservation and the service they provide are alarming (Winfree *et al.*, 2009). Oilseed rape (*Brassica napus*, hereafter called OSR), a common crop in European cropping systems, has been suggested as possibly able to counteract bee decline in agroecosystems, providing a highly rewarding food resource during the flowering period (Westphal *et al.*, 2003). However, little is known about the temporal influence of OSR on bees in SNH such as permanent grasslands. Many wild bees are attracted by OSR flowering, but this attraction has been found to depend on bee sociality (Rollin *et al.*, 2013). Eusocial species (i.e. highly social, represented here by bumblebees) and other social species (*Halictidae* family) need to collect high amounts of nectar and pollen for the colony while solitary bees do not. The aim of this study was to understand the effects of the proportion of OSR (%OSR) and SNH (%SNH) in the landscape on wild bees in permanent grasslands, regarding species richness and abundance of three different sociality functional groups. More specifically, we studied the temporal evolution of the effects throughout the season: during OSR flowering (April and May) versus after (June to September).

Materials and methods

Field work

The study was carried out in 2015 in the county of Moselle, Lorraine, France. The region mainly comprises forest (33.7%), annual crops (28.7%) and permanent grasslands (21.9%). We selected twenty mown and little-fertilized permanent grasslands according to a gradient of %OSR. For each plot, we quantified the surrounding landscape in a circle of 1 km radius, by implementing field surveys. The %SNH ranged from 17.3 to 58.3% and %OSR ranged from 0 to 17.6%. Bees were captured with fifteen pan traps left active during 48h once every month from April to September. Overall, 1,800 pan trap contents were analysed. To evaluate local floristic and nesting opportunities for bees in each plot, we estimated visually the percentage of bare ground (%bare ground) and assessed the flowering plant species richness in ten 1 m^2 quadrats (flowering plant species richness).

Data analysis

We assessed the effects of %SNH and %OSR on abundance and species richness of each group of sociality, using generalized linear models with Poisson distribution. Firstly, models were realised across the season. Secondly, models were built for two periods (during OSR flowering and after) because bees may have contrasted responses towards %SNH and %OSR throughout the season, not observed in across season analysis. All data were aggregated at the plot level for each capture. Explanatory variables included in each model were %SNH, %OSR, flowering plant species richness, %bare ground, date of capture (Julian day), Julian day squared, the interaction between flowering plant species richness and %bare ground and the interaction between %SNH and %OSR.

Results and discussion

We collected 2,600 wild bee individuals of 88 species during the entire season. Parasitic species (34 individuals; 10 species) were removed from further analysis because of their low abundance. Of the remaining individuals 96.8% were polylectic.

No signs of correlation were found between local and landscape variables ($|r_{pearson}| \leq 0.21$ in all cases). Furthermore, there were no spatial autocorrelation pattern for both species richness and abundance of eusocial, other social and solitary bees (P>0.16 for all Mantel tests).

Across season, %OSR positively enhanced eusocial bee abundance but not species richness (Table 1). The %SNH and %OSR had a positive effect on eusocial bees abundance only after OSR flowering. This result highlighted the importance of SNH to provide food resources in the late season for eusocial bees. Furthermore, our results on %OSR effects were partially consistent with previous studies which observed a negative effect of %OSR on eusocial bee abundance in SNH during OSR flowering (Holzschuh *et al.*, 2011; Kovács-Hostyánszki *et al.*, 2013) and a positive effect after OSR flowering (Kovács-Hostyánszki *et al.*, 2013). After OSR flowering, eusocial bees may benefit from the food resources collected and stored during OSR flowering.

Table 1. Results of across-season models on abundance and species richness of three bee sociality functional groups.

		df	Abundance						Species richness					
			Eusocial		Other social		Solitary		Eusocial		Other social		Solitary	
			LR	Effect	LR	Effect	LR	Effect	LR	Effect	LR	Effect	LR	Effect
Across season	OSR	1	2.5	+ **	221.4	– ***	0.3		0		6.1		2.1	
	SNH	1	0.3		46.7	– ***	19.8		0.8		4.3		6.5	
	OSR:SNH	1	5.6	– *	0.5		2.3		1.2		0		0	
During OSR flowering	OSR	1	0.5		124.7	– ***	0		0.3		1.1		1.1	
	SNH	1	0.4		1.6	– **	0.5		0		0.1		0	
	OSR:SNH	1	0.5		9.3	+ **	0.0		0.8		0.2		0.3	
After OSR flowering	OSR	1	2.5	+ ***	85.7	– **	2.1	+ *	0	+	3.1		0.4	
	SNH	1	0.2	+ *	22.9	– ***	11.6		0.7		3.4		6.5	
	OSR:SNH	1	8.6	– **	0.9		4.3	– *	3.0	–	0		0.1	

[1] OSR = % oilseed rape in a 1 km radius; SNH = % semi-natural habitats in a 1 km radius. LR = Likelihood-Ratio Chi-square. * P<0.05; ** P<0.01; *** P<0.001.

Across season, %OSR and %SNH negatively impacted on other social bee abundance but not species richness (Table 1), illustrating a clear competition for bees between OSR and SNH plots. Similarly, %OSR and %SNH had a negative effect on other social bee abundance in permanent grasslands but not species richness, during and after OSR flowering. This suggested that OSR fields attracted social bees during OSR flowering, but did not contribute to enhance abundance in permanent grasslands after OSR flowering as for eusocial species. Agricultural practices in OSR may explain this result but were not studied here. Notably, the benefit of the mass rewarding resource may be outweighed by pesticides exposure in OSR fields. Consistently, Winfree *et al.* (2009) found that social bees were more sensitive to disturbances (including pesticides) compared to solitary bees. Furthermore, the global negative effect of %SNH on abundance may illustrate a dilution effect suggesting that in SNH-rich landscapes, SNH plots may compete with each other for other social bees.

After OSR flowering, solitary bee abundance but not species richness was enhanced by high %OSR values (Table 1). The abundance of these bee groups was found to be unrelated to %OSR (Holzschuh *et al.*, 2011; Kovács-Hostyánszki *et al.*, 2013). Since solitary bees seem to be little attracted by OSR fields, especially after OSR flowering, they may therefore concentrate in other resourceful habitats such as permanent grasslands when %OSR is high (positive effect of %OSR). The %SNH had no effect on solitary bee abundance or species richness, potentially because they may find all their food requirements locally in the permanent grassland or in close vicinity.

Conclusions

Our results highlight the relevance of temporal approaches. We found that in SNH-rich landscapes (%SNH >17%), %SNH had contrasting effects on abundance, depending on bee sociality groups: %SNH reduced social bee abundance while it enhanced eusocial bee abundance after OSR flowering in permanent grasslands (no effect on solitary bees). Similarly, %OSR effects also greatly depended on bee sociality groups: %OSR had a global positive effect on eusocial species abundance and solitary bee abundance (only after OSR flowering) but a negative effect on other social bee abundance in permanent grasslands, illustrating a competition between SNH and OSR for other social bees. OSR fields had no effect on species richness and therefore its possible interest for conservation purposes in SNH appears to be quite weak.

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Harvesting seeds of an *Arrhenatherion* meadow as a source of propagation material for grassland restoration

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Abstract

The seeds necessary for the restoration of species-rich grassland can be obtained through direct harvesting in semi-natural communities. This opportunity was tested in 2009-2010 by studying an *Arrhenatherion elatioris* meadow within boundaries of the Natura 2000 area near the Brody Experimental Station of PULS. The experiment was established in a random block design with four replications on 60 m^2 plots. We analysed the seed yield potential of the community and assessed the harvestable seed yield as a whole as well as the selected dominant species in relation to several methods of seed harvesting. The seed yield potential was described by: fertile stems density, the number of seeds per inflorescence, and seed mass of the control treatment. The harvestable seed yield of the meadow was analysed using three methods: (1) green hay collection; (2) on site threshing – using a combine harvester to thresh mature seeds from the sward; and (3) seed stripping – application of a hand-held seed stripper to harvest seeds from the growing plants. It was concluded that green hay was the most efficient harvesting method, as nearly all seeds present can be harvested with it. The other methods had lower efficiencies, but the remaining biomass can be used as hay for animal feeding.

Keywords: *Arrhenatherion* meadows, grassland restoration, harvestable seed yield

Introduction

Arrhenatherion elatioris communities are species-rich extensively managed hay meadows that provide many ecosystem services. These communities are becoming rare in Central Europe (Scotton *et al.*, 2009) due increased use of fertilizers and cutting frequencies (Dietl, 1995). Due to the gradual decline of *Arrhenatherion* meadows, effective methods for their restoration should be elaborated. Seed yield of well-preserved *Arrhenatherion* meadows should be assessed in terms of additional income for the owners of donor sites. Also, the opportunities for harvesting seed from this community with reference to the methods applied should be examined (Kiehl and Wagner, 2006). The aim of the study reported here was to determine the seed yield potential of the *Arrhenatherion* meadows and to assess the harvestable seed yield as a whole, as well as of selected dominant species, in relation to the methods of seed harvesting.

Material and methods

In 2009-2010, studies were carried on *A. elatioris* meadow (52°27'11'N, 16°15'33'E, 84 m a.s.l.) in NATURA 2000 area 'Zgierzynieckie Lake' near the Brody Experimental Station of Poznań University of Life Sciences (PULS). The studied meadow had been cut 1-2 times per year. From 2004 neither organic nor mineral fertilization has been used there. The investigated area (960 m^2) had a rich botanical composition: 48 species of vascular plants were found, 39 of which were forbs. The dominant plants were (abbreviations for species names are given in Table 2): *Ae* (10.1%), *Ap* (8.8%), *Hl* (4.6%), *Ml* (4.6%) and *Dc* (21.7%). The vegetation was classified as *Arrhenatherion* alliance (Kącki *et al.*, 2013) developed on poor soil. The experiment was established in a randomized block design with four replications on 60 m^2 plots. An analysis of the seed potential of the community and an assessment of the seed yield as a whole, as well as of the selected dominant ten species, were performed in relation to several methods of seed harvesting from first regrowth. The seed yield potential was assessed on the area of the control treatment on which permanent, small areas measuring 1×1 m were established in five replicates. The indicators of seed yield potential were: fertile stem density, the number of seeds per inflorescence, and seed mass. For each species 30 fertile stems at a stage of middle-late seed maturity were collected and biometric analyses were performed. The harvestable seed yield of the meadow was analysed using three methods: (1) green hay collection (GH) – collection of the fresh seed-containing biomass by mowing at the end of June each year; (2) on site threshing (OST) – using a combine harvester to thresh mature seeds from the cut plants; and (3) seed stripping (SS) – application of a hand-held seed stripper to harvest seeds from growing plants. The collected GH biomass cut with a mower and raked from the whole plots was weighed and sampled to determine seed yield. Threshing and stripping were carried out directly on the field on standing grass at the same date as for GH harvesting using a Wintersteiger Classic plot combine 1.5 m, and Prairie Habitats Inc. hand held seed stripper, respectively. Statistical analysis was performed on the data aggregating all species, grasses and forbs (species of botanical families other than grasses). The efficiency of the harvesting methods was calculated as the ratio of the number of mature seeds harvested to the number of mature seeds in standing seed yield. One-way ANOVA was used to determine the significance of differences between treatments.

Results and discussion

The seed potential of the *A. elatioris* meadow reached the level of 29,728 mature seeds per m^2 (Table 1). The seed material consisted mainly of forbs. Grasses accounted for only 17.6% of all collected seeds. The seed potential was lower in comparison with the results reported by Scotton *et al.* (2009) who reported the standing seed yield at seed maturity of an *A. elatioris* hay-meadow in the eastern Italian Alps was about 33,100 seeds m^{-2}. The mass of mature seeds, in our own investigations, reached ca. 250 kg ha^{-1}. Harvestable seed yield differed significantly among the methods of seed harvesting. The number of seeds harvested using the GH method was 9513. Compared with GH, the efficiency of OST was 44.3% and SS only 24.4%. Nowadays, in Poland there is demand for local provenance seed mixtures for species-rich grassland restoration (Czerwiński *et al.*, 2016), e.g. carried out to compensate for habitat loss or degradation during construction works. The remaining biomass after harvesting seeds can be used as hay in the feeding of herbivores. With the GH method, the whole harvested biomass is used for the restoration process.

The seed potential of the ten most dominant species reached the level of 47.2% total seeds. *Dc, Php, Ml, Am* and *Hl* were found to have the highest seed potential of all the analysed taxa (Table 2). In terms of seed harvesting efficiency, GH was the best method and SS was the worst. With respect to individual plant species, the best harvest effectiveness (above 50%) was recorded in the case of *Rs* using the GH and OST methods. The GH method was also found very effective (30-50%) when harvesting *Hl* and *Dg*. Also, the SS method resulted in good efficiency (25-35%) of *Ap, Dg* and *Hl* harvesting. The plant species *Hl* is harvested with an efficiency above 20% by each method. The performed analysis of seed harvest from this *Arrhenatherion elatioris* meadow revealed the effect of phenological specificity of species. The reason is a more variable phenological development of forbs compared with that of grasses, which formed

Table 1. Characteristics of the potential and harvestable seed yields of *Arrhenatherion* meadow.

Item	Species group	Seed yield potential	Method of seed harvesting			Significance of difference
			GH	OST	SS	
No. mature seeds (seeds m^{-2})	Total	29,728	9,513	4,022	2,323	P<0.01
	Grasses	5,228	2,222	837	384	P<0.01
	Forbs	24,500	7,291	3,185	1,939	P<0.01
Mass of mature seeds (g m^{-2})	Total	24.95	8.30	3.57	1.90	P<0.01
	Grasses	2.62	1.11	0.42	0.24	P<0.01
	Forbs	22.33	7.19	3.15	1.66	P<0.01

Table 2. Seed potential and harvested seed numbers of selected species of *Arrhenatherion* meadow by using different methods.[1]

Species	Seed potential (seeds m^{-2})	Harvested seeds (seeds m^{-2})			Efficiency of harvesting (%)		
		GH	OST	SS	GH	OST	SS
Arrhenatherum elatius (Ae)	513	116	37	72	22.6	7.2	13.9
Avenastrum pubescens (Ap)	504	89	27	185	17.7	5.3	36.7
Dactylis glomerata (Dg)	774	255	40	201	33.0	5.2	26.0
Holcus lanatus (Hl)	1,068	489	263	291	45.8	24.7	27.3
Phleum pratense (Php)	2,328	338	15	5	14.5	0.6	0.2
Medicago lupulina (Ml)	1,659	466	431	16	28.1	26.0	0.9
Trifolium pratense (Tp)	210	44	15	4	21.1	6.9	1.8
Achillea millefolium (Am)	1,345	64	5	0	4.7	0.3	0.0
Daucus carota (Dc)	5,531	828	98	5	15.0	1.8	0.1
Rhinanthus serotinus (Rs)	109	70	93	5	64.2	85.4	4.2

[1] GH = green hay collection; OST = on site threshing; SS = seed stripping.

generative shoots mainly in the first regrowth (Kiehl and Wagner, 2006). Delaying harvesting time of the meadow increased the seed production and harvesting effectiveness of some forbs species, such as *Ml*, *Dc* or *Galium* sp., but caused the loss of other species with earlier seed maturation.

Conclusions

At the end of the first regrowth it is possible to harvest considerable amounts of mature seeds developed on *A. elatioris* meadows for species-rich grassland restoration purposes. GH was the most efficient harvesting method, as nearly all seeds present can be harvested by this method, but the entire biomass must be used for restoration process. The methods OST and SS had lower efficiencies, but the remaining biomass can be used as hay for animal feeding. The best efficiencies were found for *Rs* with the GH and OST method, although only a small number of seeds were present in the meadow.

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Incorporating daffodil-derived galanthamine production into upland grassland systems

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Abstract

Galantamine is a pharmaceutical compound that has been an approved Alzheimer's disease treatment since 1998. Galantamine can be produced from the alkaloid galanthamine extracted from plants, but supplies are limited. Daffodils (*Narcissus* spp.) are the only economically feasible plant source for cultivation in many parts of the world. An initial study tested an innovative approach for producing daffodil-derived galanthamine based on integrating daffodil growing into existing marginal pasture; avoiding the need to plough the land. Experimental lines of daffodils were established under upland permanent pasture. Over 80% of bulbs successfully established at each site. There was no effect of planting site or planting density on galanthamine concentrations within vegetative tissues, which were higher than anticipated. The results confirm that planting daffodils under grass in upland areas could offer a novel and sustainable source of plant-derived galanthamine. The findings have the potential to increase the economic sustainability of farming communities in less favoured areas by providing farmers with a high value crop while maintaining traditional farming systems.

Keywords: galanthamine, daffodils, grassland, upland area

Introduction

The number of people suffering from dementia is considerable and growing at a significant rate. Around 46.8 million people worldwide were living with dementia in 2015, with this figure predicted to nearly double every 20 years (Alzheimer's Disease International, 2015). Alzheimer's disease (AD), a disorder associated with progressive degeneration of memory and cognitive function, currently accounts for between 60 and 80% of these cases. Galantamine (galan/t/amine) is a long acting, selective and reversible acetylcholinesterase inhibitor that has been a licenced treatment for AD in the USA, across Europe and into Asia since 2000. The main source of galantamine for the pharmaceutical industry has been the alkaloid galanthamine (galan/th/amine) extracted from plants. Galanthamine occurs in several species of the *Amaryllidaceae* family, but with the exception of daffodils (*Narcissus* spp.), the source plants are wild flowers not suitable for agricultural exploitation. Upland areas of the UK and northern Europe are characterised by poor growing conditions brought about by a combination of climate and a shortage of major nutrients. Consequently, agricultural production in these areas is generally limited to grassland-based ruminant systems. However, it has been reported that daffodils grown at altitude may yield higher concentrations of galantamine compared with bulbs grown under lowland conditions (Morris *et al.*, 2006). The aim of our study was to test the feasibility of an innovative dual-cropping approach to producing daffodil-derived galanthamine based on integrating daffodil growing into existing marginal pasture and harvesting green above-ground growth rather than bulbs.

Materials and method

Daffodil bulbs were planted into long-term improved permanent pastures at the Pwllpeiran Upland Research Platform, Ceredigion, Wales. Four locations from 253 m a.s.l. to 430 m a.s.l. were selected on a transect up a typical hillside (Table 1). At each site, replicate lines of daffodils were planted at three different inter-bulb intervals: 5 cm, 10 cm or 15 cm apart. Each line of daffodils was 8 m long, and lines

Table 1. Details for the four experimental sites where daffodils were established.

	Site 1	Site 2	Site 3	Site 4
Altitude (m a.s.l.)	253 m	284 m	398 m	430 m
Exposure	Comparatively sheltered	Comparatively sheltered	Poor protection from wind	Exposed hilltop
Soil analysis results				
Phosphate-P (ppm)	9.37	4.91	3.74	11.00
K (meq %)	0.42	1.67	0.34	0.63
pH	5.25	5.49	5.55	5.94
Date harvested	01-04-15	03-04-15	07-04-15	10-04-15

were spaced 1 m apart. Planting lines were created using a single bolt-on tooth (15 cm × 10 cm wide) on the front bucket of a mini-digger. Bulbs were planted at the prescribed densities by hand, with the tops of the bulb the treatment distance apart.

A 500 g soil sample was collected for each site by bulking 10 × soil cores cut to bulb planting depth taken at random between daffodil lines. Sampling of the daffodil biomass was undertaken when the majority of flowers at a site reached the 'gooseneck' growth stage – i.e. were bent downwards to an angle of approximately 45° but were unopened. The number of daffodil plants growing was counted along a 6 m length in the centre of each line. The corresponding growth was then harvested to a height of 3 cm using grass shears and bagged separately. The material cut from each line was weighed to determine fresh matter (FM) weight. A sample of approximately 100 g was oven dried to constant weight to determine dry matter (DM) content. A separate sub-sample of approximately 100 g was taken for subsequent analysis to determine alkaloid concentrations, determined by high-performance liquid chromatography following extraction.

Results and discussion

The plant counts prior to harvest showed over 80% of the bulbs had successfully established (Table 2), demonstrating that planting under long-term pasture on comparatively poor soils is feasible. Planting distance inevitably had a significant effect on the biomass of herbage harvested ($P<0.001$) but there was no effect of altitude on total DM yield or DM yield per bulb planted.

Galanthamine concentrations in daffodil leaves have been found to increase until flowering, then decrease (Lubbe *et al.*, 2013). Although higher concentrations of alkaloids could be obtained from the daffodil

Table 2. Effect of altitude of planting site on the establishment, yield and galanthamine (GAL) concentration of daffodil biomass harvested at the goose-neck growth stage.[1,2]

	Altitude				SED	Site
	253 m	284 m	398 m	430 m		
No plants/bulbs planted	0.86	0.83	0.87	0.83	0.033	ns
FM yield (g m^{-1})	95^a	126^b	100^a	96^a	6.4	<0.001
DM content (g kg^{-1} FM)	148^b	119^a	135^b	139^b	7.3	<0.05
FM yield per bulb (g)	6.7^a	9.5^b	7.4^a	7.2^a	0.50	<0.001
DM yield (g m^{-1})	13.2	15.0	13.8	13.3	0.96	ns
GAL concentration (% FM)	0.045	0.036	0.049	0.043	0.0050	ns

[1] Means within rows of treatments with different superscripts are significantly different at $P<0.05$. SED = standard error of the difference.

[2] FM = fresh matter; DM = dry matter.

leaves at an earlier growth stage than the gooseneck stage, the total amount of biomass and thus total yields of alkaloids would be expected to be lower. This is an aspect that may warrant further research. The galanthamine concentrations achieved during the current experiment were substantially higher than those recorded during the earlier study that focussed on bulbs (Morris *et al.*, 2006), and higher than concentrations previously reported for above-ground daffodil biomass (Kreh, 2002). Furthermore, by cutting green material there is potential for a single planting of bulbs to deliver harvests over multiple years. There was no effect of planting distance on galanthamine concentrations. Thus, the results suggest that higher planting densities which would favour biomass yield would maximise galanthamine yield. However, monitoring over multiple harvest years is required to determine whether further nutrient depletion from already poor quality soils becomes a factor over time.

Conclusions

The feasibility of establishing daffodils under permanent pasture in upland areas as a means of producing plant-derived galanthamine has been shown. Further research is now required to verify the commercial viability of this supply route.

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Grassland in Wallonia: expected location based on key ecosystem services

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Abstract

In Wallonia, grassland covers about 48% of the utilised agricultural area. This proportion is around 20% at the European scale. One challenge is to allow these agroecosystems to produce enough food but also to supply regulation and cultural services to improve human well-being. As ecosystem services depend on the location of the ecosystem in the landscape, we mapped, at the Wallonia scale, areas where grasslands are the best choice as agricultural land use in regard to soil and environmental constraints, in particular to limit erosion risk. To this end, we selected three criteria. The first one is the erosion issue due to tillage practices. The second one focuses on soil quality constraints limiting tillage ability. The last one is based on environmental priorities for biodiversity conservation. These surfaces and their locations, defined through the development of a GIS model, are compared to current agricultural use to define potential improvement. The results underline that the actual proportion of grassland would allow preserving the ecosystem services targeted by this study. Nevertheless, the location of 20% of these grasslands should be adapted to optimise regulation services; this was especially true in agricultural areas specialised in crop production.

Keywords: ecosystem, erosion, soil quality, biodiversity conservation, mandatory grasslands

Introduction

One of the main agricultural challenges is to maintain and/or improve the capacity of agro-ecosystems to deliver ecosystem services (ES), from production; including food, feed and biomass production; to regulation, support or cultural services. Nevertheless, some key ES such as erosion regulation, pollination support or cultural services, rely on the location of agro-ecosystems in the landscape (Villamagna *et al.*, 2013). Therefore, the use of a spatialized approach is necessary to objectify the ES potentially delivered by an agro-ecosystem. Among the different agro-ecosystems, grasslands play a key role in terms of fodder production, carbon sequestration, erosion and water leaching regulation, pollination support and cultural services. The key role played by grassland in the limitation of soil erosion is related to permanent soil cover with living vegetation. This induces a reduction of the volume and speed of water running off. In order to mobilize regulation ES delivered by grasslands in terms of nutrients or pesticides leaching risk reduction, implementation of this agroecosystem as buffer zone all along streams and in alluvial area is promoted. Such buffers lead to a reduction of leaching of 30 to 80% when well located. Grasslands have also the ability to value soils unsuitable for crop production, such as high rocky content or superficial soil, to support animal production without competition with man for the natural resources. In this context, based on these constraints and ES optimization, this research aims to quantify the surface of 'mandatory grasslands' (MG) in the Walloon area and to compare it with the current grassland surface and location in the regional utilised agricultural area (UAA).

Material and methods

Walloon Region, the southern part of Belgium, covers 16,903 km^2. Agriculture valorizes 45% of this surface, currently 52% as crop and 48% as grassland productions. In order to model MG areas two levels of constrains were integrated: (1) areas in which grasslands are necessary to limit soil erosion within an acceptable level and (2a) soils with a limited depth and/or a high rocky rate, and (2b) soils of high ecological value and/or alluvial soils – river banks area.

1. Mandatory grasslands to limit soil erosion risks

Soil losses risks were modelled through the universal equation of Wischmeier and Smith (1978): E = R * K * LS * C * P [Equation 1], where: E is the long term, annual soil losses [10^3 kg $ha^{-1}year^{-1}$]; R is the rain erosivity [MJ mm ha^{-1} h^{-1} an^{-1}]; K is the soil sensitivity to rain erosivity [10^3kg ha^{-1} mm^{-1} MJ^{-1}]; LS is the topographic factor taking into account slope length and intensity (≥ 0, without dimension); C is the cropping factor ([0,1], without dimension); P is a factor reflecting the effect of measures aiming to limit erosion ([0,1], without dimension). In this model, the possible hydric inter-connectivity existing between the parcels is not taken into account. Potential erosion (R * K * LS) was calculated from equation 1, simulating an absence of plant cover (C=1) (GISER, 2014). For a given parcel, the suitable cropping factor (sC) was defined based on the hypothesis that erosion must be lower than an acceptable level. This factor is positively linked to the soil 'reserve', thus positively linked to soil depth and negatively connected to stone content (Maugnard *et al.*, 2013). On this basis a shape file was generated. The ratio of acceptable erosion on potential erosion gave a raster of sC factor connected to 100 m^2 pixels. This raster was superposed to the shapefile of agricultural parcels from Wallonia to obtain the average sC value of each parcel. As sC directly depends on land use (Maugnard *et al.*, 2013), it was possible to determine, in order to reach these sC values, the parcels that must be covered by permanent grasslands (sC<0.10) or with grassland for two years (sC<0.23) or one years (sC<0.37) out of three, on average. We defined that parcels that must be covered for a minimum of two years out of three; grasslands that must be permanent grasslands (sC<0.23).

2. Mandatory grasslands due to soil cultivation limitation

Three parameters, extracted from numeric soil map of Wallonia, were taken into account to quantify soil cultivation limitation: stone content (three classes: 5-15%; 15-50%; >50%), soil depth (three classes: <20 cm; 20-40 cm; ≥40 cm) and soil series drainage class. Some of these parameters being mobilized to evaluate erosion risks, there is an overlap between these two constraints. Soils characterized by a poor drainage (gley soils) are MG. Crossing stone content and soil depth led to nine combinations, ranked, following the consultation of experts, on the basis of their ability to support crops. Soils with more than 50% of stone or with a depth lower than 20 cm are also MG. Conversely, soils with a stone content lower than 15% and deeper than 20 cm could be used for any crop. For soils with a stone content ranging from 15 to 50%, grassland cover is recommended whether soil depth is lower than 40 cm. When soils are deeper than 40 cm, with a stone content ranging from 15 to 50%, we considered that the current soil occupation took into account the local constraints.

3. Mandatory grasslands in area of high biological value

Alluvial soils and soils with huge carbon content (peat soil) are classified as MG. 12 m-width buffers of MG are also delimited all along Walloon's rivers network, and was determined from the map of hydrological network in Wallonia.

4. Integration of these levels of constraints and comparison to the current situation

Soil and biological constraints were superposed to the shapefile of agricultural parcels from Wallonia to calculate the proportion of each parcel that is under constraint. On this basis, three options were developed. The first one fixed that a parcel must be covered by grassland as soon as 25% of its surface is under constraint. For the second and the third options this limit was set to 50 and 75%, respectively. These three options were superposed to the surfaces of MG aiming to limit soil erosion risks (sC<0.23) in order to characterize three final scenarios. These results were compared to the current permanent grassland coverage. These approaches were performed with ArcGIS© software (version 10.3).

Results and discussion

While the proportion of surface under soil and/or ecological constraints necessary to identify a parcel as MG decreases from 75 to 25%, the surface of MG increases from 291,480 ha to 358,107 ha (+23%). The surface of MG aiming to limit soil erosion risks, 254,491 ha, represents 87 to 71% of this surface (Table 1). The proportion of these 254,491 ha also constrained by other soil and ecological parameters shifts from 47 to 71% as the proportion of parcel surface under soil and/or ecological constraints necessary to identify it as MG decreases from 75 to 25%. Current surface of permanent grasslands is 314,139 ha in Wallonia. Whatever the proportion of parcel surface under soil and/or ecological constraints necessary to identify this parcel as MG, 21 to 22% of MG surfaces are currently cultivated with another crop. This proportion of discrepancy is strongly correlated to the importance of grassland in the UAA as illustrated while considering nine Walloon agricultural zones characterized by contrasted permanent grasslands occurrences: the highest the grassland occurrence, the lowest is the discrepancy (Surface discrepancy= -0.561*% Grassland on the UAA+0.591; R^2=0.949; n=9). Nevertheless, the absolute importance of surface discrepancy is a function of both discrepancy proportion and grassland occurrence in the UAA. These two parameters allow us to identify the areas offering the highest opportunity to improve grasslands location in order to improve the ES they offer.

Conclusions

Considering that parcels with high soil erosion risk or with superficial/rocky soils and/or of high ecological value would be mandatory grasslands, we can underline that current permanent grassland surfaces are, in absolute value, near to being optimal. Nevertheless, the location of 20% of them could be optimized in order to maximize the delivery of the ES analysed. Both discrepancy proportion and grassland occurrence in the UAA allow identifying the agricultural area we have to focus on. Nevertheless, aside soil and ecological constraints, it would have been interesting to include climatic constraints in the model.

Table 1. Evolution of the surfaces of mandatory grasslands (ha) in link to the constraints taken into account and of these surfaces that are currently cultivated with other crops.

	Proportion of parcel surface under soil and/or ecological constraint to identify it as MG[1]		
	75%	50%	25%
Soil and ecological constraints	155,849	216,129	284,920
Erosion risk limitation	254,491		
Total	291,480	317,973	358,107
Only due to soil and ecological constraints	36,989	63,482	103,616
Cumulating the different constraints	118,860 (76%)	152,647 (71%)	181,304 (64%)
Surface of MG currently cultivated (% of MG)	62,130 (21%)	67,282 (21%)	79,589 (22%)

[1] MG = mandatory grassland.

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Drought response of proanthocyanidins in sainfoin (*Onobrychis viciifolia* Scop.) is affected by the plant's ontogenetic stage

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Abstract

Sainfoin (*Onobrychis viciifolia* Scop.) is a traditional forage legume well adapted to extensive agriculture and widely cropped until the end of the 19th century. Renewed interest in sainfoin derives from its benefits with regards to climate change and animal health: it is drought tolerant (adaptation) and its content in proanthocyanidins (PAs) reduces greenhouse gas emissions from ruminants (mitigation). In addition it acts as an anthelmintic agent against gastrointestinal parasites. To identify the impact of drought on the PA concentration and composition in the vegetative and generative stage of sainfoin, a rain exclosure experiment was carried out near Zurich. Leaves were sampled repeatedly and analysed for PAs by UPLC-MS/MS. While vegetative plants showed a strong response to drought in their leaf PA concentration (+34%), generative plants showed no response (+0%). The PA composition of both ontogenetic stages was relatively stable across environments. The results show that the ontogenetic stage at which drought occurs significantly affects the plant's response in PA concentration and that sainfoin can be an appropriate choice in a context of climatic change.

Keywords: *Onobrychis viciifolia*, sainfoin, tannins, drought, ontogenetic stage

Introduction

Proanthocyanidins (PAs, syn. condensed tannins) are plant secondary metabolites which are considered to provide benefits for ruminant production by improving animal health (bloat reduction and anthelmintic effects) and reducing environmental burdens (direct reduction of methane emissions and indirect reduction of nitrous oxide emissions by shifting N to a more stable form in dung) reviewed by Lüscher *et al.* (2014). For this, however, the structural characteristics of tannins need to be considered, namely the procyanidin (PC) to prodelphinidin (PD) ratio and the mean degree of polymerization (mDP) (Waghorn, 2008). Sainfoin (*Onobrychis viciifolia* Scop.) is an interesting forage plant because it has a natural concentration of PAs with suitable structural characteristics and is bioactive (Häring *et al.*, 2007; 2008; Malisch *et al.*, 2015). Our aim was to test the drought effect on sainfoin's PA concentration and composition. For this, five accessions, mainly cultivars available on the European seed market, were subjected to an18-week drought period in a field experiment using rainout shelters.

Materials and methods

The field experiment was located north of Zurich, Switzerland (47°44' N, 8°53' E; 482 m a.s.l.). For this analysis, a subset of an existing experiment was used. The experimental setup was described in Malisch *et al.* (2016). Briefly, each treatment was replicated twice and contained nine individual plants per replicate, with a 0.25×0.5 m distance to each other (within and in between rows). Drought conditions were induced by installing rainout shelters. These were designed to maintain environmental conditions (temperature, irradiance) which resembled the unsheltered control. Drought lasted for 127 days, from 12 June to 17 October 2013 and reached a soil water potential of below -1.5 MPa. Half of the plants were cut at week 7 of the drought period, shifting their ontogenetic stage back to vegetative, while the uncut plants continued generative growth. Except where mentioned otherwise, all results presented here are the mean values taken from two sampling events at peak drought, sampled at weeks 10 and 14 of the drought period. Phenolic compounds were analysed using a UPLC-MS/MS chain and with multiple reaction monitoring (MRM) methods as developed by Engström *et al.* (2014). Linear mixed regression models were conducted using the R software.

Results and discussion

When plants were in their vegetative growth, severe drought resulted on average in a 34% increment (P=0.06) in the concentration of PAs in leaves, whereas PA concentration was unaffected in the generative growth (P=0.88; Figure 1A,D). This was because over the drought period (time), PA concentration developed differently within the four treatments (drought × cut × time: P<0.001). There were also large differences in PA concentration among the five accessions (acc: P<0.001). At peak drought 'CPI 63750' had the highest (22.0 mg g^{-1} dry matter, P<0.001) PA concentration which was 45% higher than for 'Perly', the accession with the lowest PA concentration. Importantly, PA concentration of accessions did not react differently to drought (acc × drought: P=0.92; Figure 1A,D). Despite the trend of 'Taja', this is true even for the generative stage alone (P=0.25).

The structural characteristics of the PAs showed a high stability across treatments: the mDP was little affected (+10%) by drought in the generative stage (P=0.12), and not at all in the vegetative stage (P=0.96; Figure 1B,E). The accessions differed significantly in their mDP (acc: P<0.001) with 'Taja' having the highest mDP (11.1) but, importantly, accessions did not differ in their response of mDP to drought (acc × drought: P=0.18). Finally, accessions differed in their PD share (acc: P<0.001, Figure 1C,F) but, again, the response of PD share to drought did not differ among accessions (acc × drought: P=0.99).

Our results clearly show that the ontogenetic stage was a major determinant for the plant's response to drought stress. It is remarkable that the same pattern of response was confirmed with all five accessions examined. The increased PA concentration in the vegetative stage due to drought could result in increased

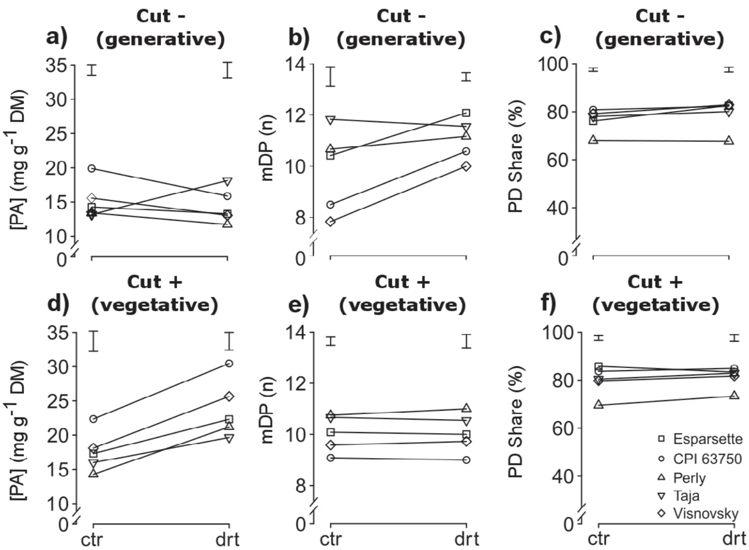

Figure 1. Concentration of extractable proanthocyanidins (PA; (A), (D)), their mean degree of polymerization (mDP; (B), (E)) and prodelphinidin (PD) share ((C), (F)) in sainfoin leaves as affected by drought and additional cut (ontogenetic stage) for each of the five accessions. The treatments consist of the factor cut, with (Cut+) or without (Cut-) an additional cut at week seven, and the factor drought, with the levels control (ctr: rainfed) and drought (drt: rain exclusion). Error bars are standard errors of the mean (adapted from Malisch *et al.*, 2016).

bioactive effects. While the extent of bioactive effects from sainfoin is a function of both concentration and structural characteristics of PAs, the mode of bioactivity is primarily defined by the structural characteristics only, with PCs being more likely to improve nitrogen use efficiency and PDs being more likely to enhance anthelmintic and antimethanogenic effects (Waghorn, 2008). The lack of differences in the accessions response to our drought treatment indicates a stability of the desired mode of bioactivity across environmental growing conditions. Together with the big differences in PA properties among the five accessions, which are in accordance with a previous study based on a much broader selection of accessions (Malisch *et al.*, 2015), this suggests a large potential for further improvements of sainfoin PAs by breeding.

Conclusions

Our results illustrate that sainfoin can be an appropriate choice in the context of climate change. The PA concentration increased under drought, thus potentially increasing the bioactive effects in ruminants. However, these effects are limited to plants in the vegetative stage.

Acknowledgements

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Pea-based fodder crops: a promising alternative to fallow in cereal-livestock systems of the Algerian High Plains

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Abstract

Fallow is commonly used in rotation with cereals in the High Plains region of inner Algeria. It is the main feed resource for smallholder livestock, which provide important income for farmers. Replacing fallow by protein-rich, legume-based forage crops could increase the quantity and quality of animal feed, while improving the soil fertility for the following cereal. Mixtures of annual legumes with winter cereals are promising fodder crops that can combine forage yield and quality. Vetch-oat mixtures are grown in Algeria and other regions of Maghreb, but farmers are less familiar with crops including field pea (*Pisum sativum* L.). This three-year study compared pea mixtures with oat or triticale with mixtures including common vetch (*Vicia sativa* L.) or Narbon vetch (*Vicia narbonensis* L.) and pure stands of component species at one site of the High Plains, recording dry-matter yield and legume and weed contents. The results highlighted the potential of pea as a feed resource for dryland cereal-livestock systems. In particular, three pea-cereal mixtures did not differ from cereal monocultures in mean forage yield, and two of them combined high forage yield with good legume proportion in the forage. Pea-based mixtures out-performed vetch-based mixtures for all recorded traits.

Keywords: *Pisum sativum*, forage yield, pea, vetch, oat, triticale, mixture

Introduction

Rain-fed cereal cropping is widespread in the High Plains region of north-eastern Algeria. Fallow is a constant practice in regional farming systems as it also represents a grazing resource for small farmers that rear livestock as an additional economic resource and as a buffer against economic shocks and crop failures. Heavy reliance on fallow exploitation for animal feeding, however, results in poor animal production levels, and may entail serious risk of overgrazing and land degradation. Replacing fallow by protein-rich, legume-based forage crops could increase the quantity and quality of animal feed while improving the soil fertility for the following cereal. Mixtures of annual legumes with winter cereals can provide resource complementarity (Hauggaard-Nielsen and Jensen, 2001), thereby enhancing the system sustainability. Vetch-oat mixtures are well known as forage crops in rain-fed Mediterranean environments (Lithourgidis *et al.*, 2006), whereas farmers are less familiar with pea-based mixtures including tall or semi-dwarf (grain type) pea. The development of leafless tall-statured pea can increase the pea crop standing ability while ensuring good competitive ability against cereals and weeds (Annicchiarico *et al.*, 2012). The aim of this study was to compare several pea-based and vetch-based mixtures and their respective pure stands for forage dry-matter yield (DMY), forage composition, and competitiveness to weeds in the High Plains region.

Materials and methods

Six pure-stand crops (two pea genotypes of contrasting plant stature, one common vetch, one Narbon vetch, one oat and one triticale cultivars) and the eight cereal-legume mixtures (Table 1) were grown in three seasons (2013-2014 to 2015-2016) under rain-fed conditions in Sétif, Algeria (36°11'N, 5°24'E), in plots of 4×3 m in a randomized complete block design with four replications. While the tall semi-leafless pea was bred by CREA-FLC, the remaining cultivars were elite material chosen on the basis of prior investigations in Mediterranean environments (Annicchiarico and Iannucci, 2008) for the semi-dwarf pea. Ordinary seeding rates of 70 germinating seeds m^{-2} for pea and Narbon vetch, 140 seeds m^{-2} for common vetch and 280 seeds m^{-2} for cereals were halved for the mixtures. Sowing was in autumn following a wheat crop. Forage was harvested in April, at late heading/early milky stage for cereals and waxy stage for legumes. DMY (dry matter yield) of sown species, weed content out of total biomass, and legume content in mixtures, were assessed on oven-dried samples. An analysis of variance (ANOVA) assessed the variation between mixtures or sets of mixtures on data averaged across the three seasons. A two-tailed Dunnett's test compared individual mixture yield values versus the average yield value of the two cereal pure stands. Differences for DMY and weed percentage among the six pure stands were assessed by a separate ANOVA.

Results and discussion

Oat and triticale monocultures were the highest ranking for DMY, confirming the high cereal potential of the target area (Table 1). However, three pea-based mixtures did not differ in DMY from the average yield of the two cereals (Table 1). The mixtures including the tall pea line (P2), in particular, combined high yield with legume proportion exceeding 20% (reaching almost 30% in the association with triticale), along with weed content comparable to that of the cereal pure stands (Table 1).

The tall pea line was slightly more competitive against the cereal companion in mixtures (as well as against weeds in the monoculture) than the semi-dwarf pea cultivar, confirming the findings of an earlier study (Annicchiarico *et al.*, 2012). Both vetch species tended to have low forage yield when grown in pure stand, and gave a modest contribution in terms of legume presence when grown in mixtures (Table 1). There was a slight, overall yield advantage of oat-based over triticale-based mixtures (+13%), consistent with previous results on mixtures of these cereals with vetch or pea (Thami Alami *et al.*, 2016). Pea-based mixtures were definitely more interesting than vetch-based mixtures in terms of forage DMY,

Table 1. Mean values over three growing seasons of dry matter yield (DMY), weed percentage out of total biomass and legume percentage in mixtures, for eight cereal-legume binary mixtures and the respective pure stands grown in the High Plains region, Algeria.

Crop		DMY (Mg ha^{-1})	Weed (%)	Legume (%)
Mixture[1]	P2-O	4.79	16.6	21.0
	P2-T	4.43	18.6	29.7
	P1-O	4.22	15.0	16.2
	V-O	4.07 *	23.9	10.2
	P1-T	3.72 *	23.7	21.3
	N-O	3.58 *	18.9	14.1
	N-T	3.39 *	18.7	13.1
	V-T	3.20 *	23.1	11.6
	LSD (P<0.05)	0.99	5.5	5.6
Pure stand[2,3]	O	5.52 [a]	15.8 [d]	-
	T	4.93 [a]	20.5 [cd]	-
	P2	3.28 [b]	27.3 [bc]	-
	P1	2.35 [bc]	33.4 [ab]	-
	N	2.07 [cd]	32.8 [ab]	-
	V	1.42 [d]	38.1 [a]	-

[1] Yield value different from the average value of cereal pure stands (O and T) according to two-tailed Dunnett's test at P<0.05.

[2] N = Narbon vetch, cv. Bozdag; O = oat, cv. Genziana; P1 = semi-dwarf semi-leafless pea, cv. Kaspa; P2 = tall semi-leafless pea, new line; T = triticale, cv. Vivaciò; V = common vetch, cv. Barril.

[3] Column, pure stand values followed by different letters differ according to Duncan's test at P<0.05.

weed competition and forage composition (Table 2). Benefits resulting from legumes in mixed swards (including those on forage nutritive value) are considered most effective at a legume proportion of at least 30% (Lüscher *et al.*, 2014). It is noteworthy that the legume proportion of our pea-based mixtures was in most cases between 20 and 30%. Adopting much higher legume seeding rate than the current one, Lithourgidis *et al.* (2006) found higher legume contribution in common vetch-based mixtures. Given the unquestioned adaptive advantage of cereals in the study region, one could wonder in retrospect if a lower seeding rate for cereals than the current one may give more balanced proportion of legumes and cereals in the harvested forage.

In conclusion, this study highlights the potential of pea-based mixtures as a key forage resource for dryland cereal-livestock systems of Algeria. This role can be increased by a regional breeding effort aimed to select pea material with better local adaptation. While a tall, semi-leafless pea is an optimal legume companion, the semi-dwarf pea type can be convenient for fodder production while having potential also as a feed grain crop in monoculture.

Table 2. Comparison among pea-based mixtures, common vetch-based mixtures and Narbon vetch-based mixtures for dry matter yield (DMY), weed percentage out of total harvested biomass, and legume percentage in mixtures (see Table 1 for mixture compositions).[1]

Crop	DMY crop (t/ha)	Weed %	Legume %
Pea-based mixtures	4.29 ^{a}	18.5 ^{b}	22.0 ^{a}
Common vetch-based mixtures	3.63 ^{ab}	23.5 ^{a}	10.9 ^{b}
Narbon vetch-based mixtures	3.49 ^{b}	18.8 ^{b}	13.6 ^{b}

[1] Column mean values followed by different letters differ according to Duncan's test at P<0.05.

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Silphium perfoliatum L. silage as alterative to lucerne and maize silage in dairy cow rations

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Abstract

Silphium perfoliatum L. (hereafter referred to as silphium) thrives in marginal landscapes too wet for lucerne (*Medicago sativa* L.) or maize (*Zea mays* L.), but its usefulness as forage for dairy cattle is not known. Lactating dairy cows were fed diets substituting a portion of a lucerne and maize silage mixture, with silphium silage in two experiments in Wisconsin, USA. In trial 1, performance of lactating cows given a diet substituting one-half of an alfalfa silage and maize silage mixture with silphium silage was compared with the performance of cows fed a control diet (46:54 forage to concentrate without silphium). Voluntary intake of the silphium-containing diet was lower, but milk production was similar to cows on the control diet. In trial 2, substitution levels of the lucerne silage and maize silage mixture with silphium silage were zero (50:50 forage to concentrate without silphium), one-third, and two-thirds. Ration intake and milk yield decreased linearly with increasing substitution rate. We conclude that silphium silage can replace at least 30% of the conventional silage in the ration without sacrificing dairy cow performance.

Keywords: forage, milk production

Introduction

Silphium perfoliatum L. is a tall-growing member of the *Asteraceae* family, indigenous to mesic regions of the original North American prairie. It is still commonly found in remnants of native vegetation, and has been intentionally or unintentionally introduced to other areas. Silphium was introduced to Europe in the 18th century as an ornamental plant, and recently has been promoted as a bioenergy feedstock in Germany (Gansberger *et al.,* 2015) while in USA, interest has been primarily as a forage crop (Albrecht and Goldstein, 1997). Ability to tolerate wet soils and frigid winters could make silphium an alternative to maize or lucerne for forage or biomass on marginally wet soils. The idea of using silphium as a dedicated forage crop or as a dual-purpose forage/biomass crop is therefore intriguing. The plant is known to be consumed by livestock on rangeland or from fence rows; however, the potential of silphium as a forage crop in livestock rations has not been systematically evaluated. The goal of this research was to determine whether silphium could replace a portion of lucerne and maize silage without reducing milk production of dairy cows.

Materials and methods

Silphium (a synthetic population of seed collected in central USA) was grown on silt loam soil in a 0.5 ha field at the University of Wisconsin Arlington Agricultural Research Station near Arlington. Seed was sown at a rate of 7.9 kg ha^{-1} two years before the feeding trials were initiated. Soil pH was 6.8, nitrogen fertilizer was applied at a rate of 150 kg ha^{-1} each spring and P and K were maintained at levels recommended for maize silage on that soil type. Silphium was cut and conditioned with a disk mower at the early bloom stage of maturity in mid-July, wilted for 30 h in the field, and ensiled in plastic bag silos. All forages were analysed for dry matter (DM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), and acid detergent lignin (ADL). Digestibility was calculated using ADL as an internal marker from faecal and silage samples.

Two feeding trials were conducted with multiparous cows housed in a tie-stall barn. Total mixed rations of control diets consisted of equal amounts of lucerne and maize silages as forage sources and balanced with concentrate feed ingredients, minerals, and vitamins. Lucerne was harvested at bud stage and ensiled after wilting. Maize was harvested at physiological maturity in late September and ensiled. Forage to grain ratios were fixed at 46:54 and 50:50 in trials 1 and 2, respectively. Trial 1 was arranged in a cross-over design with two treatments. Six Holstein cows in early lactation were randomly assigned to each treatment. The control diet (0 silphium) was compared with a diet with silphium substituting 1/2 of the lucerne/maize silage mixture (1/2 silphium) for two 14-day periods. Trial 2 was designed as a 3×3 Latin square with four squares and three 21-day periods. Twelve Holstein cows in mid-lactation were randomly assigned to one of the three treatment diets. The control diet (0 silphium) was compared with diets where 1/3 (1/3 silphium), or 2/3 (2/3 silphium) of the lucerne/maize silage was replaced by silphium silage. Diets were fed in the morning and afternoon and one week was allowed for adaptation to diets. Milk yield and composition were measured in the final week (trial 1) or final two weeks (trial 2) of each experimental period.

Results and discussion

Standing silphium contains only about 15% DM and requires wilting to ensure an appropriate environment for fermentation. Silphium was chopped and ensiled at marginally low levels of DM (Table 1) because of impending rain, and sensory evaluation revealed a slight butyrate odour in silage. Han *et al.* (2000b) also reported that difficulty in moisture reduction could affect fermentation of silphium. NDF and ADF concentrations were greater in silphium silage than in either maize or lucerne silages, a potential limitation to intake. However, the high fibre concentration creates digestibility characteristics different than alfalfa silage because lignin concentration is constant, even at broad ranges of NDF, resulting in relatively low ADL to NDF ratios and greater fibre digestibility (Han *et al.*, 2000a). Crude protein concentration in silphium was intermediate between lucerne and maize silages.

In trial 1, body weight loss for both control and silphium containing rations indicated that energy intake did not meet energy requirements and mobilization of energy from the body was required to maintain lactation performance (Table 2). The proportion of concentrate in rations was increased in trial 2, providing a baseline for the proportion of silphium in the diet of lactating dairy cows. Milk yield was not affected until the substitution level rose to 2/3 of the total forage portion for cows producing approximately 45 kg milk per day. Thus, silphium comprised 1/3 of the total forage portion in the diet without a significant sacrifice in cow performance. The lower milk yield without significant concentration changes in milk fat and milk protein for cows fed 2/3 silphium as forage source indicated that decreased performance was mostly due to lower intake of silphium silage. The body weight loss at the greatest

Table 1. Chemical composition of silages used as the forage portion in the lactating cow diets.[1]

	Silage					
	Trial 1			Trial 2		
	Silphium	Lucerne	Maize	Silphium	Lucerne	Maize
DM (g kg^{-1})	275	480	358	295	444	317
NDF (g kg^{-1} DM)	454	412	382	493	396	385
ADF (g kg^{-1} DM)	363	343	227	349	268	203
ADL (g kg^{-1} DM)	36	69	22	40	51	14
ADL/NDF (%)	8	17	6	15	19	8
CP (g kg^{-1} DM)	98	216	74	118	250	79
Ash (g kg^{-1} DM)	133	107	33	150	121	37

[1] Dm = dry matter; NDF = neutral detergent fibre; ADF = acid detergent fibre; CP = crude protein; ADL = acid detergent lignin.

Table 2. Intake and performance of cows fed a control diet and test diets that substituted various proportions of maize/lucerne silage with silphium silage.[1,2]

	Experimental diet				
	Trial 1		Trial 2		
	0 Silphium	1/2 Silphium	0 Silphium	1/3 Silphium	2/3 Silphium
Intake (kg DM d^{-1})					
DM	24.1^a	21.4^b	32.1^a	28.7^b	25.1^c
CP	4.9^a	4.1^b	6.7^a	5.7^b	4.7^c
ADF	3.4^a	2.7^b	5.3^a	4.2^b	3.7^c
NDF	5.9^a	5.1^b	8.3^a	7.4^b	6.3^c
DM digestibility (%)	61.5	62.3	64.0^a	57.6^b	56.1^c
Lactation performance					
Milk yield (kg d^{-1})	43.1	41.1	46.4^a	44.2^a	41.3^b
Milk fat (%)	3.5	3.4	3.6	3.5	3.4
Milk protein (%)	2.9	2.8	3.1	2.9	2.8
Milk fat yield (kg d^{-1})	1.5	1.4	1.6	1.5	1.5
Mean body weight (kg)	589	584	656	649	635
Body weight change (kg 21 d^{-1})	-34.3	-41.7	24.4^a	6.6^b	-14.2^c

[1] Dm = dry matter; NDF = neutral detergent fibre; ADF = acid detergent fibre; CP = crude protein; ADL = acid detergent lignin.

[2] Means in the same row within a trial with different letters differ at $P<0.05$.

dietary silphium proportion in trial 2 may be due to silage fermentation characteristics or secondary plant compounds in silphium. Silphium is a plentiful source of sesqui-, di-, and triterpenoids (Pcolinski *et al.*, 1994) that are non-toxic but could affect palatability.

Conclusions

The performance of cows fed diets containing silphium silage was similar to that of control diets at 33% substitution level, but the documented difficulty in producing well fermented silage is important is determining the proportion of silphium in rations for dairy cattle. In this research, we have shown that the forage portion of diets for high producing lactating cows can contain about 1/3 silphium silage, with the remainder a mixture of maize and lucerne silage. In areas with marginally wet soil conditions, silphium is a feasible substitute for maize or lucerne silage for dairy cows.

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Seasonal variation of nutritional characteristics of some Mediterranean shrub species

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Abstract

The aim of this study was to determine the chemical composition and *in vitro* dry matter digestibility of eight browse species (*Arbutus unedo*, *Calicotome villosa*, *Cistus salvofolius*, *Erica arborea*, *Myrtus communis*, *Phillyrea angustifolia*, *Pistacia lentiscus* and *Quercus suber*) harvested in spring and summer from uplands in the province of Nefza (Norwest Tunisia). Species varied widely in their crude protein content (CP, 38-221 g kg^{-1} dry matter (DM)), cell wall content (NDF, 325-630 g kg^{-1} DM), condensed tannins (1-570 g kg^{-1} DM) and *in vitro* dry matter digestibility (IDM) (430-681 g kg^{-1} DM). CP content and digestibility coefficients tended to decrease from spring to summer ($P<0.001$), whereas NDF and tannin contents followed the opposite trend. NDF fraction may have a negative influence on browse digestibility. Based on these results, it is concluded that some of these species may represent a high quality feeding resource for grazing ruminants in the rangelands, particularly when the herbage availability is reduced.

Keywords: chemical composition, *in vitro* digestibility, browse

Introduction

In Tunisia, livestock production represents an important activity mainly in the rural zones. In these areas woody and herbaceous plants are an important contributor to grazing animal nutrition, especially during long periods of drought. They are supposed to meet the nutritional requirements of many animals, although there is little information on the nutritive value of tree and shrub leaves commonly browsed by small ruminants. Chemical analysis, particularly in combination with *in vitro* digestibility can help in the preliminary evaluation of the nutritive value of shrub species. The objective of this study was to assess the potential nutritive value of some Tunisian browse species and their seasonal fluctuation based on chemical composition and *in vitro* dry matter digestibility.

Materials and methods

Samples (leaves) of browse from eight shrub species (*Arbutus unedo* L., *Calicotome villosa* (Poir.) Link, *Cystus salvifolius* L, *Erica arborea* L., *Myrtus communis* L., *Phillyrea angustifolia* L., *Pistacia lentiscus* L. and *Quercus suber* L) were collected in spring and summer from the uplands of Taref (northwest of Tunisia). Ground samples (1 mm) were subjected to chemical analysis. Crude protein (CP) was determined by AOAC (1999), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined by Van Soest *et al.* (1991). Free condensed tannins (FCT) were measured using the butanol-HCl assay (Porter *et al.*, 1986) using purified quebracho tannin as standard. A similar technique was used to quantify the bound condensed tannins in the solid residue remaining after extraction. Concentration of total condensed tannins (TCT) was the sum of FCT and bound condensed tannins. Concentrations of tannins were expressed in g kg^{-1} DM, standard equivalent. For the determination of the *in vitro* dry matter digestibility a sample of rumen was fluid collected from four Merino sheep fitted with rumen cannula. The technique of Goering and Van Soest (1970) was followed to determine the *in vitro* dry matter digestibility (IDM). All obtained data were subjected to one-way analysis of variance (Steel and Torrie, 1980).

Results and discussion

Chemical composition of shrubs is presented in Table 1. Highest CP concentration (22%) was observed for *C. villosa*, while the lowest (3.8%) was for *A. unedo*. CP declined from spring to summer. High temperatures and water shortage during the summer bring about a more intense lignification of the cell wall as a response to tissue ageing. Relatively high cell wall contents and its lignification were observed especially in *A. unedo, E. arborea* and *P. lentiscus*. Moreover, cell wall contents followed an opposite trend to that for CP content. It appears therefore that as plants mature, photosynthetic products are converted more rapidly to structural components, thus having the effect of decreasing protein and soluble carbohydrate and increasing the structural cell wall components. It is pertinent that, with the exception of *C. salvifolius*, *C. villosa* and *Ph. Angustifolia*, the other studied species revealed high levels of total condensed tannins (Table 1). Thus CP content per se should not be the sole criterion for judging the relative importance of a particular feedstuff. The *in vitro* dry matter digestibility coefficients (IDM) of the different shrubs (Table 2) collected during spring was found to vary between 52% (*P. lentiscus*) and 68% (*C. salvifolius, E. arborea and Ph. angustifolia).* For all studied species IDM was higher than expected. This may be due to the fact that extraction with the neutral detergent removes bacterial cell walls and other endogenous products, and this could result in an over-estimation of the undigested residue, thus resulting in higher values of IDM. On the other hand, IDM was subjected to seasonal variation and has shown a downward trend from spring to summer with significant differences for all studied species ($P<0.001$). It is well reported that with advancing maturity the parenchyma is progressively replaced by vascular and supporting tissues characterised by a greater development of secondary plant cell wall (Wilson and Hatfield, 1997). It is noteworthy that *E. arborea* had higher TCT and FCT than *C. villosa* and *C. salvifolius*, even though its foliage was more digestible (Table 1). It appears, therefore, that the effect of condensed tannins on the digestibility of nutrients depends mainly on their structure and biological activity and not on their concentrations (Ammar *et al.*, 2004).

Table 1. Chemical composition (g kg^{-1} dry matter (DM)) and condensed tannins (g kg^{-1} DM, standard equivalent) of foliage of shrub species.[1]

Species		DM	OM	CP	NDF	ADF	ADL	TCT	FCT
A. unedo	Spring	440b±2.0	947b±2.6	55±1.7	410±7.5	250±2.6	135±4.6	232±8.2	177±8.7
	Summer	450a±1.7	960a±5.0	38±1.0	520±2.6	330±2.0	276±1.7	260±4.4	190±4.4
C. salvifolius	Spring	470±1.7	932±2.6	95±1.7	325±2.6	230±2.6	90±1.7	6±1.0	1±1.0
	Summer	560±3.5	960±4.6	64±1.0	580±5.2	301±1.7	201±2.6	12±1.0	1±0.6
C. villosa	Spring	261±3.0	948±2.6	221±1.0	572±1.7	204±3.0	112±6.1	10±0.5	1±0.9
	Summer	312±7.8	962±8.7	180±3.6	630±9.5	250±3.6	130±2.6	20±1.2	5±2.0
E. arborea	Spring	528±11.5	965±6.2	80±2.6	524±1.4	398±6.6	297±5.3	279±12.1	220±11.8
	Summer	535±5.3	972±7.0	62±4.3	620±3.0	420±5.6	382±7.8	570±19.0	505±18.2
P. lentiscus	Spring	478±5.3	946±4.6	60±4.6	433±6.6	252±7.8	240±5.6	383±12.5	324±14.2
	Summer	498±12.8	945±6.1	48±3.6	519±4.6	458±6.6	320±7.0	405±20.5	363±22.4
Ph. angustifolia	Spring	533±9.6	953±7.0	110±4.4	445±5.3	354±5.3	260±6.2	11±1.7	2±1.8
	Summer	505±8.9	962±4.6	100±6.1	560±7.5	450±1.7	340±5.3	20±3.0	11±3.1
M. communis	Spring	200±5.6	957±3.6	130±5.3	383±7.9	200±10.4	110±2.6	104±2.1	49±3.5
	Summer	225±8.2	956±5.3	95±3.6	522±7.8	287±5.3	204±7.2	201±9.1	165±10.0
Q. suber	Spring	490±7.5	959±3.6	70±4.4	551±6.6	395±6.1	360±4.0	151±5.5	103±3.7
	Summer	545±2.6	966±3.6	58±3.6	530±8.7	430±5.3	390±6.2	178±5.5	129±2.6

[1] DM = dry matter; OM = organic matter; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; TCT = total condensed tannin; FCT = free condensed tannin.

Table 2. *In vitro* dry matter digestibility (g kg^{-1}) of the foliage of shrub species.[1]

	A. unedo	*C. salvifolius*	*C. villosa*	*E. arborea*	*P. lentiscus*	*Ph. angustifolia*	*M. communis*	*Q. suber*
Spring	648.7^a	679.7^a	652.0^a	680.7^a	523.3^a	680.7^a	674.3^a	542.7^a
Summer	541.3^b	520.3^b	580.7^b	610.0^b	429.7^b	598.7^b	590.3^b	500.7^b
SEM	0.53	1.20	1.60	2.36	1.94	3.36	3.33	2.96

[1] $P<0.001$; in the same column, different letters indicate significant differences between values of *in vitro* dry matter digestibility coefficients.

Conclusions

Based on their CP, condensed tannin contents and IDM, shrubs can be classified into three groups: the most nutritional group (*C. villosa, C. salvifolius* and *Ph. angustifolia)*, the intermediate (*M. communis* and *Q. suber*) and the least nutritional group (*A. unedo*, *E. arborea* and *P. lentiscus*). Other parameters need to be evaluated to assess the feeding value of these resources and justify their importance to meet the nutritional requirements of grazing animals, in particular when the herbage availability is reduced.

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Can we differentiate growth regulation of red fescue (*Festuca rubra*) and forecast yield based on early measurements of normalized difference vegetation index (NDVI)?

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Abstract

Red fescue (*Festuca rubra*) is a grass species used in many different seed mixtures for pastures. It is drought tolerant and abrasion resistant due to its ability to produce rhizomes. The climate in Denmark with mild winters and frequent rain during spring and summer is well suited for producing seeds of red fescue. The species is exposed to lodging at flowering and therefore plant growth regulators (PGR) are generally used in order to keep plants upright at flowering, thereby ensuring pollination. The objective of the study was to investigate if Normalized Difference Vegetation Index (NDVI) measured early in the season can be used to estimate biomass and seed yield at harvest and if it could be used to apply PGR site-specifically. A field experiment was established in 2014 and harvested in 2015. Four different crop densities were sown and four different PGR applications were applied. NDVI was measured with a handheld device and Norm Excess Green Index was measured from a drone. Seed yield at harvest was not correlated to NDVI and Norm Excess Green Index measured in May. The PGR applications did not affect seed yield significantly. However, further field trials will be carried out in order to study yearly variations.

Keywords: *Festuca rubra,* lodging, seed production, Moddus M, trinexapac-ethyl

Introduction

Several grass species grown for seed production are prone to lodging at flowering. Red fescue (*Festuca rubra*) is one of them. Lodging disables flowering and pollination, resulting in reduced seed production. Well-fertilized, dense and vigorous crops are especially exposed to lodging (Elgersma, 1985), and as a consequence there has been an increasing interest to use plant growth regulators (PGR) in grass seed production on order to reduce plant elongation. Reducing stem length at the right growth stage reduces risk of lodging, thereby keeping the plants upright at flowering and to ease pollination, seed production and harvesting. Fertilizers and chemicals like PGR are usually applied evenly throughout the field even though the grass may not have the same growth potential all over the field. Consequently, it might be cost effective to apply chemicals like PGR site-specifically, based on expected potential yield. Yield potential may be estimated early in the season by measuring normalized difference vegetation index (NDVI). The index reflects the near infrared (NIR) light used by the plants for photosynthesis. The higher the index the more NIR light is used by the plant biomass (Rasmussen *et al.*, 2016). We hypothesize that we would be able to estimate seed potential of red fescue early in the season based on NDVI measurements. If we succeed, we would also be able to base trinexapac-ethyl application (PGA) on the measurements site-specifically and reduce the farmer's costs, increase the seed yield and avoid unnecessary application of PGR for the benefit of the environment.

Materials and methods

A field trial was established in 2014 at Højbakkegård Taastrup Campus, University of Copenhagen and harvested in 2015. Red fescue cv. Maxima was undersown in spring barley cv. Odyssey on 15 April 2014. The experiment had a randomized split plot design with four different sowing densities (2 kg ha^{-1}, 4 kg ha^{-1}, 6 kg ha^{-1}, and 8 kg ha^{-1}, four Moddus M (250 g L^{-1} trinexapac-ethyl) dosages (0 l ha^{-1}, 0.3 l ha^{-1}, 0.6

l ha^{-1}, and 1.2 l ha^{-1}) with four replicates of each treatment. Each plot had a width of 1.5 m and a length of 12 m. One half of the plot was treated with PGR and the other half was untreated. PGR treatments were applied on 11 May 2015. The harvest took place on 15 July 2015. NDVI measurements were taken with a handheld bi-directional narrow band spectrometer (Spectrosense 2) (Skye Instruments, Llandrindod Wells, UK) recording reflectance in red (647 nm) and NIR (799 nm) and with the incident light as a reference. Images were also taken from a drone (Norm Excess Green Index). Biomass samples were taken from 0.1 m^2 in each plot on 8 May and dry weights were measured. Hypotheses were tested using linear mixed models with sowing density and PGR dosage as factors and blocks and plots as random effects.

Results and discussion

Relation between NDVI and biomass in May was tested and the results are displayed in Figure 1A. Only NDVI for the seed density of 2 kg ha^{-1} was significantly different from the other densities (4 kg ha^{-1}, 6 kg ha^{-1}, and 8 kg ha^{-1}) (P=0.0003). At the time NDVI was measured (8 May) the plots sown with 4, 6 and 8 kg ha^{-1} were difficult to distinguish by eye and the canopy was uniform from above. One reason could be that the plants compensated for a low sowing rate by producing more tillers and at the time of measurement covered the ground at a similar extent, resulting in the same NDVI. Figure 1B shows that there was no correlation between NDVI in May and the seed yield (kg ha^{-1}) at harvest. The NDVI was not a good indicator of yield potential at the time it was measured. Measurements earlier in the season might give better indication and need to be investigated in the future. Surprisingly, there was no significant effect of PGR. The yield varied a lot (Figures 1B, D) and even for dense stands no lodging was observed and PGR seems to have been unnecessary in 2015. In Figure 1C the Norm Excess Green Index, calculated from images taken by a drone, shows low correlation with dry weight biomass (kg ha^{-1}) in May. Sowing density was not correlated to the Norm Excess Green Index and there was no correlation between Norm Excess Green Index and seed yield (kg/ha) at harvest (Figure 1D). In Figure 1 no linear correlation had R^2-values >0.1392.

Conclusions

Our results showed that it was not possible to use a measurement of NDVI in early May as a tool for estimating the need for applying Moddus M to red fescue and to forecast the seed yield. However, we are going to repeat the experiment to investigate seasonal variations.

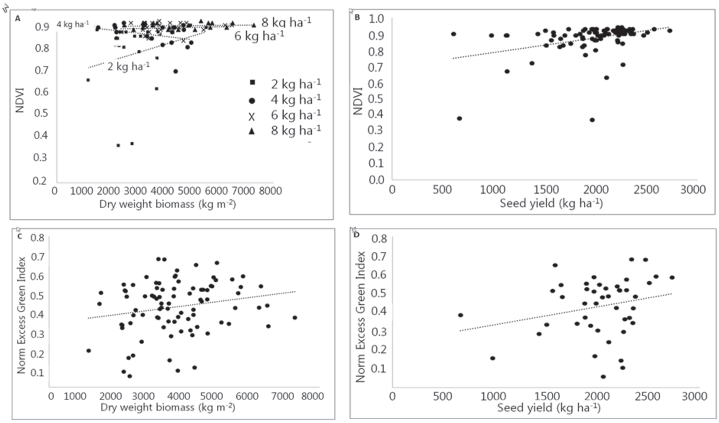

Figure 1. (A) NDVI measurements correlated with dry weight biomass (kg m^{-2}) on 8 May 2015. The symbols illustrate sowing densities equivalent to 2 kg ha^{-1}, 4 kg ha^{-1}, 6 kg ha^{-1} and 8 kg ha^{-1}. (B) NDVI measures correlated with seed yield (kg ha^{-1}). (C) Norm Excess Green Index (measured by the drone) correlated with dry weight on 11 May (kg ha^{-1}). (D) Norm Excess Green Index (measured by the drone) correlated with seed yield (kg ha^{-1}).

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Fractional harvest of perennial legumes can improve forage quality and their exploitation

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Abstract

Lucerne (*Medicago sativa* L.) is a widely used forage crop, but fodder galega (*Galega orientalis* Lam.) is grown only in northern environments. Galega is recognized as a long-lived, low-input crop; however, coarse stems decrease its fodder value. Fractionation of lucerne and galega leaves and stems at harvest could allow ruminant rations to be tailored for optimum economic return. Harvest fractionation was accomplished by stripping leaves and stem tops at harvest using a modified flail harvester. The stripped fraction consisted of about 70% leaf tissue for galega and 60% for lucerne. In harvested leaves and stem tops of galega the concentration of crude protein (CP) was 226, neutral detergent fibre (NDF) 492 and acid detergent fibre (ADF) 332 g kg^{-1} dry matter (DM); and *in vitro* digestibility was 79.2%, whereas whole plants contained 220, 493, 338 g kg^{-1} DM, and 77.5%, respectively. In harvested leaves and stem tops of lucerne, concentration of CP was 194, NDF 424, ADF 288 g kg^{-1} DM, and *in vitro* digestibility 80.4%, whereas whole plants contained 167, 507, 363 g kg^{-1} DM, and 74.3%, respectively. The proposed fractional harvest seems to be more promising for lucerne than for galega. Regardless of harvest method or maturity, galega has potential to be a protein and energy source for livestock in northern Europe.

Keywords: ADF, flail harvester, galega, *in vitro* true digestibility, lucerne, NDF

Introduction

Perennial legumes are a fundamental and relatively cheap source of protein and digestible fibre for ruminants, especially in areas with challenging environments. Lucerne and galega show promise as reliable crops in northern and central Europe in areas with light soil, relatively low precipitation and low winter temperatures (Ignaczak, 1999). A disadvantage of both species is rapid decline in nutritive value with maturity resulting from changing proportions of leaves and stems. Moreover, typical leaf loss during hay drying results in reduced nutritive value. A solution to both of these problems may be harvest fractionation of the plants, consisting in separate harvest of leaves and stems. Methods for fractional harvest of lucerne were tested in Sweden (Grajewski *et al.*, 1996) and in the USA (Shinners *et al.*, 2007). They involved adapting a mower-loader of the Fortschritt type (Sweden) or a combine harvester for harvest of green bean pods (USA).

The leaf portion of fractional harvest may constitute a raw material for production of protein concentrates and dried leaf meal, or blended with other materials and ensiled. Stems may be used as roughage, as substrate for bioethanol or biogas production, or as organic matter for soil amendment. This harvest method could allow for a delay in cutting time with no loss of nutritive value. In Poland, a flail harvester has been adapted for fractional harvest (Ignaczak and Andrzejewska, 2016). The objective of this work was to perform nutritive value assessment of fodder from galega and lucerne harvested using a modified flail harvester at early and late bloom stage.

Materials and methods

The study was carried out at the UTP Bydgoszcz, Mochełek Research Station (53°12'N; 17°51'E) in 2016. A 25-year-old plantation of galega and 3-year-old plantation of lucerne cultivated on fine-sandy loam, mixed mesic, Ustic, Typic Hapludalf with pH 6, were used in the study. First spring growth was harvested at early bloom or late bloom stage with a modified flail harvester (Ignaczak and Andrzejewska, 2016) in four replicates and a 500 g subsample was collected and hand separated into leaf and stem components. Before each machine-harvest, forage was also harvested by hand from four 1 m^2 quadrats to determine forage yield. After weighing, a subsample of about 500 g was used for hand separation into leaf and stem components. Plant material was dried at 55 °C and dry matter content, crude protein (CP), neutral and acid detergent fibre (NDF, ADF) and *in vitro* true digestibility (IVTD) were determined. Data were subjected to analysis of variance separately for each harvest method. The significance of differences was determined with Tukey's test at the probability $P<0.05$.

Results and discussion

Regardless of harvest time and method, dry matter yields of both species were similar. Yield harvested with the use of a modified flail harvester accounted for 59 and 54%, respectively, of the yield of galega and lucerne harvested by hand (Table 1). This resulted from the fact that stems with a length of 30-40 cm were left in the field (unpublished data). The proportion of leaves in yield of galega harvested by hand was on average significantly higher than in the yield of lucerne. With hand-harvest, a delay in time until late-bloom caused a significant reduction in leaf proportion in the yield of lucerne. The application of fractional harvest caused an increase in the proportion of leaf in yield, and additionally in lucerne it eliminated a difference resulting from the harvest time. In yield harvested with a modified flail harvester the proportion of galega leaves increased by 21 percentage points, and that of lucerne leaves by 49 percentage points, in comparison with harvest by hand (Table 1).

Galega harvested by hand contained more protein than plants of lucerne, but with fractional harvest these differences underwent considerable reduction (Table 2). Crude protein content in galega yield from fractional harvest was greater by 3%, and in lucerne yield by 16% compared with the crude protein content in the yield of plants harvested by hand (Table 2). The content of NDF in forage of both species harvested by hand was similar. Lucerne from fractional harvest contained significantly less NDF than galega (Table 2), and additionally, no significant increase in NDF content was observed in fodder from lucerne with a delay of the harvest time when fractionally harvested. Performing plant harvest using the fractionation method had a greater effect on reduction in ADF content in fodder from lucerne than from galega. Fodder from both species, regardless of the time of harvest, did not differ in *in vitro* true digestibility. A delay in harvest time always caused a significant decrease in the fodder digestibility. Galega from fractional harvest had the same NDF level as galega harvested by hand, whereas it contained 5% less ADF, and its digestibility was 2% greater. However, fractional harvest had a larger effect on lucerne fodder quality – the content of NDF decreased by 16%, the content of ADF by 21%, and digestibility increased by 8% compared to hand harvested forage (Table 2).

Table 1. Yield and proportion of leaves in yield of galega and lucerne as affected by harvest method and development stage.[1]

Harvest method and development stage	Dry matter yield (g m^{-2})		Proportion of leaves in yield (%)	
	Galega	Lucerne	Galega	Lucerne
By hand (H)				
Early bloom	547	567	59.9	45.9 a
Late bloom	646	638	54.3	36.1 b
Mean H	596	602	57.1 A	41.0 B
Fractionation (F)				
Early bloom	273	277	70.5	65.8
Late bloom	428	366	67.4	56.2
Mean F	351	322	68.9	61.0
% (F/H)	59	54	121	149

[1] Within each harvest method (H or F) significant differences between species are marked with different capital letters, and between harvest times with different small letters.

Table 2. Nutritive value of galega and lucerne depending on harvest method and development stage.

Harvest method and development stage	Crude protein (g kg^{-1})		NDF (g kg^{-1})		ADF (g kg^{-1})		IVTD (%)	
	Galega	Lucerne	Galega	Lucerne	Galega	Lucerne	Galega	Lucerne
By hand (H)								
Early bloom	257^{a}	195^{a}	450^{b}	457^{b}	309^{b}	321^{b}	81.8^{a}	79.7^{a}
Late bloom	183^{b}	138^{b}	537^{a}	557^{a}	366^{Ba}	405^{Aa}	73.2^{b}	68.9^{b}
Mean H	220^{A}	167^{B}	493	507	338	363	77.5	74.3
Fractionation (F)								
Early bloom	258^{a}	221^{a}	444^{b}	395^{a}	271^{b}	257^{b}	84.4^{a}	84.2^{a}
Late bloom	194^{b}	166^{b}	541^{Aa}	453^{Ba}	372^{Aa}	320^{Ba}	74.0^{b}	76.5^{b}
Mean F	226	194	492^{A}	424^{B}	322^{A}	288^{B}	79.2	80.4
% (F/H)	103	116	100	84	95	79	102	108

[1] Within each harvest method (H or F) significant differences between species are marked with different capital letters, and between harvest times with different small letters.
[2] NDF = neutral detergent fibre; ADF = acid detergent fibre; IVTD = *in vitro* true digestibility.

Values of fodder quality indexes from fractional harvest are not as high as those reported by Grajewski *et al.* (1994) and Shinners *et al.* (2007). This resulted from a relatively late harvest time of the plants and consequently, from a smaller share of leaves in yield. The modified flail harvester used in the study allows for harvesting nearly all leaves without damaging them. No leakage of juices from the leaves was observed, which occurred in the study by Grajewski *et al.* (1996). In the present study, the proportion of stem in yield accounted for 30-40%, but there is a possibility of further reduction of their contribution to yield by increasing the height of cutting.

Conclusions

Fractional harvest performed using a modified flail harvester is more promising for lucerne than for galega. Harvesting leaves and top parts of stems may be particularly justified when, due to the weather conditions, lucerne harvest cannot be performed at early development stages, when the fodder quality is the highest. Regardless of the harvest method, the nutritive value of fodder galega from the first spring regrowth is similar to the fodder value of lucerne.

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Wild species flowering margins for improved biodiversity in intensive agricultural areas

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Abstract

The experiment was carried out during the period 2013-2016 at the Joniskelis Experimental Station of the Lithuanian Research Centre for Agriculture and Forestry (LAMMC). The aim of the study was to determine the establishment of autochthonous species measured by their density in field margins, on land used intensively for agriculture. The seeds of 24 flowering species of plants from natural swards were collected during expeditions to river valley meadows. In experimental field margins these plants were grown with a mixture of wild meadow grasses. It was found that the seeds of *Thalictrum lucidum* L., *Gladiolus imbricatus* L. and *Gentiana cruciata* L. did not germinate. Autochthonous species, such as *Medicago falcata* L., *Medicago lupulina* L., *Achillea millefolium* L., *Galium boreale* L., *Galium mollugo* L., *Hypericum perforatum* L., *Trifolium pannonicum* L., *Centaurea jacea* L., *Heracleum sibiricum* L. and *Plantago lanceolata* L. formed a sufficient density of swards and plants grew well. *Medicago falcata* L. formed a greater sward density than *M. lupulina* and *G. mollugo* formed a higher density than *G. boreale.* The plant species density in the swards and their survival rate was influenced by the amount of mature seeds and their viability, competition within plants and environmental conditions. Most of the meadow plant species bloom from June to August.

Keywords: establishment of species, flowering time, seeds, sward density

Introduction

Areas of intensive agriculture have a tendency to expand, so the fragments of natural habitats decrease. Trees, shrubs and natural meadows mostly disappear. With decreasing plant diversity, species abundance and the occurrence of animals that have trophic relationships with plant diversity also decreases, and the general ecological state becomes worse. In this situation, and in these areas, many bugs, butterflies, worms and other small fauna disappear also (Karpavičienė and Marcinkonis, 2009). In Lithuania the natural native systems have been disrupted mostly in regions of intensive agriculture; here there are places with only 17% of required quantity of natural native fragments (Jonaitis *et al.*, 2002). In order to optimize the agro-ecosystems, it is necessary to preserve and create new natural fragmentation as an integral part of crop production. The natural grassland, especially in forests and river valleys, has a large variety of plant species. There are more than 30 kinds of natural communities of herbal plants, special areas with melliferous and officinal plants. Their reduction and deterioration due to abandonment or agricultural intensification are among the recent negative environmental changes in Central Europe (EEA, 2004). The aim of the present study was to investigate the establishment of autochthonous species of natural swards and formation density in field margins on land used intensively for agriculture.

Materials and methods

The experiment was carried out during 2013-2016 at the Joniskelis Experimental Station of the LAMMC. The soil of the experimental site is an Endocalcari Endohypogleyic Cambisol (CMg-n-w-can) with a clay loam topsoil texture. The seeds of 24 species of flowering plants were collected from natural communities during expeditions to river valley meadows. A plant strip (80×6 m) was established in an intensive agriculture area. A mixture of *Poaceae* grasses (*Holcus lanatus* L., *Poa compressa* L., *Poa pratensis* L., *Festuca* *trachyphylla* (Hack.) Krajina, *Festuca ovina* L., *Phleum phleoides* (L.) H. Karst. and *Agrostis capillaris* L.) was sown in 2014 at a seed rate of 16 kg ha^{-1}. The strip was divided into 72 plots (3×2 m) and 24 species (Table 1) were sown randomly in each plot (one species per plot with 3 replications). Measurement of plant density was carried out using a 0.25 m^2 (50×50 cm) frame, in spring and autumn of every investigated year. Plant density was assessed each time in the same two spots per plot. The germination capacity of the collected seeds was not tested. Flowering time for each species was determined from the beginning of flowering (10% and more deployed flowers) to the end of flowering (10% and less flowers). The Lithuanian climate is classified as boreal climate zone. In the territory of Central Lithuania there is sufficient precipitation (570 to 700 mm), but the low temperatures (Growing Degree Days with a base temperature of 10 °C are about 2,060-2,200) are the most important limiting factor for plant growth. Data were statistically processed by a one-factor analysis of variance using the program package Selekcija.

Results and discussion

In this study the seeds of meadow plants germinated slowly and in different intensity (Table 1). The density of *H. perforatum* (156 plants m^{-2}), *M. lupulina* (108 plants m^{-2}), *M. falcata* (106 plants m^{-2}), *A. millefolium* (76 plants m^{-2}) and *G. mollugo* (56 plants m^{-2}) was highest in the first observation year in 2014. A proportion of the seeds presumably germinated only in the second year.

No establishment of *T. lucidum, G. imbricatusa* and *G. cruciata* was observed. Swards of *A. millefolium* and *H. perforatum* had the highest density during the entire study period. The seeds of species such as *A. ursinum*, *C. persicifolia*, *D. borbasii* and *G. rivale* germinated and established in the second year and *A. vulneraria, I. salicina* in the third year. The same family species comparison shows that *M. falcata* formed a 56-72% greater sward density than *M. lupulina* and *G. mollugo* formed a 25-40% higher density than *G. boreale.* Other meadow plants such as *A. glycyphyllos*, *T. pannonicum*, *A. eupatoria*, *A. vulgaris, C. jacea*, *H. sibiricum, P. lanceolata*, *V. cracca*, *A. ursinum, V. teucrium* germinated at an intermediate rate and formed a sward of medium density. Malinauskaitė and Kukauskienė (2013) reported for instance that the germination of seeds of *Gentiana cruciata* depends on their collection time and seed dormancy. If harvested too early (end of August) seeds are sterile. Further changes in the density of plant species could be influenced by the quantity of mature seeds and intraspecific competition. Many of the investigated meadow plant species began blooming in June, mostly in the second half of the month and they flowered in June-August (Figure 1). The earliest flowering was of the meadow

Table 1. Natural meadow plants and their density (plants m^{-2}). Standard error of the means are given in brackets.

Plant species	Assessment date			Plant species	Assessment date		
	19/09/2014	25/05/2015	20/05/2016		19/09/2014	25/05/2015	20/05/2016
Anthyllis vulneraria L.	0	0	8 (0)	*Dianthus borbasii* Vandas.	0	4 (2)	0
Astragalus glycyphyllos L.	4 (0)	12 (5)	12 (0)	*Galium boreale* L.	42 (3)	36	54 (5)
Medicago falcata L.	106 (15)	86 (15)	64 (2)	*Galium mollugo* L.	56 (9)	60 (5)	84 (18)
Medicago lupulina L.	108 (25)	24 (2)	28 (1)	*Gentiana cruciata* L.	0	0	0
Trifolium panonicum L.	32 (9)	28 (9)	38 (13)	*Geum rivale* L.	0	12 (1)	4 91)
Vicia cracca L.	18 (1)	22 (13)	2 (1)	*Gladiolus imbricatus* L.	0	0	0
Achillea millefolium L.	76 (16)	488 (23)	264 (23)	*Heracleum sibiricum* L.	20 (2)	24 (7)	18 (4)
Agrimonia eupatoria L.	9 (3)	20 (3)	12 (3)	*Hypericum perforatum* L.	156 (35)	134 (27)	136 (51)
Allium ursinum L.	0	8 (1)	16 (2)	*Inula salicina* L.	0	0	2 (1)
Aquilegia vulgaris L.	26 (12)	44 (21)	12 (0)	*Plantago lanceolata* L.	14 (1)	14 (1)	30 (10)
Campanula persicifolia L.	0	4 (1)	4 (0)	*Thalictrum lucidum* L.	0	0	0
Centaurea jacea L.	36 (7)	36 (9)	28 (7)	*Veronica teucrium* L.	4 (3)	19 (7)	5 (4)

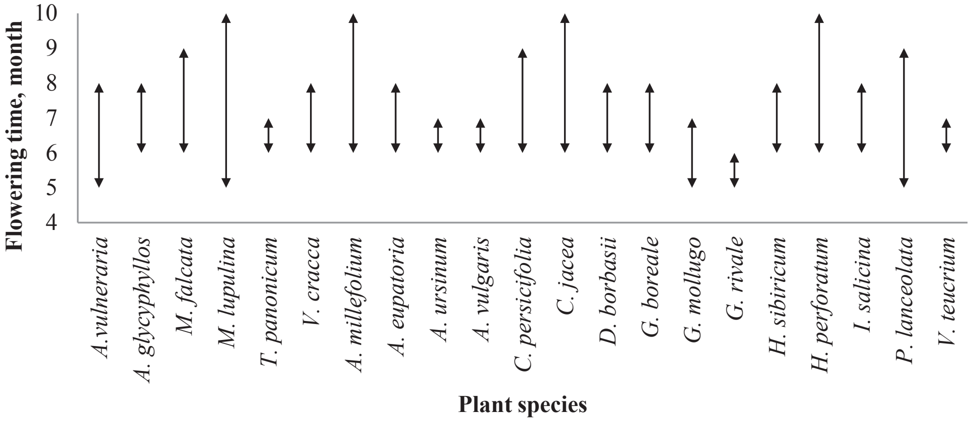

Figure 1. Plant species flowering time (↓ – beginning of flowering, ↑ – end of flowering).

plant species *A. vulneraria*, *M. lupulina, G. mollugo, G. rivale* and *P. lanceolata*. *Medicago lupulina*, *A. millefolium, C. jacea* and *H. perforatum* can still bloom in October.

Conclusions

Autochthonous species, such as *M. falcata, M. lupulina*, *A. millefolium*, *G. boreale*, *G. mollugo, H. perforatum, T. panonicum, C. jacea*, *H. sibiricum* and *P. lanceolata* formed a sufficient density of swards and plants grow well. Most of the meadow plant species bloom from June to August. *Medicago lupulina, A. millefolium, C. jacea, H. perforatum* and *P. lanceolata* bloom for the longest period of time. These species form dense swards with a long flowering period, and are suitable for establishment of field margins on land used intensively for agriculture.

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Herbage yield and quality of Persian clover and annual ryegrass mixtures harvested at different growing stages

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Abstract

This research was conducted to determine the effects of mixture rates and harvest periods on hay yield and quality of Persian clover (PC) and annual ryegrass (RG) mixtures. Experiments were conducted in the Black Sea Region during the years 2012 and 2013, using a randomized complete block design. Pure stands of PC and RG and their mixtures (80% PC + 20% RG, 70% PC + 30% RG, 60% PC +40% RG, 50% PC + 50% RG, 40% PC + 60% RG) were investigated. Hay yield, crude protein content, acid detergent fibre (ADF) and neutral detergent fibre (NDF) ratios were determined in different harvest periods (beginning of budding and 25% flowering). Average hay yields were between 4,732 and 8,121 kg ha^{-1} at the beginning of budding, and between 7,261 and 11,042 kg ha^{-1} at 25% flowering stage. At beginning of budding crude protein varied between 13.26 and 17.82%, ADF between 37.03 and 41.46% and NDF between 46.55 and 57.74% at beginning of budding; the values at 25% flowering varied, respectively, between 10.50 and 15.22%, between 41.66 and 47.63% and between 50.93 and 61.86%. It was concluded that harvests should be performed between budding and the beginning of flowering and mixture ratios should be selected as either 20RG+80PC or 30RG+70PC.

Keywords: Persian clover, annual ryegrass, harvest period, hay yield, crude protein

Introduction

Soil and climate conditions exhibit great diversity in Turkey and such diversity potentially allows farmers to cultivate several forage crops of those grown worldwide. However, the actual number of cultivated forage crops is quite low in Turkey. Therefore, there is a quite high deficit in forage supply in the country. Intercropped cultivation of legumes and grasses may provide higher yields and a more stable forage source rich in carbohydrate and protein for livestock feeding. However, to have higher yields and more stable herbage from legumes + grasses in intercropping systems, the mixture rates of each component should be well-adjusted (Shoaib *et al.* 2016). For the Samsun region, Persian clover (*Trifolium resupinatum* L.) and annual ryegrass (*Lolium multiflorum* L.) are recommended as alternative crops to meet the forage deficit (Hatipoğlu *et al.*, 2005). Persian clover (PC) (*Trifolium resupinatum* L.) and annual ryegrass (RG) (*Lolium multiflorum* L.) can be grown for hay production until the end of May on land left uncultivated after the harvest of summer crops in October, without detriment to the main crop. The present study was conducted to determine the best mixture ratio for hay yield and nutrient composition at different harvest periods in Persian clover + annual ryegrass mixtures in Samsun (Humid Mediterranean climate).

Materials and methods

Persian clover cv. Demet-82 and annual ryegrass cv. Efe-82 were used in the experiments. Besides pure stands of PC and RG, various mixtures (80% PC + 20% RG, 70% PC + 30% RG, 60% PC + 40% RG, 50% PC + 50% RG, 40% PC + 60% RG) were investigated in this study. The experimental soils had clay texture (54.75% clay), were neutral in pH (6.86), and medium in organic matter (2.93%). During the vegetation period (from November to May), total precipitation was 369.9 mm and mean temperature was 9.4 °C. Experiments were conducted using a randomized complete block design during the years 2012 and 2013. Hay yield, crude protein content (%), Acid detergent fibre (ADF) and neutral detergent fibre (NDF) ratios were determined for different harvest periods [(1) beginning of budding and (2) 25% flowering)]. Plots were not irrigated. Crude protein, ADF and NDF analyses of dried, milled samples were performed in Foss NIR Systems (Hoy *et al.*, 2002) Model 6500 Win ISI II v1.5 device by using the IC-0904FE calibration program. Data were subjected to statistical analyses with SPSS 17.0 software.

Results and discussion

Hay yields, crude protein contents, ADF and NDF ratios of Persian clover and annual ryegrass mixtures harvested at different periods are provided in Table 1. Significant differences were observed in dry hay yields within and between harvest periods. Dry hay yield was measured as 6,619 kg ha^{-1} at the beginning of budding and 9,788 kg ha^{-1} at 25% flowering. While the lowest yields were observed in pure stands of PC and RG at the beginning of budding, the greatest yield was obtained from all treatments at the 25% flowering stage, except in pure stands of PC (Table 1). Lodging was observed in these plots at 25% flowering. Soya *et al.* (1981) reported increasing yields in PC until the beginning of flowering and decreasing yields later on. Shoaib *et al.* (2016) reported higher yields for intercropping systems than for pure stands. The differences in crude protein contents of the harvest periods were also found to be significant and protein ratios decreased with the progress of plant growth stages (respectively as 15.11

Table 1. The results obtained from Persian clover and annual ryegrass mixtures.[1,2]

Traits	Harvest period		Mixing ratio (%)							
			20 RG+80 PC	30 RG+70 PC	40 RG+60 PC	50 RG+50 PC	60 RG+40 PC	100 PC	100 RG	Average
Hay yield	1	PC	5,710.0	3,911.9	3,645.4	3,625.8	3,049.2	4,732.0	-	6,619.3^B
(kg ha^{-1})		RG	2,411.4	3,120.4	3,400.7	3,651.2	3,716.1	-	5,361.7	
		Total	8,121.4^a	7,032.3^{ab}	7,046.1^{ab}	7,277.0^{ab}	6,763.5^{abc}	4,732.0^c	5,362.7^{bc}	
	2	PC	6,093.3	6,234.7	5,196.4	4,967.9	6,308.5	7,269.7	-	9,877.7^A
		RG	4,534.7	4,550.4	4,396.63	6,026.2	4,733.7	-	8,840.9	
		Total	10,628.0^a	10,785.1^a	9,593.0^{ab}	10,994.1^a	11,042.2^a	7,260.7^b	8,840.9^{ab}	
	Average		9,374.7^a	8,908.7^a	8,319.3^{ab}	9,135.5^a	8,903.7^a	5,995.8^c	7,101.2^{bc}	
Crude protein	1	PC	17.4	16.4	15.8	17.4	16.8	17.8	-	15.1^A
(%)		RG	12.3	13.3	12.8	13.1	12.0	-	13.3	
		Average	15.9^a	15.0^a	14.4^{ab}	15.2^a	14.2^{ab}	17.8^a	13.3^b	
	2	PC	13.8	13.9	15.5	14.2	14.1	15.2	-	13.0^B
		RG	10.4	11.5	11.2	11.9	10.9	-	10,5	
		Average	13.4^b	12.9^b	13.5^b	12.9^b	12.7^b	15.2^a	10.5^c	
	Average		14.1^b	13.9^b	13.9^b	14.1^b	13.4^b	16.5^a	11.9^c	
ADF (%)	1	PC	35.0	35.7	35.4	39.2	35.9	37.2	-	41.3^B
		RG	41.9	40.7	44.9	39.5	43.4	-	41,5	
		Average	37.0^c	37.9^{bc}	39.9^{ab}	39.4^{abc}	40.1^{ab}	37.2^c	41.5^a	
	2	PC	42.1	40.2	43.7	41.4	41.1	43.6	-	43.7^A
		RG	44.0	43.7	44.8	43.8	46.5	-	47,6	
		Average	42.9^{bc}	41.7^c	44.2^b	42.8^{bc}	43.5^{bc}	43.6^{bc}	47.6^a	
	Average		39.9^c	39.8^c	42.1^{bc}	41.0^b	41.7^b	40.4^{bc}	44.5^a	
NDF (%)	1	PC	42.4	45.0	46.3	46.9	46.0	47.6	-	50.8^B
		RG	56.5	54.7	57.4	56.5	55.2	-	57,7	
		Average	46.5^d	49.3^{bc}	51.6^b	51.7^b	51.1^b	47.6^{cd}	57.7^a	
	2	PC	51.7	46.3	50.2	48.6	47.9	50.9	-	55.4^A
		RG	59.9	60.4	65.4	63.2	61.5	-	61,9	
		Average	55.2^{bc}	52.3^d	57.1^b	56.6^{bc}	53.7^{cd}	50.9^d	61.9^a	
	Average		50.9^{de}	50.80^{de}	54.4^b	54.2^{bc}	52.4^{cd}	49.3^e	59.8^a	

[1] Means with the same letter in the same row and in the same column are not significantly different at 0.05.

[2] ADF = acid detergent fibre; NDF = neutral detergent fibre; PC = Persian clover; RG = rye grass.

and 13.02%). While the average ADF ratio of hay harvested at the beginning of budding was 41.35%, the value was measured as 43.72% at 25% flowering. ADF ratios at the beginning of budding varied between 37.03 and 41.46% and the ADF ratios at 25% flowering varied between 41.66 and 47.63%. With regard to ADF ratios, hay obtained from all plots, except for the pure stand of RG plot, had a good quality according to AFGC (2009). While the average NDF ratio of hay harvested at the beginning of budding was 50.81%, the ratio was measured as 55.40% at 25% flowering. As the average of both harvest periods, the lowest average NDF ratio was observed in pure stand of PC plots and the greatest NDF ratio was observed in pure stand of RG plots. The mixtures with the lowest NDF ratio were identified as 20RG+80PC and 30RG+70PC. Overall, crude protein ratios of herbage decreased, whereas ADF and NDF ratios increased with the progress of ripening, in line with the results of Kaya (2008).

Conclusions

It was concluded in this study that, based on hay yield, crude protein content, ADF and NDF ratio of hay, harvest should be performed between budding and the beginning of flowering and mixture ratios should be selected as either 20RG+80PC or 30RG+70PC.

Acknowledgements

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Can diverse forage mixtures optimise ruminant animal production, resilience and environmental impact?

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Abstract

Greater sustainability, biodiversity and resilience of ruminant livestock production systems may be achieved through the harnessing of diverse forage mixtures. These mixtures have greater potential productivity under variable climates, include species with N-fixation capabilities, and contribute to greater N use efficiency at an animal and farm scale compared with the conventional swards typically grazed in the UK. This new study funded by SARIC, BBSRC and NERC aims to assess three diverse forage mixtures against a control of perennial ryegrass with mineral fertiliser N across four sites in southern England over three years. The three diverse forage mixtures comprise six (Smartgrass), twelve (Biomix) and seventeen (Herbal Ley) species, including grasses, legumes and other forbs. In addition to the main agronomy trial sites, the mixtures are being trialled on ten commercial farms over the three years. Measurements will include forage quality and quantity throughout each year; production resilience in drought-prone and waterlogged soils; growth and N-use efficiency of growing cattle grazing or fed conserved forage; and finally performance and practicalities of inclusion of diverse forage mixtures in current farming systems across the South and West of the UK. This paper outlines the main experimental components of this new project.

Keywords: diverse, productivity, sustainable, resilience, nitrogen use efficiency

Introduction

Grassland accounts for 72% of agricultural land in the UK (Defra, 2016) and forage is predominately comprised of perennial ryegrass (*Lolium perenne* L.) or perennial ryegrass and white clover (*Trifolium repens* L.). Both of these species require moist, cool conditions to flourish and ryegrass monocultures need nitrogen (N) fertiliser inputs to produce optimum productivity. With climate change and more frequent extreme weather events such as flooding and drought predicted, there are concerns regarding the persistency of perennial ryegrass. Moreover, there is increasing urgency to improve nitrogen use efficiency (NUE) in pasture systems to reduce production costs and the negative environmental effects associated with the production and application of inorganic N fertilisers, including greenhouse gas emissions and acidification and eutrophication of land and water bodies. Diverse forage mixtures offer potential advantages over conventional perennial ryegrass monocultures and binary perennial ryegrass-white clover mixtures. These potential benefits include greater agronomic value in terms of productivity and forage quality, improved soil structure and fertility, increased faunal diversity, improved NUE and resilience to atypical weather events. Studies have shown that diverse plant communities often produce more biomass (Cardinale *et al.*, 2007) and improve resource capture due to species-niche complementarity and positive interspecific interactions (Lüscher *et al.*, 2014) than monocultures. Including plants containing tannins and other bioactive compounds within the sward can reduce enteric methane emissions (Hammond *et al.*, 2014). Swards containing species known to enhance the efficiency of protein utilisation in ruminants and to reduce N losses in silo, such as birds-foot trefoil (*Lotus corniculatus* L.) and red clover (*Trifolium pratense* L.) have the potential to deliver substantial benefits to the environment. The inclusion of legumes in a sward can benefit non-N_2 fixing plants through the transfer of symbiotically fixed N and improve NUE by reducing reliance on inorganic N inputs.

The main objective of this new study is to examine the productivity, nutritional value, resilience, and environmental impact of diverse forage mixtures as an alternative to conventional pasture species. Three diverse forage mixtures with increasing species-richness have been established in southern England in a series of experiments and will be monitored to test if they deliver more sustainable ruminant production over multiple seasons than conventionally managed ryegrass.

Materials and methods

The project duration is for five years, year one (2016) to establish three diverse forage mixtures at a number of sites followed by three years of monitoring and then data analysis and dissemination in the final year. The forage mixtures were developed by reviewing literature for key agronomic and functional attributes of pasture plant species and at an initial workshop attended by project and industry partners. The three forage treatments are: (1) 'Smartgrass' comprising two grass, two legumes and two non-legume forbs (forb) species; (2) 'Biomix' comprising five grasses, five legumes and two forbs; (3) 'Herbal Ley' comprising six grasses, six legumes and five forbs. The project consists of five work packages (WP).

WP 1 (agronomy) aims to quantify the agronomic performance and environmental footprint of the three diverse forage mixtures in comparison with perennial ryegrass (PRG) under varying climates and soil type. A replicated set of plots (four replicates per mixture) were sown in autumn 2016 at four sites: University of Reading Centre for Dairy Research, Arborfield, Berkshire; Duchy College, Stokes Climsland, Cornwall; University of Reading Crop Research Unit, Sonning, Berkshire; and Rothamsted Research, North Wyke, Devon. The PRG mixture was sown at a rate of 34.6 kg ha^{-1} and the other three mixtures at 32.1 kg ha^{-1}. Recommended rates of N fertiliser for each site will only be applied to the PRG control plots. Treatment swards will be cut and herbage removed based on best practice for diverse mixtures. Each treatment is expected to grow at a different rate hence the more productive swards will be cut more frequently. WP2 (forage utilization) aims to evaluate the performance of the four treatment forages when grazed, fed fresh indoors and fed ensiled forage indoors through assessment of livestock growth rate, digestibility and enteric methane emission. For this purpose, a 20 ha paddock was established in autumn 2016 at Arborfield, Berkshire with five replicate plots of each treatment mixture. Holstein Friesian steers, aged approximately 15 months, will rotationally graze the forage treatments for the duration of the growing season. Steers in one indoor feeding trial will be fed surplus fresh forage cut from the treatments throughout the grazing season and in the second indoor trial steers will be fed conserved forage at the end of the grazing season. WP3 (on-farm evaluation) aims to assess the performance of the two most species-rich seed mixtures, Biomix and Herbal Ley, on ten farms in southern England representing a range of soil types and climatic conditions. Each mixture was established in 0.5 ha plots in autumn 2016 and will be compared with the farmer's own preferred seed mix. Recommendations regarding seed-bed preparation, establishment and sward management were given; however, farmers have complete sward management control for the duration of the experiment. WP4 (resilience) aims to determine the resilience of the four forage mixtures to environmental stress. An experiment with four replicates per treatment was established in autumn 2016 at two sites in Sonning, Berkshire; one that is historically prone to drought and one prone to water logging. Management of this experiment will be the same as WP1. A wide range of measurements will be undertaken across WPs 1 to 4 to assess plant performance, productivity and nutrient cycling of the forage treatments. Species presence, biomass and developmental status, forage quality and productivity (dry matter, protein, sugars, fibre and ash), soil structure and nutrient content and temperature and rainfall will be monitored. WP4 will have additional measurements using methodology outlined in a former study (Hammond *et al.*, 2014). Faeces samples will be collected to determine total tract digestibility and urine and faeces samples will be analysed to assess N utilisation. Methane emissions will be measured using SF_6 tracer.

WP5 (Nitrogen cycling and ecosystems services modelling) will integrate the results from the first four WPs using an existing field scale model, SPACYS (Wu *et al.*, 2007) and farm scale model, SIMS(DAIRY) (del Prado *et al.*, 2011) of nutrient balance and ecosystem services to determine the potential benefits of diverse swards. The greenhouse gas and ammonia emission inventory models for UK agriculture will be used to upscale the potential impacts of diverse grasslands at a national-scale. Results will be disseminated through workshops.

Discussion

Diverse forage mixtures have the potential to deliver more sustainable, productive and profitable ruminant livestock agriculture during future climate change scenarios than conventional monoculture or binary swards. This project aims to validate that diverse pastures can be productive, resilient and persistent over four years and have positive effects on the wider ecosystem across a range of environments. We envisage the project will demonstrate increased floristic diversity improves NUE through reduced N fertiliser inputs and the effects on animal use of N. Results from this project will have wide-scale impact on farm sustainability in terms of livestock production systems, soils and the environment.

Acknowledgements

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Biomass allocation to shoots and roots, and nutrient content in herbage legumes

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Abstract

Specialisation on organic agriculture and environmentally sound production methods have led to a need for higher proportions of herbage legumes in crop rotations. There are additional purposes of species cultivation, and some non-native species have been introduced. With the aim of elucidating the production potential of legume biomass, and its allocation and chemical composition, a field experiment comprising six herbage legume species was carried out at the Estonian Crop Research Institute. White melilot (*Melilotus albus* Medik.) produced the highest biomass yield (14.9 Mg ha^{-1}) of which the total nitrogen yield was 326.8 kg ha^{-1} and the C/N ratio 19.9. The respective figures for red clover (*Trifolium pratense* L.) resembled white melilot and the difference between these species was not significant. The biomass of annual clovers amounted to 42.8-43.3% that of red clover. Aboveground parts of the studied species comprised 75.4-83.0% of the biomass, which if harvested at full bloom, contained 78.8-88.4% of fixed nitrogen, 81.1-86.3% of carbon, 72.7-84.6% of phosphorus and 86.0-92.1% of potassium. Bigleaf lupine (*Lupinus polyphyllus* Lind.) was distinguished by its biomass allocation, of which 55.7% was roots and stubble. An effective strategy for enhancing the soil fertility is to cultivate white melilot or red clover on one field of a crop rotation and to plough in the entire biomass.

Keywords: red clover, alsike clover, white melilot, bigleaf lupine, crimson clover, berseem clover, biomass

Introduction

In organic plant production it is necessary to include a leguminous crop into a rotation in order to maintain soil fertility and crop productivity. The same requirement is stated in the EU legislation that regulates environmentally sound crop production. There are no prescriptions about crop species and utilization of the produce. The following species serve this purpose in Estonia: red clover (*Trifolium pratense* L.), alsike clover (*Trifolium hybridum* L.), white melilot (*Melilotus albus* Medik.) and bigleaf lupine *(Lupinus polyphyllus* Lind.). In case of lack of domestic seed of native species, the seed of crimson (*Trifolium incarnatum* L.) and berseem clover (*Trifolium alexandrinum* L.) can be imported from Mediterranean countries, mainly Italy. Aboveground biomass of the clovers can be used as forage of superior quality. The herbage of white melilot and bigleaf lupine is not used as cattle feed because of coumarin content in the former and alcaloids in the latter. Nevertheless, white melilot and bigleaf lupine have been recently regarded as suitable raw materials for biogas production (Jasinskas *et al.*, 2008; Kryzeviciene *et al.*, 2008; Hensgen and Wachendorf, 2016).

There are two alternative uses that may be applied to all these named species: either (1) a part, just the roots and stubble, or (2) the entire biomass, is ploughed into the soil with the aim of soil fertility maintenance and improvement. The purpose of the current research was to compare the biomass production potential of the aforementioned herbage legumes, elucidate the allocation between shoots and roots and nutritional composition, and to quantify the return of the elements into the soil by the two alternative ways of ploughing.

Materials and methods

Red clover cultivar (cv. Jõgeva 433; 2n), alsike clover (cv. Jõgeva 2; 2n), bigleaf lupine (cv. Lupi), white melilot (cv. Kuusiku), crimson clover (cv. Contea) and berseem clover (cv. Alex), were seeded into a field trial at the Estonian Crop Research Institute. The first three species are perennials, white melilot is biennial and the two latter are annuals. All the species were seeded into the trial in pure stands at 15 cm drill space, except bigleaf lupine (at 30 cm). Each species was seeded onto an area of 432 m^2. Plots of 8.4 m^2 were randomly located within this area in four replicates. Biennial and perennial species were sown in July 2011, annuals in April 2012. The seeding rate (in kg ha^{-1}) of red clover was 11, alsike clover 8.8, bigleaf lupine 25, white melilot 23.8 and annual clovers 25 kg. No mineral or organic fertilisers were applied. The trial site was on a calcaric cambisol (K_0), clayish loam in texture, with the following agrochemical characteristics: pH_{KCl} 6.7, phosphorus (P) 65, potassium (K) 102 mg kg^{-1}, calcium (Ca) 4.2, organic carbon (C_{org}) 35 and total nitrogen (N) 2.6 g kg^{-1}.

Weather conditions favoured the growth of forage crops in 2011 and 2012. Precipitation in July and August in the sowing year (2011) was greater than the long-term average, and warm weather prevailed. The year 2012 was also rainy (110 mm in June, i.e. 163%), but air temperatures remained lower than average.

In crop rotations in Estonia a green manure crop precedes winter cereals, primarily rye (*Secale cereale* L.), for which the optimal sowing time is 25-31 August. An interval of one month should remain between the ploughing of green manure and sowing of winter cereals. Therefore the biomass characteristics were determined at full bloom of the stands on 1 August 2012 in the current trial, using a plot harvester (Hege 212). In fast-developing bigleaf lupine the herbage yield was measured at full bloom on 20 June and the aftermath was harvested on 1 August. The dry matter yields of the two harvests were summed for this species. The samples were collected from an area of 15×30 cm and a depth of 25 cm on one occasion before ploughing in of all species for the determination of the amount of roots and stubble in the topsoil. The roots were washed with a sieve (mesh size 3 mm), dried and weighed. All measurements were performed in four replicates and the data are presented as dry matter.

Herbage dry matter from aboveground biomass and roots was analysed for the contents of N, C, P and K. Statistical analyses (ANOVA and Fisher´s LSD) were carried out by Agrobase 20™.

Results and discussion

Approximately equal quantities of total biomass and C were ploughed in on the second-year stands of white melilot, red and alsike clover (Table 1). The bigleaf lupine produced significantly less biomass than these three species. Prior to ploughing, annual clovers made up just 42.8-43.3% of the red clover biomass. White melilot and red clover excelled in having the highest quantities of N in the biomass, with 327 and 319 kg ha^{-1} respectively. Their respective C/N ratios (19 and 20) in the biomass were the most favourable among the species. The benefit of white melilot over the remaining species consisted in it having higher quantities of P (48 kg ha^{-1}) and K (280 kg ha^{-1}) in the biomass to be ploughed into the soil. The tested species have maximum effect on soil fertility maintenance and improvement only if the entire biomass is ploughed in.

The aboveground part constituted the major portion of total biomass (75.4-83.0%) in most of the studied species. Bigleaf lupine was exceptional in allocating 55.7% of its total biomass into the roots and stubble. Removal of the harvestable biomass takes away 78.8-88.4% of the total N, 72.9-82.9% of P and 85.8-92.1% of the K quantity within the biomass. The figures again differ somewhat for bigleaf lupine: viz. 62.3% of N, 58.3% of P and 67.7% of K. Lupine is invasive and for this reason it cannot be recommended for use in organic farming.

Table 1. Biomass production of herbage legumes (DM, Mg ha^{-1}), its allocation and nutrient stocks (kg ha^{-1}).

	Species	Dry matter	N	C	C/N	P	K
Aboveground biomass	*Trifolium pratense*	11.20^{ab}	259^{a}	5,147^{ab}	20	29^{b}	211^{b}
	Trifolium hybridum	10.80^{b}	195^{b}	4,912^{b}	25	27^{b}	217^{b}
	Lupinus polyphyllus	4.57^{c}	99^{c}	2,009^{c}	20	14^{c}	111^{c}
	Melilotus albus	12.34^{a}	286^{a}	5,609^{a}	20	35^{a}	258^{a}
	Trifolium incarnatum	5.02^{c}	98^{c}	2,240^{c}	23	14^{c}	108^{c}
	Trifolium alexandrinum	4.76^{c}	98^{c}	2,101^{c}	21	14^{c}	109^{c}
	LSD 0.05	1.20	46	567		5	38
Root biomass in the 0-25 cm soil layer	*Trifolium pratense*	3.53^{b}	60^{a}	1,086^{b}	18	6^{c}	25^{b}
	Trifolium hybridum	2.99^{c}	51^{b}	970^{bc}	19	7^{c}	23^{b}
	Lupinus polyphyllus	5.74^{a}	59^{a}	1,966^{a}	33	10^{b}	53^{a}
	Melilotus albus	2.53^{c}	41^{c}	894^{c}	22	13^{a}	22^{b}
	Trifolium incarnatum	1.36^{d}	13^{e}	426^{d}	33	3^{d}	15^{c}
	Trifolium alexandrinum	1.55^{d}	27^{d}	490^{d}	19	3^{d}	18^{bc}
	LSD 0.05	0.50	7	166		2	4
Total biomass	*Trifolium pratense*	14.73^{ab}	319^{a}	6,233^{ab}	19	35^{b}	236^{b}
	Trifolium hybridum	13.79^{b}	246^{b}	5,882^{b}	24	34^{b}	240^{b}
	Lupinus polyphyllus	10.31^{c}	159^{c}	3,975^{c}	25	24^{c}	164^{c}
	Melilotus albus	14.87^{a}	327^{a}	6,503^{a}	20	48^{a}	280^{a}
	Trifolium incarnatum	6.38^{d}	111^{d}	2,666^{d}	24	17^{d}	123^{d}
	Trifolium alexandrinum	6.31^{d}	125^{cd}	2,591^{d}	21	17^{d}	127^{d}
	LSD 0.05	1.00	39	485		3	33

[1] Means within each column with a letter in common are not significantly different according to Fisher's LSD test.

Conclusions

In the second year the biomass production potential of red clover equals that of white melilot, a traditional green manure crop. Also, the amount of N taken into the soil with the biomass and the C/N ratio are close. The biomass of white melilot returns significantly more P and K to the soil compared with red clover. Alsike clover resembles red clover in biomass production capacity and the quantity of P and K, but it contains significantly less N in the biomass. By 1 August the annual clovers made up less than half of the red clover's biomass, partly due to their shorter growing period. They return 111-125 kg ha^{-1} N into the soil, i.e. just a third of the respective figure for red clover. Among the studied species, bigleaf lupine was distinguished by having a vigorous root system that can comprise over half of the total biomass.

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Whole soybean grain decreases fungal development in rehydrated corn grain silage

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Abstract

In silages, the type of substrate affects the development of microorganisms and ultimately the conservation efficiency. Thus, our objective was to evaluate the development of yeasts and moulds on rehydrated concentrate containing different protein sources and it effects over aerobic stability. The concentrates were formulated with ground corn grain and a protein source (whole soybeans or soybean meal). Water was added onto concentrates to increase the moisture content to 30%. A commercial inoculant containing *Lactobacillus plantarum* and *Propionibacterium acidipropionici* was applied to supply 1.25×10^5 cfu g^{-1}. Afterwards, the treated material was packed into three 200 litre plastic barrels and stored for 60 d. At silo opening, samples were collected for microbial counts and aerobic stability test. Serial dilutions (10^{-2} to 10^{-5}) were prepared (3 repetitions per dilution) and plated on to Petrifilm®YM count plates. The plates were incubated at 28 °C for 5 d. For the aerobic stability test 3 kg of each silage were placed in plastic buckets (4 repetitions per treatment) and allocated to a climate controlled chamber maintained 25°C for 144 h. The temperature of silages was measured using a digital thermometer twice a day and samples were collected to determinate the pH daily. Grain silage containing whole soybeans showed lower counts of yeasts and moulds and higher aerobic stability upon air exposure.

Keywords: aerobic deterioration, grain silage, mould, yeast

Introduction

Ensiling cereal grains with high moisture content typically increases starch digestibility due the occurrence of proteolysis during fermentation (Hoffman *et al.*, 2011). When corn grain with its original moisture is not available, a widespread technique is rehydration of the grains to moisture contents from 10 to 30-35% before ensiling, which makes possible the process of conservation by fermentation. However, high-moisture grain silages are prone to deterioration caused by the development of undesirable microorganisms such as yeasts and moulds, which ultimately will result in dry matter losses and loss of hygienic quality. As an alternative to ensiling high-moisture corn grain as a sole substrate, mixing corn grain and concentrates rich in protein could be attractive for farmers looking for an easier feeding management. Thus, our objective was to evaluate the development of yeasts and moulds and aerobic stability of corn grain silages containing whole soybeans or soybean meal as protein sources.

Materials and methods

The ensiled concentrates were formulated with ground corn grain and whole soybeans or soybean meal. Water was added on to the ground grains to increase the moisture content to 30%. A commercial inoculant containing *Lactobacillus plantarum* and *Propionibacterium acidipropionici* was applied to supply 1.25×10^5 cfu g^{-1}. Afterwards, the concentrates were packed into three 200 litre plastic barrels and stored for 60 d. At silo opening, samples were collected for microbial counts. From each sample 25 g were weighed and diluted into 225 mL of sterile saline solution (9 g of NaCl l^{-1} of distilled water) and manually agitated to homogenize the solution. Serial dilutions (10^{-2} to 10^{-5}) were prepared (3 repetitions per dilution) and plated onto Petrifilm®YM count plates. Plates were incubated at 28 °C for 5 d. For the aerobic stability test, 3 kg of each silage were placed in plastic buckets (4 repetitions per treatment) and allocated to a climate controlled chamber maintained at 25 °C for 144 h. Silage temperature was measured twice a day (at 8:00 am and 16:00 pm) using a digital thermometer. Samples were collected to determinate the pH daily (at 16:00 pm). The assessed variables were aerobic stability (h), maximum temperature (°C), sum of temperatures (°C), mean temperature (°C), maximum pH, time to reach maximum pH (h) and mean pH. The experimental design was completely randomized and data were analysed using the MIXED procedure of SAS. Means were compared by Student *t* test (α=0.05).

Results and discussion

Silage based on corn grain plus whole soybean grain showed the highest aerobic stability (47.7 h) and maximum temperature during air exposure (50.57 °C) compared with corn grain plus soybean meal silage (15.0 h and 47.5 °C, respectively) (Figure 1). On the other hand, silage containing soybean meal showed the greater sum of temperature (620 vs 560 °C), mean temperature (41.3 vs 37.3 °C), mean pH (5.36 vs 4.73) and time to reach maximum pH (137 vs 118 h) (Table 1). Concentrate containing whole soybean showed reduced yeast counts. Yeasts consume soluble carbohydrates and organic acids, even lactic acid, increasing the dry matter losses and reducing the silage aerobic stability after silo opening (Hao *et al.*, 2015). The use of raw soybeans reduced mould contamination. Moulds have a slower development compared to yeasts. However, they are capable of producing mycotoxins which pose a risk to animal and human health (Carvalho *et al.*, 2016). The polyunsaturated fatty acids (PUFA) within soybean grain can inhibit the microbial development due to their amphipathic characteristic. The PUFA can bind to cell membranes causing a disruption in cell membrane which leads to the loss of selective permeability of protons and ions (Sikkema *et al.*, 1994).

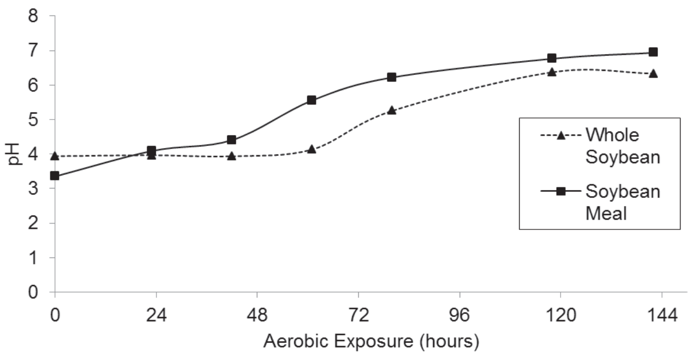

Figure 1. Influence of different protein sources on pH of rehydrated corn grain silage during aerobic exposure.

Table 1. Microbial counts and aerobic stability of concentrates based on different protein sources.

Variables	Treatments		s.e.d.[1]	*P*-value
	Corn + Soybean Meal	Corn + Whole Soybean		
Yeasts (log cfu g^{-1})	5.67	5.30	0.55	0.0026
Moulds (log cfu g^{-1})	7.22	4.98	0.15	<0.001
Aerobic stability (h)	47.7	15.0	6.31	<0.001
Maximum temperature (°C)	50.5	47.5	0.74	0.01
Sum of temperature (°C)	560	620	11.57	<0.001
Mean temperature (°C)	37.3	41.3	0.77	<0.001

[1] s.e.d. = standard error deviation.

Conclusions

The addition of crude soybeans reduces the growth of yeasts and moulds and increases aerobic stability compared with soybean meal in rehydrated corn grain silages. This is an alternative means to inhibit undesirable microbial conditions at the feedout phase of high-moisture grain silages.

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Inoculation and fertilization affect sulla performance in a new cultivation area

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Abstract

The forage legume sulla (*Sulla coronaria* (L.) Medik) establishes a host-specific nitrogen-fixing symbiotic interaction and its inoculation is mandatory where it is cultivated for the first time or not present as spontaneous species. This study aimed at investigating the effects of inoculation and fertilizer nitrogen (N) on yield performance and N-fixation ability of sulla established in a new habitat within a Mediterranean agropastoral area of Sardinia (Italy). Sulla plants, previously inoculated with peat-based inoculant, liquid inoculant and soil, and unfertilized (N0) or N100 fertilized, were sampled at full vegetative stage. Length, dry matter yield and fixed N of sulla shoots and roots were measured. Inoculation significantly affected several traits. In particular, plant DM varied from 0.5 to 3.2 g in uninoculated and inoculated plants, respectively. At N0 and using soil as inoculant, shoot fixed N reached 43 mg plant^{-1} whereas it was negligible in the uninoculated control. Under N100, N-fixation ability of sulla was decreased. Results highlighted that inoculation is essential for extending the cultivation of sulla in a new habitat.

Keywords: legume, sulla, inoculation, symbiosis, fertilization, N-fixation

Introduction

The short-lived perennial legume sulla (*Sulla coronaria* (L.) Medik syn. *Hedysarum coronarium* L.) is native to the Mediterranean basin, where it is widely grown as a rain-fed biennial forage crop in several countries. It is a non-bloating legume with remarkable adaptability to drought-prone and marginal environments and is used for hay, silage and as a pasture plant because of its great forage value and versatility (Amato *et al.*, 2016). Sulla establishes a host-specific nitrogen-fixing symbiotic interaction and its inoculation is mandatory where is cultivated for the first time or not present as a spontaneous species. In fact, it was reported that sulla cultivation away from its natural habitat requires inoculation to ensure adequate nodulation (Casella *et al.*, 1984), and a suitable matching of plant and bacterial genotypes. The strain WSM1592 of *Rhizobium sullae*, isolated from nodules recovered from *S. coronaria* in Sardinia, is highly effective at fixing N with this legume (Yates *et al.*, 2015). This strain is available as a peat-based commercial inoculant produced in Australia, representing a novel opportunity for farmers to extend the cultivation area of the sulla crop. In fact, previous sulla inoculation methods such as 'assullamento', based on the addition of soil containing bacteria, or the use of liquid inoculants, proved to be unsuitable methods for supporting the extension on a large scale of sulla cropping in new environments, so limiting the exploitation of this valuable legume. The main objective of this research was to assess the effects of different inoculation methods and inorganic N fertilization on growth, yield performance and N-fixation ability of a sulla crop, when introduced in a new area of cultivation outside its traditional habitat.

Materials and methods

The research was carried out during 2013-2014 on a private agro-pastoral farm (40°49'74"N, 8°28'14"E) located in North Sardinia (Italy). The soil, classified as Lithic Xerorthents has neutral pH (7.2) and loamy texture with adequate contents of nitrogen and phosphorus, and had been left uncultivated for the last 40 years. The sulla crop was established in September 2013, after soil ploughing and seedbed preparation, at a sowing density of 300 seeds m^{-2} in 15 m length rows. Before sowing, the soil was fertilised with 100 kg ha^{-1} of P_2O_5. The following inoculation methods were compared: (1) PEA, peat inoculation with the current Australian commercial inoculant strain for sulla (*R. sullae*, group C, strain WSM 1592); (2) LIQ, using a liquid inoculant, kindly supplied by University of Sassari; (3) SOI, ancient inoculation method (assullamento) by mixing seed with soil from a field where sulla is usually grown and/or native; and (4) CON, uninoculated control.

Each plot was split into two subplots: a subplot was fertilized in November with ammonium nitrate fertilizer at a rate of 100 kg N ha^{-1} (N100), and a subplot was left unfertilized (N0). The experimental design consisted of a split-plot with inoculation factor in main plot and N fertilization in subplot, and three replications. The N-fixation ability of the sulla crop was estimated by the ^{15}N isotopic dilution method (Waremburg, 2003), using oats as the non-fixing reference species (NFS). For sulla and oats, enriched ^{15}N fertilizer (10 atom% ^{15}N enriched ammonium sulphate) was applied at a rate of 4 kg N ha^{-1} at seedling emergence. Representative samples containing plants (40 cm length, 40 cm depth) were extracted in early spring at 170 days after sowing. Length, dry matter yield, fixed N of sulla shoots and roots, plus nodule number and weight were determined. Dry sub-samples were ground to pass through a 1 mm mesh and submitted by dry combustion to elemental analyser isotope ratio mass spectrometry (Cheshire, UK) to determine both N content (%N) and the atom% ^{15}N. The effect of inoculation techniques (inoculation), N fertilization rates (fertilization), and the interaction inoculation × fertilization on the measured traits were tested using PROC MIXED in SAS (version 9.02 SAS Institute, Cary, NC, USA).

Results and discussion

N0 shoot length ranged from 52 to 17 cm in SOI and CON, respectively and it was increased three-fold by fertilisation in CON (Table 1). Root length also showed significant but slight differences due to fertilisation. The number of nodules per plant was affected by inoculation and fertilisation. Nodule weight ranged from 0.6 to 4.1 mg.

Shoot DM showed a similar trend to that for shoot length, except for a reduction in SOI under N100 (Table 2). In contrast, root DM was unaffected by inoculation and fertilisation. Finally, in N0 the shoot fixed N of CON was negligible compared with that of inoculated treatments, whereas N100 decreased shoot fixed N of SOI. Root fixed N of SOI and PEA was significantly higher than LIQ and CON. Inoculated sulla produced about five-fold more shoot DM than did the uninoculated control, confirming that inoculation is essential for a successful establishment of an effective symbiotic relationship in a new area of cultivation. These findings were in agreement with previous results obtained in Spain and

Table 1. Sulla shoot and root length, nodules number and weight as affected by inoculation method and N rates.[1,2]

	Shoot length (cm)		Root length (cm)		Nodules (no. plant^{-1})		Nodule weight (mg)	
Treatments	N0	N100	N0	N100	N0	N100	N0	N100
SOI	52.4^{a}	46.5	19.8^{A}	15.5^{B}	19.7^{a}	18.0^{b}	4.1	3.0
PEA	50.6^{a}	52.3	17.0	16.0	25.0^{a}	23.7^{b}	2.4	3.3
LIQ	28.8^{b}	43.1	18.7^{A}	15.9^{B}	13.7^{a}	30.7^{b}	3.1	1.9
CON	7.0^{cB}	49.8^{A}	15.4^{B}	21.4^{A}	8.0^{bB}	45.0^{aA}	1.8	0.6
Tukey-Kramer test	Pr > F		Pr > F		Pr > F		Pr > F	
Inoculation (I)	*		n.s.		*		n.s.	
N rates (N)	*		*		*		n.s.	
I × N	*		*		n.s.		n.s.	

[1] Values with different letters are statistically different according to LSMEANS (P<0.05) in rows for each fertilisation treatment (upper case letter), and columns for each inoculation treatment (lower case letters).

[2] * significant at P≤0.05; n.s. = not significant.

Table 2. Sulla shoot and root dry matter (g per plant) and fixed N (mg per plant) as affected by inoculation method and N rates.[1,2]

	DM shoot (g)		DM root (g)		Shoot fixed N (mg)		Root fixed N (mg)	
Treatments	N0	N100	N0	N100	N0	N100	N0	N100
SOI	3.35^{aA}	1.47B	0.72	0.41	43.0^{aA}	13.5^{B}	4.0^{a}	2.7^{a}
PEA	3.26^{a}	3.27	0.96	0.56	25.5^{a}	16.4	7.3^{a}	3.1^{a}
LIQ	1.46^{a}	2.30	0.53	0.42	20.7^{a}	17.3	0.1^{c}	1.1^{b}
CON	0.71^{bB}	3.24^{A}	0.20	0.81	3.0^{b}	9.3	0.8^{b}	0.1^{bc}
Tukey-Kramer test	Pr > F		Pr > F		Pr > F		Pr > F	
Inoculation (I)	*		n.s.		*		*	
N rates (N)	*		n.s.		*		n.s.	
I × N	*		n.s.		n.s.		n.s.	

[1] Values without common letters are statistically different according to LSMEANS ($P<0.05$) in rows for each fertilisation treatment (upper case letter), and columns for each inoculation treatment (lower case letters).

[2] * significant at $P\leq0.05$; n.s. = not significant.

Australia (Ewing *et al.*, 2001; Rodriguez-Navarro *et al.*, 1991). Under N100, compared with N0, the N fixation ability of sulla decreased only for treatment SOI, with no differences in treatments PEA and LIQ.

Conclusions

Our research allowed for elucidating effects caused by inoculation and N fertilization on the N-fixation ability of a sulla crop established for the first time in a new habitat. Results also highlighted that inoculation is essential for extending the cultivation of sulla.

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Silvopastoral systems with birch and pine saplings under mixed grazing of sheep and goats

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Abstract

Silvopastoral systems may be beneficial by combining forestry with livestock grazing. In this work, preliminary results on tree survival and animal performance from a new project on silvopastoral systems under mixed grazing of sheep and goats are presented. A factorial design of three types of sapling plantation, i.e. birch (*Betula celtiberica*), Scots pine (*Pinus sylvestris*) or both mixed, and two stocking rates (6.7 or 10 animals ha^{-1}) with three replicates (0.6 ha plots) was established in 2015. Three other plots (one per plantation treatment) were excluded from grazing. Initially, saplings were protected with 1.5 m high cylindrical plastic mesh anchored with pine rods, but this system was ineffective against animals' pushing and scratching. The protectors were reinforced in 2016 with 30 cm high rigid tubes anchored with chestnut rods, but again goats managed to overthrow them, damaging about 60% of saplings. One year after planting, birches were higher and had a greater shoot diameter than pines. Goats presented greater body weight and body condition gains than ewes. Changes in body condition were more favourable at the lower stocking rate. New plantations are difficult to establish in heathlands under goat grazing, so new protection systems should be designed.

Keywords: agroforestry, heathland, small ruminants, sapling

Introduction

Silvopastoral systems may be beneficial both economically and ecologically by combining the utilization of forest resources with livestock grazing. Cantabrian heathlands in northern Spain are very susceptible to wildfires, causing soil erosion and loss of biodiversity. Grazing by livestock, especially by goats, has been proven to reduce accumulation of combustible woody phytomass in these plant communities (Celaya *et al.*, 2007a). Although reforestation is hard to be accomplished on those poor and shallow soils, the presence of trees (once they are established) could benefit soils, plants and grazing animals, promoting environment-friendly and sustainable extensive systems (Rigueiro-Rodríguez *et al.*, 2009). These effects may differ between evergreen and deciduous trees (Benavides *et al.*, 2009), and may interact with the grazing management imposed (Rigueiro-Rodríguez *et al.*, 2012). In 2015, a new project on silvopastoral systems established on heathlands with mixed grazing of sheep and goats was started, aiming to study three types of plantation (using a conifer, a broad-leaved tree or both mixed) under different grazing intensities. In this work, preliminary results on tree growth and survival, and animal performance are presented.

Materials and methods

The experiment is being conducted in a mountain area (850-1000 m a.s.l.) in Asturias (N Spain). A factorial design of three types of sapling plantations, i.e. downy birch (*Betula celtiberica*), Scots pine (*Pinus sylvestris*) and both mixed, and two stocking rates (6.7 or 10 animals ha^{-1}) with three replicates (0.6 ha plots) was established in 2015. In addition, 3 other plots (one per plantation treatment) were excluded from grazing. Previously, heathland vegetation was mechanically cleared in all 21 plots. Seedlings that germinated in the preceding year (about 10 cm high) were planted in the spring of 2015 at a density of 400 seedlings ha^{-1} (grids of 5×5 m). Approximately 120-130 seedlings were planted per plot. In the plots to be grazed, saplings were protected with 1.5 m high cylindrical plastic mesh anchored to soil with 2 m high pine rods (2×2 cm section). Chemical analyses of soil samples collected in March 2015 revealed their acidity and low availability of phosphorus, potassium and magnesium.

Non-lactating crossbred (Gallega × Latxa) ewes (34.8±1.5 kg body weight-BW) and Cashmere goats (36.6±0.8 kg BW) grazed during the autumn of 2015 and spring-summer of 2016 (from late April to late September). Two sheep and two goats per plot, and three sheep and three goats per plot were managed at low and high stocking rate treatments, respectively. As the protection system was inefficient against animals' lunge and scratch in the autumn grazing, protectors were reinforced in 2016 with 30 cm high rigid plastic microperforated tubes anchored with tougher chestnut rods (3×3 cm section).

After the first growing season (November 2015), the height and shoot basal diameter of 25-30 randomly selected saplings per plot were measured. Once the grazing season of 2016 finished, damaged saplings (partially or totally defoliated or uprooted) were counted in October in all grazed plots. All animals were periodically weighed and their body condition score (BCS) assessed on a scale of 1 (emaciated) to 5 (fat).

All statistical analyses were performed with plots as experimental units. Sapling data were subjected to GLM to examine the fixed effects of species (birch or pine), plantation type (single or mixed), grazing management (high or low stocking rate or ungrazed) and their interactions. For animal data, the model included the effects of plantation type (birch, pine or mixed), stocking rate, livestock species as a within-subject factor, and their interactions.

Results and discussion

After the first growing season, birch saplings were taller (35.4 vs 12.4 cm; standard error of the mean (SEM) = 1.49; $P<0.001$) and had a greater shoot diameter (5.54 vs 5.00 mm; SEM = 0.153; $P<0.05$) than pines. Neither the grazing management nor the type of plantation nor any of the interactions had any effect on sapling growth. Birches generally grow faster than pines at early stages, and may act as natural pioneer species on acid soils. After the 2016 spring-summer grazing season, undamaged saplings (with no signs of defoliation) accounted for 39% of the total, with no differences between stocking rates, plantation types or tree species. A greater percentage of birches eaten but alive were found compared to pines (60 vs 6%; SEM = 6.7; $P<0.001$). However, the number of eaten and dry pines exceeded that of birches (29 vs 6%; SEM=5.4; $P<0.01$), as well as the number of uprooted saplings (16 vs 6%; SEM = 2.1; $P<0.01$). In addition, uprooting was more frequent in mixed than in monospecific plantations (16 vs 6%; $P<0.01$), and under high than under low stocking rate (14 vs 7%; $P<0.05$). Ultimately, survival rate was greater in birches than in pines (88 vs 63%; SEM = 6.7; $P<0.01$). Although the experimental design does not allow for the comparison between sheep and goat grazing effects, goats were most likely the cause of pine browsing (Celaya *et al.*, 2007b), and were also seen pulling up the protectors with their horns.

Plantation type did not affect animal performance. During spring, body weight (BW) and BCS changes did not differ between livestock species or stocking rates (means of 7 g day^{-1} and 0.02 BCS). During summer, goats showed greater BW and BCS gains than ewes (40 vs -20 g day^{-1}; 0.09 vs -0.07 BCS), resulting in better overall performance during the whole season in goats than in sheep (27 vs -10 g day^{-1}; 0.18 vs -0.13 BCS; Table 1). Overall BCS changes were more favourable at the lower stocking rate (0.12 vs -0.07;SEM = 0.039; $P<0.01$; Table 1).

Table 1. Body weight (BW) and body condition score (BCS) changes of non-lactating Gallega × Latxa sheep and Cashmere goats grazing together during spring-summer under two stocking rates (low: 6.7 animals ha^{-1}; high: 10 animals ha^{-1}) on heathlands planted with birch and pine seedlings.

	Stocking rate (SR)	Low		High		s.e.m.	Significance[1]		
	Species (Sp)	Sheep	Goat	Sheep	Goat		SR	Sp	SR × Sp
Initial BW (kg)		38.2	37.0	33.3	36.3	3.13	ns	ns	ns
BW change (g day^{-1})	Spring (29 April – 29 June)	-3.7	6.6	11.9	12.0	14.39	ns	ns	ns
	Summer (29 June – 19 Sept.)	-22.8	43.6	-16.6	36.9	8.42	ns	***	ns
	Overall (29 April – 19 Sept.)	-14.6	27.9	-4.4	26.3	8.66	ns	***	ns
Initial BCS (scale 1-5)		2.51	2.71	2.79	2.66	0.105	ns	ns	ns
BCS change (scale 1-5)	Spring (29 April – 29 June)	0.00	0.10	-0.12	0.08	0.095	ns	ns	ns
	Summer (29 June – 19 Sept.)	0.02	0.12	-0.16	0.06	0.061	ns	**	ns
	Overall (29 April – 19 Sept.)	0.02	0.22	-0.28	0.14	0.080	**	**	ns

[1] ** $P<0.01$; *** $P<0.001$; ns: non-significant ($P>0.05$).

Conclusions

These preliminary results show there was a better performance for goats than for sheep, as previously found in similar heathland communities (Osoro *et al.*, 2013). New plantations with young saplings are difficult to establish in these heathlands under goat grazing, given the difficulties in fixing the protectors to the ground. If sheep and goat grazing is desirable for establishing silvopastoral systems on heathlands whilst controlling excessive shrub encroachment, more efficient protection systems should be designed.

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Prediction of *in vivo* digestibility of pasture-based diets in dairy goats from faecal indicators

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Abstract

For estimating quality and intake of pasture herbage by grazing goats, estimation of herbage digestibility is often required. The aim of this work was to predict *in vivo* organic matter digestibility (OMD) of diets based on temperate pasture forage from faecal composition (CP mainly). A series of indoor experiments was carried out with goats fed on grass hay or with fresh herbage cut daily and given either *ad libitum* or in restricted amounts, to mimic the effect of pasture herbage availability. Concentrate supplementation level and regrowth age of the herbage were also studied in order to achieve a wide range of diets composition and quality. Intake of each feed, and of faecal output (total collection), were measured precisely during 5 consecutive days in each experimental period. For each dataset (fresh pasture herbage only or fresh pasture herbage + hay), there was a strong and inverse relationship between OMD and faecal crude protein (CP) concentration. Including dietary CP concentration in the regression allowed increased accuracy of the prediction. The proposed equations, relevant for good quality pastures, show a good continuum with previous published equations on goats fed on much less digestible tropical, Mediterranean or Sahelian forages.

Keywords: goat, digestibility, pasture, faecal nitrogen, prediction

Introduction

Reliable methods for determining individual daily intake of herbage from pasture are needed for predicting variations of nutrient intake in grazing dairy goats. One of the main techniques is the 'faecal output/digestibility' method (Penning, 2004), intake being calculated from the ratio between daily faecal output and the indigestible fraction of the diet. Faecal output is often estimated from the dilution of an indigestible external marker, while digestibility is often determined *in vitro* or from faecal composition, mainly from faecal nitrogen concentration (Penning, 2004). Several predictive equations of *in vivo* digestibility exist for cattle (Penning, 2004; Ribeiro Filho *et al.*, 2005) and sheep (Chenost *et al.*, 1985) fed on good quality forages, for meat goats fed on tropical (Boval *et al.*, 2003) or sahelian (Schlecht and Susenbeth, 2006) forages, but not for dairy goats fed on good-quality pastures. The objective of this work was to develop an accurate predictive equation of *in vivo* diet digestibility based on faecal indicators in dairy goats fed on good-quality forages, including fresh pasture.

Materials and methods

A series of 4 trials was carried out at the INRA-PEGASE experimental farm of Méjusseaume (Le Rheu, Brittany, France) between spring 2014 and spring 2016 to determine voluntary intake, *in vivo* digestibility and faecal composition of Alpine goats fed under a large range of diet quality, diet composition, and intake level, which mimic what could happen under grazing conditions. The forage fed was grass hay of medium quality in trial 1 (Spring 2014), fresh herbage from a multi-species pasture including grasses, clovers, chicory and dandelion in trial 2 (Autumn 2015) and fresh grass-based pasture herbage in trials 3 and 4 (Spring 2016). Fresh herbage was cut once daily before feeding as 4 meals per day to goats. Three to four nutritional treatments were compared within each experiment, varying by the level of allocation of the forage (130 to 80% of *ad libitum* intake level determined before the start of the experiment), by the level of supplementation (no supplement to 600 g/day of a pelleted concentrate containing 220 g of crude protein (CP) kg^{-1} dry matter (DM)), and by the regrowth age of the freshly cut pasture herbage (from young and leafy to aged and stemmy). Experimental designs were Latin squares repeated 2 to 3 times, with 3 to 4 successive periods of 14 days, and six Alpine goats per trial. Goats were dry in trial 1, milked once-a-day in trial 2, and milked twice-a-day in trials 3 and 4. Goats were maintained in digestibility boxes allowing individual measurement of intake and total faecal collection. The amount of forage and concentrate DM offered and refused, and the amount of faecal DM output were measured during the last 5 days of each period. Amount of DM, organic matter (OM), N, neutral detergent fibre (NDF) and average daily gain (ADF) eaten (offered minus refused) and excreted in faeces were determined, allowing to calculate *in vivo* diet OM digestibility (OMD) and faecal and diet nutrients concentrations. Multiple regressions were performed on data averaged per trial × treatment × period to find the best equations predicting OMD from faecal and diet indicators. Two datasets were considered: hay + fresh pasture herbage (HP dataset, n=39), or fresh pasture herbage only (P dataset, n=23). Specific effects of concentrate supplementation (with or without) or intake level (*ad libitum* or restricted) were tested through covariance analyses.

Results and discussion

The OMD averaged 0.694 for hay-based diets and 0.796 for fresh pasture-based diets. Large ranges in OMD, intake, faecal output, and diet and faeces CP concentrations were observed (Table 1). There was a strong and inverse relationship between OMD and faecal CP concentration (Table 2 and Figure 1), with 0.021 and 0.013 of residual standard error of the regression when this variable is considered alone, in the HP and P databases, respectively. Taking also into account in the regression of the ratio diet/faecal CP concentrations (Penning, 2004; Ribeiro Filho *et al.*, 2005) allowed increased the accuracy of the prediction in both databases. These regressions established with temperate forages showed a good continuum with existing regressions on goats fed on tropical (Boval *et al.*, 2003), Mediterranean or Sahelian forages (Schlecht and Susenbeth, 2006); nonetheless these forages are much less digestible than those of this study (Figure 2). Faecal ADF concentration had no significant effect on OMD provided that diet CP concentration is taken into account, contrary to previous studies with cattle (Penning, 2004; Ribeiro Filho *et al.*, 2005), but in good agreement with studies on meat goats (Boval *et al.*, 2003; Schlecht and Susenbeth, 2006). The effects of concentrate supplementation level or of intake level were never significant. This suggests that the predictive equations are sufficiently generic to be used in grazing

Table 1. Description of the dataset relating diet and faecal characteristics to *in vivo* diet organic matter (OM) digestibility in dairy goats fed on pasture-based diets (n=39).[1]

Variable	Unit	Mean	Min	Max	SD	CV
Total intake	g DM d^{-1}	1712	808	2597	518	30%
Diet OM digestibility	g g^{-1}	0.754	0.636	0.826	0.057	8%
Diet CP concentration	g kg^{-1} OM	166	94	235	41	25%
Faecal CP concentration	g kg^{-1} OM	199	127	262	47	24%

[1] CP = crude protein; SD = standard deviation; CV = coefficient of variation.

Table 2. Parameters of main equations predicting *in vivo* diet OM digestibility from faecal and diet characteristics in dairy goats fed on pasture-based diets.[1]

Dataset		Intercept	1/FCP	DCP/FCP	R^2	RSD
Hay + fresh pasture (n=39)	Eq. 1	0.959	-3.83		0.86	0.021
	Eq. 2	1.012	-3.65	-0.074	0.88	0.019
Fresh pasture (n=23)	Eq. 3	0.895	-2.24		0.61	0.013
	Eq. 4	0.939	-2.44	-0.0445	0.69	0.012

[1] FCP and DCP are the faecal and diet crude protein concentration, in g 100 g^{-1} organic matter, respectively). RSD = relative standard deviation.

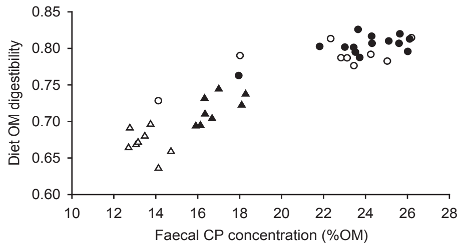

Figure 1. Relationship between faecal crude protein (CP) concentration and organic matter (OM) digestibility on dairy goats fed on pasture (triangles: hay; circles: fresh pasture herbage) with (black symbols) or without (white symbol) concentrate supplementation.

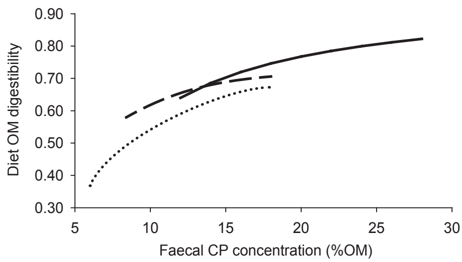

Figure 2. Relationship between faecal crude protein (CP) concentration and organic matter (OM) digestibility on goats fed on forages (——: this study, Eq. 1; - - -: tropical pastures, Boval *et al.*, 2003; ····: sahelian forages, Schlecht and Susenbeth, 2006).

situations with various concentrate supplementation levels and various grazing pressure. The fact that concentrate supplementation does not affect the relationship between faecal CP concentration and digestibility has been already observed by Chenost *et al.* (1985) on sheep fed on diets based on fresh pasture herbage.

Conclusions

The inverse relationship observed between OMD and faecal CP concentration seem generic enough to be used under a large range of grazing situations, but larger databases are needed to achieve more precision, particularly considering the large range of pasture type and supplement type used. The proposed equations, developed and relevant for temperate pastures, show a good continuum with previous published equations on goats fed on tropical, Mediterranean or Sahelian forages, nonetheless much less digestible.

Acknowledgements

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Morphological and nodulation variation among local populations of *Medicago truncatula* Gaertn. collected in Djelfa area (Algeria)

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Abstract

The aim of this work is to identify annual legumes of the genus *Medicago* L. that can be used to improve forage production in Algeria. Populations of *Medicago truncatula* collected were evaluated for their morphological traits. The experiments were conducted at the experimental farm of the National Institute of Agronomic Research of Algeria. Analysis of variance was performed on the complete set of data. The results obtained show that there is high genetic variability among populations of *M. truncatula*. The variance analysis showed highly significant differences for the majority of measured traits and not significant for number of internodes, number of primary branches and plant weight. The large variability available for all studied traits provides opportunities for the exploitation of local plant genetic resources to improve forage production in Algeria.

Keywords: *M. truncatula*, plant genetic resources, variability, nodulation.

Introduction

The genus *Medicago* L. (*Leguminosae*) comprises a large number of species of annual herbs, herbaceous perennials and rare shrubs, mostly native to the Mediterranean region (Lesins and Lesins, 1979). Annual species are found in all bioclimatic zones from wet to Sahara. Some have a wide spectrum of distribution, whereas others have a more defined spatial distribution (Abdelkefi and Marrakchi, 2000). *Medicago* and *Trifolium* are the most important genera. They are represented in Algeria by annuals and perennial species.

Legumes of the genus *Medicago* are of special ecological and agropastoral importance in the Mediterranean regions. They include forage species expressing high levels of N-fixation and protein production per hectare (Huguet *et al.*, 1994). Annual medics are mainly used as pasture species in extensive farming systems, especially to improve low quality natural pastures. They are grown as regenerating pasture in the agro-pastoral Mediterranean systems or in cereal farming systems, and are an important feed resource not only as green forage throughout the growing season but also as stubbles and pods in summer and early autumn (Porqueddu, 2001). In addition to their ability to fix atmospheric nitrogen and to improve soil fertility, medics can also be used in semi-arid and arid areas to regenerate degraded rangelands.

The objective of the present study was to characterize morphological variation of local populations of *M. truncatula* collected from different area of Djelfa.

Materials and methods

Five *Medicago truncatula* populations were used. These populations were collected in the Djelfa region in 2008 by the National Institute of Agronomic Research of Algeria. The origin sites of these populations are: Population from Mliliha (MtMli) (34°49'N; 3°49'E); Population from Oued Touil (MtOT)

(35°16'N; 2°33'E); Population from Bouiret Lahdab (MtBL) (35°12'N; 3°02'E); Population from Ain Oussera (MtAO) (35°17'N; 2°57'E); Population from Charef (MtCh) (34°40'N; 2°43'E). The trial was conducted at the experimental farm of the Research Center for Plant Sciences (INRAA). This region is characterized by a sub-humid climate with mild winters and hot summers.

Scarified seeds were sown in pots filled with clay at a rate of five seeds per pot. At emergence, three of the seedlings were removed, leaving two plants per pot. Pots were arranged in a completely randomized design with five replicates. At flowering stage, the following traits were measured on ten plants per population: onset of flowering (OF) (days after emergence), length of the branch that brought the first flower (LB1F) (in cm), number of internodes of the branch bringing the first flower (NIN), number of primary branches (NPB), plant fresh weight (leaves and stems) (PFW) (in g), root weight (RW) (in g) and number and weight (in mg) of nodules (NND, WND).

Analysis of variance was performed on the complete data using the GenStat software (Discovery Edition). Comparisons between means were made using least significant difference (LSD) at 5% probability level.

Results and discussion

We noted the existence of substantial genetic variability among the studied *Medicago truncatula* populations (Table 1). The variance analysis showed highly significant differences between populations for most of the traits studied, except for number of internodes, number of primary branches and plant weight, for which differences were not significant. El Hansali *et al.* (2007) indicated the existence of an important variability among nine natural populations of *M. truncatula* from Morocco, sampled in areas with different climates. Similarly, Arraouadi *et al.* (2006) reported a high degree of quantitative polymorphism within and between natural Tunisian populations of *M. truncatula*.

Onset of flowering ranged from 94 to 98 days after emergence. The population from Mliliha (MtMli) was the earliest; the Bouiret Lahdab (MtBL) population was the latest (Table 1). Del Pozo and Aronson (2000) indicated that annual legumes show both ecotypic differentiation and a high degree of plasticity in flowering time. Sulas *et al.* (2000) reported that flowering of *Trifolium subterranean* and annual medics occurs in response to day length and temperature, but cultivars vary considerably in their response to these factors. For morphological traits, the population from Oued Touil (MtOT) displayed the longest branches that brought the first flower (17.7 cm), while LB1F was the shortest (13.2 cm) for plants of the Bouiret Lahdeb population (MtBL). Chebouti *et al.* (2006) reported that *M. truncatula* presented the

Table 1. Means value of the morphological traits measured in five *Medicago truncatula* populations.[1]

Populations	OF	LB1F	NIN	NPR	PFW	RW	NND	WND
MtMLi	94.0[c]	17.1[ab]	5.8	5.7	31.0	25.8[a]	61.8[bc]	79.4[bc]
MtOT	95.0[c]	17.7[a]	6.7	5.9	30.4	20.2[bc]	58.2[c]	80.4[bc]
MtBL	98.0[a]	13.2[c]	6.2	6.0	30.7	15.4[d]	79.8[b]	118.3[b]
MtAO	97.0[b]	16.4[ab]	6.5	6.3	32.1	23.7[ab]	53.6[c]	46.0[c]
MtCh	96.0[b]	15.6[b]	6.3	6.4	29.7	17.3[cd]	106.4[a]	182.2[a]
Mean	96.0	16.0	6.3	6.0	30.8	20.5	72.0	101.4
Sign.	***	***	ns	ns	ns	***	***	**
P-value	0.001	0.001	0.068	0.089	0.948	0.001	0.001	0.002
LSD	0.857	1.937	0.596	0.548	6.133	4.268	20.69	0.061
C.V.	1.1	14.8	11.6	11.0	16.8	17.25	21.8	45.6

[1] Morphological traits OF ... WND, see Methods section.

[2] LSD = least significant difference; CV = Variation coefficient.

longest branch compared to *M. orbicularis* and *M. aculeata*. Badri *et al.* (2002) mentioned that natural populations of *M. truncatula* tend to develop their plagiotropic axis.

The mean root weight across all populations was 20.5 g, and varied from a minimum of 15.4 g for the Bouiret Lahdab (MtBL) population to a maximum of 25.8 g for the population from Mliliha (MtMli). No significant differences were noted between populations for fresh above ground plant weight (leaves and stems together).

For nodulation traits, the population from Charef (MtCh) produced the highest number of nodules (106.4) with a weight of 182.8 mg of nodules, while the population from Ain Oussera presented the lowest number of nodules (53.6) with a weight of 46.0 mg.

Conclusions

It can be concluded that there is a large variability among the populations of *M. truncatula* for most studied traits. This variability is of a great interest in terms of forage resources, providing opportunities for better exploitation of local plant genetic resources in order to improve forage production in Algeria. Further research is needed to isolate and characterize rhizobia strains in local populations of *M. truncatula* with the aim of identifying strains with high nitrogen-fixing potential that are able to effectively nodulate annual medics.

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Effect of cutting and fertilization on temporal differentiation of semi-natural grassland vegetation

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Abstract

In a split-plot field experiment with four replicates, combinations of cutting and fertilizing regimes were compared with regard to their effects on vegetation composition and diversity of a semi-natural meadow (*Arrhenatherion* grassland) located in Slovenia. Treatments on main plots were: (A) 2-cut system, (B) alternating 3- and 4-cut systems and (C) 4-cut system. Treatments on the sub-plots were: (1) zero fertilizer (control), (2) PK fertilizer, (3) slurry, (4) slurry plus reduced NPK fertilizer rate and (5) NPK fertilizer. In total 270 vegetation relevés were made in the spring time over five years (2011-2015) using the Braun-Blanquet method. Fertilizer regimes affected the vegetation composition over the years and its changes showed quite high tendency of grouping. Temporal changes of the vegetation composition caused by different cutting regimes were weak. Number of species (range 16 to 37) was affected only by fertilizer regimes while Shannon diversity index (range 2.12 to 2.34) was affected by both factors and by their interaction.

Keywords: semi-natural grassland, vegetation temporal change, cutting regime, fertilization

Introduction

Semi-natural grasslands are a valuable source of forage while also providing other ecosystem services, e.g. supporting diverse grassland biocoenoses. During the last 70 years many European semi-natural grasslands have been intensified leading to decrease in species diversity (e.g. Bosshard (2015) for *Arrhenatherion* grassland). Intensified grasslands are generally considered as less sustainable due to high rates of nutrients required, low nitrogen use efficiency and low resilience capacity of the swards in adverse growth conditions. The question regarding semi-natural grasslands, therefore, is what is their optimal management regime on order to assure reasonably high forage productivity at low expense of plant diversity. This seems a tricky task because two aspects of grassland intensification (cutting frequency and fertilization) co-act on sward community composition (Socher *et al.*, 2012). This paper summarises short-term effects of different combinations of cutting and fertilizing treatments on composition and diversity of an *Arrhenatherion* grassland. The emphasis is in the assessment how differences in sward composition between management regimes slowly accumulate over the years, which has been rarely studied before. We also compared cutting and fertilizing effects in their relative importance as drivers of sward community change.

Materials and methods

The study was carried out using field experiment located in the pre-Alpine region of Slovenia (46°03'N, 14°28' E, 300 m a.s.l.) during five years (2011-2015). The soil at the experimental site is sandy loam with a moderately gleyed layer at a depth of 30-70 cm. The initial soil characteristics were as follows: pH ($CaCl_2$) 6.4, 25 mg P and 104 mg K kg^{-1} of dry soil (extracted in ammonium lactate). Climatic characteristics of the site (annual average for 1981-2010) are: mean air temperature 10.9 °C, precipitation sum 1,362 mm. Mesic semi-natural grassland on the experimental site belongs to *Arrhenatherion* alliance. Previously it has been extensively managed (two to three cuts per year plus approximately 60 kg N, 26 kg P and 50 kg K ha^{-1} $year^{-1}$ applied as a mineral fertilizer in early spring). The treatments were arranged in a split-plot design with four block replicates. Three cutting regimes and five fertilizer treatments were assigned to main plots and sub-plots, respectively. The sub-plot size was 5.0×2.5 m. A description of the treatments is shown in Table 1.

Over the experimental period the relevé was carried out for each sub-plot in the spring time of each year (270 relevés in total). Species cover-abundance in the sward was visually estimated using the Braun-Blanquet scale with 6 classes (+ ≤1%, 1 = 1-10%, 2 = 10-25%, 3 = 25-50%, 4 = 50-75%, 5 = 75-100%). For statistical analyses these classes were converted to percentages using the respective class averages (0.5% was used for + class). Temporal differentiations of the sward composition were assessed by detrended correspondence analysis (vegan package in R-3.2.2 software), while the effects of treatments and time on the species number and Shannon diversity index were tested using linear mixed models.

Results and discussion

The study focused on temporal shift of the sward composition, its species number and plant diversity under different management treatments. Comparison of the graphs on Figure 1 reveals that fertilizing regimes temporarily affected sward composition differently and much stronger than cutting. The fertilizing effects show grouping for control (unfertilized sward), slurry and PK treatment on one side and for NPK treatments with or without slurry on the other side. The pattern of PK treatment is quite distinctive with unique development in the early years of investigation. The weak effect of slurry application in comparison with the control is surprising, although it can be explained by its relatively low nutrient content (57 kg N, 11 kg P and 52 kg K ha^{-1} year^{-1}). Organic matter, added to sward with slurry, is less relevant for grassland plants growth, therefore less relevant for sward composition.

The sward under the PK treatment had a considerably higher proportion of legumes than the others (data not shown). Adding moderate amount of nutrients, particularly mineral nitrogen, either with NPK or slurry plus NPK fertilizer strongly influenced sward composition (first ordination axis seems to be correlated with N availability). These effects were observed already in the first year (2011). The dominant nutrient availability effect on sward composition is well supported by the literature (Smith, 1993).

Composition of the sward community changed similarly under all cutting regimes over the course of the experiment. It is much more the result of averaging over fertilizing treatments than the effect of cutting regimes *per se*.

In all vegetation relevés 93 vascular plant species were identified. Species number in the sward was significantly affected by fertilizing regimes (P<0.001) but not by cutting. A species reduction occurred where mineral nitrogen was added. There was also a significant interaction between fertilizing and year (P<0.001). Addition of mineral nitrogen in both fertilizing treatments reduced species number in the

Table 1. Cutting regimes [A-C] and fertilizer treatments [1-5] applied in the experiment

[A] Traditional 2-cut system (first cut 5-10 Jun, second cut 10-20 Aug, aftermath cutting in early autumn)
[B] 4- (3-) cut system (four-cut system in each of three consecutive years, three cuts with delayed first cut in the fourth year – first cut 25-30 Jun, second cut 10-15 Aug, third cut 20-25 Oct)
[C] 4-cut system (first cut 10-20 May, second cut 25 Jun-5 Jul, third cut 10-20 Aug, fourth cut 20-30 Oct)
[1] Zero fertilizer (control, no fertilizers were used)
[2] PK (31 kg P plus 174 kg K ha^{-1} year^{-1})
[3] Slurry (22 t of cattle slurry ha^{-1} in third decade of March; 2.60 g N, 0.51 g P and 2.35 g K kg^{-1} of slurry)
[4] Slurry plus NPK (22 t of cattle slurry ha^{-1} in third decade of March plus 120 kg N plus 18 kg P plus 100 kg K ha^{-1} year^{-1}; split-application of N: 50:70 kg in two cut system, 0:40:40:40 kg in four cut system)
[5] NPK (220 kg N plus 31 kg P plus 174 kg K ha^{-1} year^{-1}; split-application of N: 110:110 kg in two-cut system, 60:60:50:50 kg in four cut system)

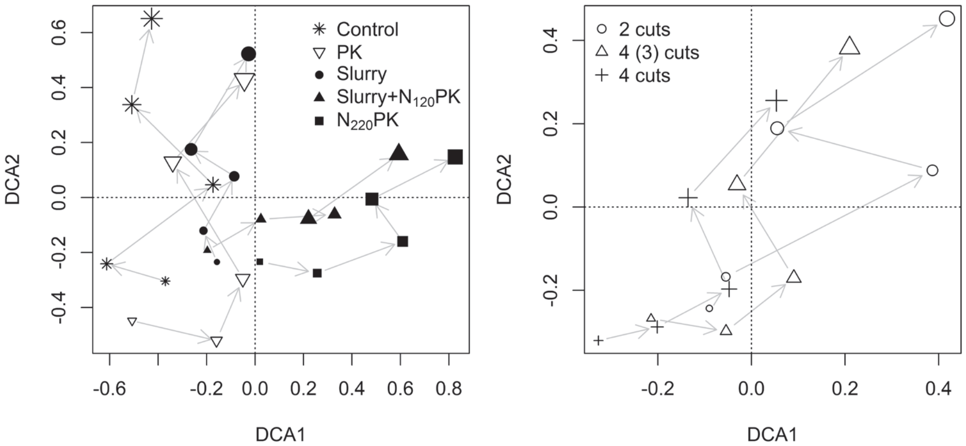

Figure 1. Temporal development of sward community composition plotted in the first two ordination axes of correspondence analysis. The relevés repeated from 2011 (smallest symbols) till 2015 (largest symbols) were averaged for fertilizing over all cutting regimes (left) and for cutting regimes over all fertilizing treatments (right).

fifth experimental year while no such temporal changes were noticed in other fertilizing treatments. On average, overall there were 27.8 species per plot, ranging from 16 to 37 species per plot of 10 m^2.

Shannon diversity index (H) of the sward was significantly affected by fertilizing (P<0.001) and cutting regimes (P=0.001) as well by interaction among fertilizing, cutting and year (P=0.01). Averaged over all experimental years, the highest differences in H appeared between 2 cuts and 4 or 4 (3) cuts (2.34 vs 2.14) and between control and NPK treatment (2.33 vs 2.12). The former difference was also the most consistent over the years at all three fertilizing regimes with higher application rate (PK and NPK treatments with or without slurry).

Conclusions

Grassland management is a complex and relevant driver of vegetation composition and structure of swards. Our research showed that short-term vegetation composition was affected largely and more quickly by fertilizing regime than by cutting regime. This was confirmed for all three variables analysed: sward temporal development, plant species number and H index.

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Maintaining upland floristic diversity whilst cutting for biomass production: the impact of the seed bank

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Abstract

An annual cutting and biomass removal management regime increased the above ground floristic diversity (as measured by the Shannon Weiner index) in five semi-natural habitats across mid Wales over a three-year period, for the purpose of bioenergy generation. There was little evidence of the seed bank contributing to an increase in above ground plant species richness in the current study. However, the seed bank may have contributed to changes in dominance in the above ground vegetation. The implications of the low impact of the seed bank upon the above ground diversity in the current study are that the seed bank alone will not provide substantial resistance to plant community dominance. The seed bank may increase the above ground dominance of particular species that create seed prolifically and with extended longevity. A cutting management regime may need to be consistently employed in order to counteract the dominance patterns that emerge from the soil seed bank, and the resultant biomass can potentially be processed for energy.

Keywords: floristic diversity, seed bank, cutting management, semi-natural habitat, species dominance

Introduction

One established technique that is used to keep dominant species at low levels of abundance when diversity conservation is a management target is to manage habitats by cutting and removing the resultant biomass (Hejcman *et al.,* 2010; Klimeš and Klimešova, 2001; Marrs and Watt, 2006). This study examines the implications for floristic diversity when semi natural landscapes are managed by cutting and the potential role of the seed bank in establishing that diversity.

Materials and methods

Six semi-natural sites were selected across mid Wales. These were categorised by the dominant species: *Molinia caerulea* (Mol); *Juncus effusus*, managed (MaJ); *Juncus effusus*, unmanaged (UnJ); *Vaccinium myrtillus* (Vac); *Pteridium aquilinum* (Bra); *Nardus stricta* (Nar). Three replicate sub plots were established on each site. Plant species richness and percentage cover (abundance) were measured by the same surveyor annually for three years. Surveying took place as soon as possible after 15 July. The plot structure consisted of a central permanent quadrat measuring 10×10 m. Species absence/presence data and percentage cover were recorded annually over a three-year period. Genstat statistical software (13th edition, VSN international) was used to analyse the data and an ANOVA was employed. The Shannon Wiener diversity index (*H'*) was used to measure plant diversity (p_i = the proportion of individuals of a certain species): $H' = -\, p_i \ln p_i$. The Jaccard coefficient (S) was used to measure the similarity between above ground species present and the seed bank species present: $S = C_{xy} / A_x + B_y$, where C_{xy} is the number of species common to the seed bank and the above ground vegetation. The variable A_x is the number of species in the above ground vegetation and B_y is the number of species in the seedbank (Jaccard, 1912). The Vac site was not included in the seedbank assessment as the sampling areas were too shallow to accept the experimental procedures. Seedbank assessment was undertaken during Year 1 and 2 only, in order to measure the impact of cutting of previously uncut habitats. During soil seed bank sampling two soil samples were obtained following randomization from a strip above and below each plot. Soil samples of approximately 1 litre were extracted at a sample depth of 5 cm using a metal trowel in mid May 2010. The four replicate samples taken for each plot were bulked together and thoroughly mixed by hand in a clean dry plastic container and processed according to Heerdt *et al.* (1996). Trays measuring 10×15 cm containing a 5 mm thick layer of sterilized potting soil (John Innes no. 1) were used to grow the seedbank samples, along with controls in a randomized design in a ventilated greenhouse. Following identification and recording, seedlings were removed and discarded. When no seed emergence had occurred for a period of a further three weeks the assessment was considered complete.

Results

The overall Shannon Wiener diversity index of the six sites (expressed collectively) increased significantly from Year 1 to Year 2 and then remained unchanged in Year 3 (Figure 1; P<0.001; standard error of differences (SED) = 0.0789).

The overall Shannon Wiener diversity index of the six sites (expressed collectively) increased significantly from Year 1 to Year 2 and then remained unchanged (P<0.001; SED = 0.0789). Similarity with regard to presence/absence data from the seed bank and the equivalent above ground data is shown in Table 1 using the Jaccard similarity index. No significant difference between the similarities of the species composition of the seed-bank and the above ground species composition were found in Year 1 compared with Year 2 on the Mol, UnJ, MaJ and Bra sites (for abbreviations see Table 1 caption). The Nardus site

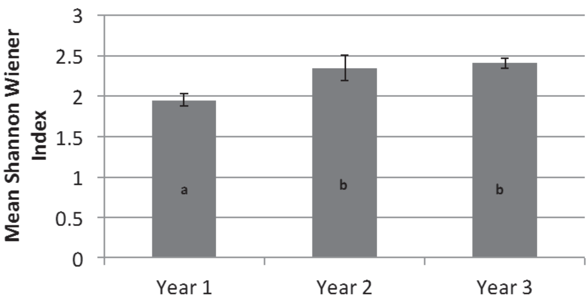

Figure 1. The impact of an annual cutting management upon the Shannon Wiener diversity index over a three-year period. The figure represents a mean diversity of six distinct semi-natural sites across mid Wales. Error bars represent the standard error of the mean. Letters represent grouping according to a post hoc multiple comparison analysis using the Student Neuman Keuls test following an analysis of variance.

Table 1. The similarity between the seed bank of Year 2 with the above ground species composition of Years 1 and 2.[1,2]

Site	Broad habitat	Dominant species	Jaccard similarity index Year 1		Jaccard similarity index Year 2		T test
			Mean	SEM	Mean	SEM	(P-value)
Mol	Neutral grassland	*Molinia caerulea, Juncus acutiflorus, Agrostis canina*	0.319	0.029	0.249	0.025	0.142
UnJ	Fen, marsh, swamp	*Juncus effusus*	0.143	0.040	0.174	0.045	0.634
MaJ	Acid grassland	*Juncus effusus*	0.260	0.031	0.288	0.044	0.623
Bra	Dense bracken	*Pteridium aquilinum*	0.142	0.040	0.118	0.029	0.657
Nar	Acid grassland	*Nardus stricta, Festuca ovina*	0.134	0.005	0.181	0.007	0.006

[1] The values are mean Jaccard index similarity scores from triplicate plots on each of five distinct sites comparing floristic species presence/absence data from the seedbank and from the above ground vegetation; SEM = standard error of the mean.

[2] Mol= *Molinia caerulea* dominant; UnJ = unmanaged *Juncus effusus* dominant; MaJ = managed *J. effusus* dominant; Bra = *Pteridium aquilinum* (bracken) dominant; Nar = *Nardus stricta* dominant habitat.

showed a significantly higher similarity between the seed bank and the above ground vegetation in Year 2 compared with Year 1. Therefore on the Nar site the seed bank may have had more influence on changes in the recorded botanical composition following cutting.

Conclusions

An annual cutting and biomass removal management regime increased the above ground floristic diversity in semi-natural habitats across mid Wales over a three-year period. There was little evidence of the seed bank contributing to an increase in above ground plant species richness in the current study. The implications of the low impact of the seed bank upon the above ground diversity in the current study are that the seed bank alone will not provide substantial resistance to plant community dominance. The seed bank may increase the above ground dominance of particular species that create seed prolifically and with extended longevity (as seen in those sites containing *Juncus* sp. that are prolific producers of seed with extended longevity, i.e. Mol; UnJ; MaJ). A cutting management regime may need to be consistently employed in order to counteract the dominance patterns that emerge from the soil seed bank.

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Peas as a cover crop for clover and lucerne installation

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Abstract

In Merelbeke (Flanders) red clover and lucerne were sown in pure stand and in combination with white clover and perennial ryegrass in a field experiment in 2015 and 2016. All these treatments were sown in spring with and without peas. Results of 2015 are presented here. Over the year, the average dry matter (DM) production of the legume-grass mixtures with peas as a cover crop was significantly higher (+1.77 Mg ha^{-1}; +18%) in comparison with the same mixtures without peas. A significant difference in crude protein (CP) yield of 0.13 Mg ha^{-1} (+7%) was noted between treatments with and without peas; i.e. 1.93 Mg CP ha^{-1} and 1.80 Mg CP ha^{-1}, respectively. Between legumes, legumes + perennial ryegrass, and perennial ryegrass the difference in CP production between a cover crop vs no cover crop was quite different: 0.03 Mg CP ha^{-1}, 0.07 Mg CP ha^{-1} and 0.68 Mg CP ha^{-1}, respectively. This can be explained by the N fixation capacity of the legumes and the low N fertilization of the grass.

Keywords: peas, cover crop, forage legumes, dry matter, protein yield

Introduction

The installation of grass, or forage legumes such as lucerne (*Medicago sativa*) and clover is more difficult when seeded in spring in comparison with seeding in August; this can affect the yield at least in the first growing season. Peas (*Pisum sativum*) sown in spring as a cover crop for new grass-clover is quite common in organic farming in Belgium and the Netherlands. Peas are harvested at early ripening as prewilted silage with use of silage additives. Yield and quality of the pea silage is measured by Latre *et al.* (2008) but the real effect of a cover crop on dry matter yield (DMY) and crude protein (CP) yield of legume (-grass) mixtures was not determined. However, these crops will be grown in the coming 2 or 3 years. In an experiment in Merelbeke (Flanders, northern Belgium) 7 mixtures of grass, clover and lucerne were sown in April with and without peas as cover crop in 2015 and 2016. In this paper the preliminary results of dry matter and protein yield of the sowing in 2015 will be discussed.

Materials and methods

The two-year experiment (2015-2016, 2016-2017) is carried out on a field in Merelbeke 50°59'N 03°46'E) characterized by 830 mm average annual rainfall, sandy loam soil with pH_{KCl} = 6.0 and C-content of 1.39%. On 16 April 2015, seven mixtures were sown with and without peas: (1) lucerne (LU, var. Arpege, 25 kg ha^{-1}), (2) lucerne (25 kg ha^{-1}) + white clover (*Trifolium repens*, WCL, var. Merlyn, 3 kg ha^{-1}), (3) red clover (*Trifolium pratense,* RCL, var. Lemmon, 15 kg ha^{-1}), (4) red clover (12 kg ha^{-1}) + white clover (3 kg ha^{-1}), (5) lucerne (12.5 kg ha^{-1}) + perennial ryegrass (*Lolium perenne*, PR, var. Melways, 20 kg ha^{-1}), (6) red clover (8 kg ha^{-1}) + white clover (3 kg ha^{-1}) + perennial ryegrass (20 kg ha^{-1}) and (7) perennial ryegrass (35 kg ha^{-1}). The lucerne seeds were inoculated with *Rhizobium meliloti,* because no lucerne had been cultivated on that field for more than 30 years. Peas (var. Rebel, 220 kg ha^{-1}) were sown 4 cm deep and the forages 1 cm deep. No herbicides were used. The fertilization in 2015 and 2016 was the same for the plots with and without peas. The field received 80 kg K_2O ha^{-1} and 240 kg K_2O ha^{-1} in 2015 and 2016, respectively. Perennial ryegrass (7) received 80 kg N ha^{-1} in 2015 and 300 kg N ha^{-1} in 2016. The grass-legume mixtures (5, 6) received no N in 2015 and 120 kg N ha^{-1} in 2016. The pure legumes (1, 2, 3, 4) received no N in 2015 and 2016. The trial design was a split plot design with mixtures in the subplots and three replicates. The Fisher's LSD test was used for multiple comparisons. Field plots of 6×1.5 m were harvested with a Haldrup forage harvester at a cutting height of 5 cm. Dry matter yields (DMY) of each replicate were calculated after drying the subsample in a forced-draft oven at 80 °C for 24 h. These samples were analysed by NIRS to determine crude protein content and other nutritional parameters. A grab subsample per plot was separated into the individual sown species.

Results and discussion

The experiment was harvested three times in 2015: 7 July, 31 August and 27 October. The average DMY of mixture (Table 1) was highly significantly influenced by the presence of the cover crop. In the first cut the effect of peas was positive while it was negative for the other cuts as well as for the total DMY. The mixtures were compared within the group (with cover crop or no cover crop) because there was always a significant effect of the interaction between the factors cover crop and mixtures (Table 1).

In the first cut a very significant difference in average DMY was observed between the treatment with and without peas: 7.39 Mg DM ha^{-1} and 3.63 Mg DM ha^{-1}, respectively. About 91% of the DMY were peas and 3% were unsown species in the cover crop treatment. Without a cover crop approximately 5% of unsown species were found in the first cut. In the other cuts, no peas and no unsown species were detected in the experiment. The effect of peas as a cover crop on the weed suppression could not be evaluated here because the number of weeds was very low. In the other cuts no peas and no unsown species were detected. The average DMY of the regrowth when no cover crop was used was much better in the second cut (+1.79 Mg ha^{-1}) and less important but still significantly better in the last cut in October (+0.3 Mg ha^{-1}). Over the year the DMY of the legume-grass mixtures with peas as a cover crop was significantly higher (+1.77 Mg DM ha^{-1} or + 18%) in comparison with the same mixtures without peas. The biggest advantage was measured on the perennial ryegrass (7) (+3.83 Mg ha^{-1}, +52%) and the smallest advantage on the pure stands of the legumes (+1.36 Mg ha^{-1}, +13%). This can be explained by the low N fertilization on the perennial ryegrass and the N fixation capacity of clover and lucerne resulting in a different catch up in DMY by the second and third cut. In the following year, 2016, five cuts were taken and peas as a cover crop in the previous year had no significant effect on the DMY. This was more or less expected because the botanical composition of the mixtures (treatments 2, 4, 5, 6) in the third cut on 29 October 2015 was the same for the mixtures with or without the cover crop (data not presented).

Legume-grass mixtures are an important protein source for cattle feeding on the farm. For this reason it is interesting to calculate the effect of the peas as a cover crop on the crude protein (CP) production per ha (Table 2). In 2015 there was a significant difference in crude protein yield of 133 kg ha^{-1} (7%) between

Table 1. Dry matter yield (Mg ha^{-1}) in the seeding year 2015 of the mixtures sown in spring with or without peas as a cover crop.[1,2]

Average mixtures	Cut 1		Cut 2		Cut 3		Total 3 cuts	
Peas	–	+	–	+	–	+	-	+
	3.62	7.39	4.20	2.41	2.05	1.85	9.88	11.65
	$P<0.001$		$P<0.001$		$P<0.001$		$P<0.001$	
LU	3.98^{a}	7.22^{b}	4.72^{a}	2.97^{a}	2.38^{a}	1.73^{cd}	11.09^{a}	11.92^{ab}
LU + WCL	3.87^{a}	6.97^{c}	4.75^{a}	2.98^{a}	2.43^{a}	1.83^{bc}	11.05^{a}	11.78^{ab}
RCL	3.82^{a}	7.50^{ab}	4.39^{b}	2.43^{b}	1.28^{c}	1.59^{d}	9.49^{c}	11.52^{bc}
RCL + WCL	3.71^{a}	7.40^{ab}	4.05^{c}	2.13^{c}	1.58^{b}	1.68^{cd}	9.34^{c}	11.20^{c}
LU + PR	3.78^{a}	7.70^{a}	4.49^{b}	2.52^{b}	2.47^{a}	1.98^{b}	10.73^{a}	12.20^{a}
RCL + WCL+ PR	3.82^{a}	7.43^{ab}	3.77^{d}	1.90^{c}	2.50^{a}	2.38^{a}	10.09^{b}	11.71^{abc}
PR	2.41^{b}	7.50^{ab}	3.21^{c}	1.93^{c}	1.73^{b}	1.75^{cd}	7.35^{d}	11.18^{c}

[1] Treatments with the same letter in a column are not significantly different at $P<0.05$.

[2] LU = lucerne; WCL = white clover; RCL = red clover; PR = perennial ryegrass.

Table 2. Crude protein yield (Mg ha^{-1}) in 2015.[1,2]

Average mixtures	Total 3 cuts	
Peas	–	+
	1.80	1.93
	P<0.001	
LU	2.21^{a}	2.09^{a}
LU + WCL	2.15^{a}	2.14^{a}
RCL	1.76^{c}	1.89^{bc}
RCL + WCL	1.77^{c}	1.88^{bc}
LU + PR	2.02^{b}	2.01^{ab}
RCL + WCL+ PR	1.74^{c}	1.88^{c}
PR	0.95^{d}	1.63^{d}
legumes	1.97	2.00
legumes-grass	1.88	1.95
grass	0.95	1.63

[1] Treatments with the same letter in a column are not significantly different (P<0.05).

[2] LU = lucerne; WCL = white clover; RCL = red clover; PR = perennial ryegrass.

the treatments with and without peas: 1.93 Mg CP ha^{-1} and 1.80 Mg CP ha^{-1}, respectively. Considering three groups of mixtures (legumes, legumes + perennial ryegrass and perennial ryegrass), the difference in CP production between a cover crop or not, is significantly different: 0.03 Mg CP ha^{-1}, 0.07 Mg CP ha^{-1} and 0.68 Mg CP ha^{-1}. This can be explained by the N fixation capacity of the legumes and the low N fertilization of the grass.

Conclusions

Over the year the DMY of the legume-grass mixtures with peas as a cover crop was significantly higher (+18%) in comparison with the same mixtures without peas. The biggest advantage was measured on the perennial ryegrass (+52%) and the smallest on the pure stand of the legumes (+13%). In 2015 there was a significant difference in CP yield of 133 kg ha^{-1} between the treatment with and without peas. Considering three groups of mixtures (legumes, legumes-perennial ryegrass and perennial ryegrass), the difference in CP production between a cover crop or not, is significantly different: 0.03 Mg CP ha^{-1}, 0.07 Mg CP ha^{-1} and 0.68 Mg CP ha^{-1}. The experiment has been sown again in 2016 to confirm these results.

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Evaluation of the feeding value of leaves of woody plants for feeding ruminants in summer

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Abstract

The nutritive value of a set of 10 woody forage resources (Italian alder, ash, chestnut, field elm, hazel, lime, black locust, white mulberry, holm oak and vine) was investigated. Leaves were collected in summer 2015. Protein and fibre concentrations, condensed tannin concentration, *in vitro* digestibility (enzymatic method), and dry matter (DM) and nitrogen *in sacco* effective ruminal degradability were determined. Results show a large variability between species: crude protein varied from 82 to 245 g kg^{-1} DM, *in vitro* digestibility from 47 to 84%, and effective nitrogen degradability from 18 to 78%. White mulberry is the most interesting species but ash, alder, elm and lime also present sufficient *in vitro* digestibility and nitrogen characteristics to be included in the diet of ruminants. Black locust, vine and holm oak present high levels of condensed tannins and low effective nitrogen degradability.

Keywords: feeding value; woody leaves, ash, mulberry, agroforestry

Introduction

Summer grazing is often limited by the lack of high quality grasslands in regions with summer droughts, i.e. currently the Mediterranean area, but also in future European oceanic regions due to climate change. Leaves from hedgerows, coppices, shrubs, or pollarded trees may become a forage resource for livestock during periods of low grassland production (summer and autumn), either directly by browsing or fed after cutting (Papanastasis *et al.*, 2008). The lack of data on the nutritive value of this unusual forage is an important limitation to its adoption in forage systems of oceanic regions. In the frame of a long term mixed crop-dairy system experiment integrating agroforestry (Novak *et al.,* 2016), a large evaluation of the feeding value of leaves from many woody resources has been initiated. This paper presents the results obtained on 10 woody species evaluated through their protein and fibre concentrations, condensed tannin concentration, *in vitro* digestibility and effective ruminal degradability.

Materials and methods

The leaves of 10 woody species were collected from 3 to 10 August 2015 in the INRA experimental farm located at Lusignan (France, WGS84: XY, 46.42 0.12). Leaves were sampled on at least 3 individuals of each species: Italian alder (*Alnus cordata*), ash (*Fraxinus excelsior*), chestnut (*Castanea sativa*), field elm (*Ulmus minor* × *resista*), hazel (*Corylus avellana*), lime (*Tilia platyphyllos*), black locust (*Robinia pseudoacacia*), white mulberry (*Morus alba*), holm oak (*Quercus ilex*) and vine rootstock (*Vitis* sp.). Alder, ash, mulberry, elm and holm oak were young trees established in 2014, while limes were adult pollarded trees, and vine, chestnut, hazel and black locust were old coppiced trees. Lucerne (*Medicago sativa*) was also collected (3 samples on a 500 m^2 plot) as a herbaceous forage control, harvested after 6 weeks of regrowth. All samples were oven dried at 60 °C during 72 h. For each species, a bulked subsample (500 g dry matter (DM)) was ground to pass through a 0.8 mm sieve. Dry matter and nitrogen effective ruminal degradability (EDDM and EDN, respectively) were determined by the incubation of nylon bags (7.5×15 cm, 46 μm pore size) containing 3 g of fresh sample in the rumen of three ruminally fistulated dry cows, during 2, 4, 8, 16, 24 and 48 h (Michalet-Doreau *et al.,* 1987). The dry cows were stall-fed 9 kg DM/day of a diet based on 70% of high-quality grass hay and 30% of a concentrate based on barley, beet pulp and soyabean. Another subsample (50 g DM ground to 1 mm) was analysed for nitrogen (N, Dumas method with a Flash 2000 CHNS/O Analyzers from Thermofisher on samples ground again with a vibro-broyeur from Retsch), crude protein (CP, calculated as N × 6.25), fibre (neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL), Goering and Van Soest method, 1970), *in vitro* DM digestibility (IVDMD) with the enzymatic method of Aufrère (1982) adapted with the DAISY Incubator from ANKOM, and ash (550 °C during 3 h in a muffle furnace). Two fresh subsamples (20 g) were also frozen to -20 °C before being freeze-dried, and the condensed tannin concentration was determined using the HCl-butanol method (Grabber *et al.,* 2013). The condensed tannin content was extracted twice and purified tannin from sainfoin was used as standard.

Results and discussion

Main characteristics of the woody leaves are given in Table 1. The leaf DM content ranges from 284 g kg^{-1} in black locust to 573 g kg^{-1} in holm oak. The CP concentration varies from less than 85 g kg^{-1} in holm oak to more than 200 g kg^{-1} in black locust, chestnut and white mulberry. The ADL concentration varies from less than 30 g kg^{-1} in mulberry to more than 150 g kg^{-1} in Italian alder and ash. The highest condensed tannin concentrations ($P<0.001$) are observed in black locust, vine and holm oak (respectively 168, 94 and 52 g kg^{-1}). The *in vitro* DM digestibility (IVDMD) ranges from less than 50% in holm oak to 76% in ash and 84% in white mulberry.

DM and CP degradation curve kinetics highlight the large differences between species. EDDM ranges from less to 50% in holm oak, chestnut, hazel and black locust to 75% in ash and 81% in mulberry. EDN varies from less than 25% in hazel and vine, to 79% in mulberry and 81% in lucerne. The lower EDN of locust, vine and holm oak could be linked to their high levels of condensed tannins, reducing protein availability for ruminal microbes. However, chestnut and hazel have few condensed tannins but low EDN suggesting that other compounds are responsible of this effect. The most effective compromise between DM digestibility, protein concentration and protein degradability is obtained with mulberry and ash, which are species traditionally fed to cattle respectively in oceanic and Mediterranean conditions. Hazel, vine rootstock and holm oak seem to be of poor nutritive quality for ruminants, at least for feeding to high-producing animals. These results agree with our first evaluation (Emile *et al.,* 2016) and with other studies (Doran *et al.*, 2007; Luske and Van Eekeren, 2015; Papanastasis *et al.*, 2008).

Table 1. Chemical composition (g kg^{-1} dry matter (DM)), *in vitro* DM digestibility (IVDMD, %), and effective degradability of DM (EDDM, %) and of nitrogen (EDN, %) of leaves of woody species during summer 2015.[1,2]

Species	DM	Ash	CP	NDF	ADL	Condensed tannin	IVDMD	EDDM	EDN
Alnus cordata	412	60	173	373	172	13[cd]	61	57	45
Fraxinus excelsior	545	95	141	251	157	2[a]	76	75	66
Castanea sativa	300	55	207	408	62	2[ab]	64	45	33
Ulmus minor × resista	463	130	148	354	33	30[e]	64	63	45
Corylus avellana	449	68	153	334	44	8[bc]	55	46	17
Tilia platyphyllos	311	119	183	380	72	23[de]	58	59	60
Robinia pseudoacacia	284	53	245	333	64	171[h]	57	48	36
Morus alba	372	123	204	173	28	2[a]	84	81	79
Quercus ilex	573	39	82	528	117	52[f]	47	40	30
Vitis sp.	296	60	128	158	30	94[g]	62	52	24
Medicago sativa (control)	355	85	176	389	66	1[a]	64	68	81

[1]Values within a column with the same superscript letter do not differ significantly.

[2] CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin.

Conclusions

The composition, nutritive value and ruminal degradability of leaves from woody resources collected during summer exhibit large variation among species. White mulberry and ash have sufficient digestibility and nitrogen degradability in summer to be included in the diet of lactating cows in mixed crop-livestock systems. These results have to be confirmed by replications over several years. Our next studies will also consider the effects of season (spring, summer, autumn) and of tree management (pollarding or pruning, mechanical or browsing) on the nutritive value of leaves. Further investigations have to be conducted to obtain more precise information on the effects of condensed tannin concentration and minerals on animal performance and health.

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Maize yield in silvoarable systems established under *Prunus avium* L. in Galicia (NW Spain)

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Abstract

In recent years, in Galicia (NW Spain) the forest area has increased gradually and therefore the availability of agricultural area has decreased. This factor could favour the establishment of silvoarable systems in this region with crops such as maize, because in Galicia maize is the main forage crop to overcome periods of shortage in summer and winter. In silvoarable systems, the production of the crops depends, among other aspects, on tree species and its density when planted. The aim of this study, in Galicia (NW Spain), was to evaluate the yield of maize at different distances from the trees (1.5, 3 and 6 m) in a silvoarable system under *Prunus avium* L., compared with an exclusively agronomic system. The results obtained showed that maize production increased significantly with the distance from the trees and that maize production combined with trees was lower than the production found in the plots without trees, because of a reduction in maize yield and also due to the surface occupied by trees.

Keywords: agroforestry, *Zea mays* L., forage, afforestation

Introduction

Silvoarable systems consist of trees intercropped with annual or perennial crops on the same unit of land (Mosquera-Losada *et al.*, 2009). This type of agroforestry system has traditionally formed important elements of the European landscape, and has the potential to make a positive contribution towards sustainable agriculture in the future (Eichhorn *et al.*, 2006). In Galicia (NW Spain), the establishment of silvoarable systems could be a solution to the low availability of the land area for agriculture. This has decreased in recent years due to the increase of forest land (IV IFN, 2011). On the other hand, Galicia is a region with high economic dependence on dairy cattle where livestock feeding is mainly based on the production of forage maize, which makes it advisable to establish silvoarable systems with this crop. When silvoarable systems are established it is important to be aware that production depends, among other aspects, on tree species and their density when planted, because the interception of light and rainfall by trees is a key factor for the understorey production. The aim of this study was to evaluate the yield of maize at different distances from the trees (1.5, 3 and 6 m) in a silvoarable system established with *Prunus avium* L. compared with an exclusively agronomic system, in Galicia (NW Spain).

Materials and methods

The experiment was established in Boimorto (A Coruña, Galicia, NW Spain) in a plot managed by the Bosques Naturales company. Bosques Naturales is a forestry company that focuses on the management, maintenance, monitoring and research of high-value hardwood species plantations, mainly walnut and cherry. In May 2015, after the soil preparation, forage maize was sown with conventional farm machinery in an alley between two trees rows. The trees were *Prunus avium* L. which had been planted in 2000. The distance between the rows of trees was 12 m and the distance between trees within a row was 5 m. The maize was sown in 10.5 m alley, leaving 0.75 m between the alley at the base of the trees (i.e. 0.75 m on both sides of the tree row). The distance between maize plant rows was 0.75 m and the distance between maize plants within a row was 0.15 m (89 plants per 10 m^2 crop land). In October 2015 the yield of the forage maize was estimated at three distances from the trees (1.5 m, 3 m and 6 m). At each distance and in three different points, ten plants of maize were collected and fresh weights recorded. To estimate the yield of the forage maize the maize final density in the rows was taken into account. Moreover, the yield of the forage maize was also estimated in a treeless area in the same conditions. In the laboratory, the plants were fractionated into the components: aborted cobs, cobs without grains, stems, leaves and grains. These components were dried and weighed to estimate the dry matter yield (60 °C for 48 h). The maize yield per hectare presented in this study was carried out considering the area occupied by the trees. Total maize yield was calculated by summing the yield of the different components. Data were analysed using ANOVA and differences between averages were shown by the least significant difference (LSD) test, if ANOVA was significant. The statistical software package SAS (2001) was used for all analyses.

Results and discussion

Figure 1 shows that the total maize yield increased with the distance from the trees ($P<0.001$). This result could be explained because the maize variety established in this study was selected for open sites, and the negative effect of shade generated by the trees on the maize production probably decreases as we move away from trees. Moreover, competitive effects for soil nutrients and water were probably also higher close to the trees. However, several studies have shown that, in some cases, there may be a moderate beneficial effect of shading for crop yield, mainly in arid areas where the trees can reduce transpiration, or in colder climates where the tree canopy may protect to the crops against ground frosts (Lin *et al.* 1999). Other studies have also shown that the deep roots of trees can draw water and the nutrients from deeper horizons of soil, then release the water into the upper horizons and recycle the nutrients via leaf litter, which could provide benefits to the crops (Jose *et al.*, 2004).

On the other hand, in general, the total maize yield and the yield per hectare of land of its components (aborted cobs, cobs without grain, stems, grains and leaves) was lower when the maize was combined with trees compared with the plots without trees ($P<0.001$). The maize yield obtained in the plots without trees was similar to the yield found in experiments in different areas of Galicia from 1999 to 2014 (22.9 t ha^{-1}) for the same variety (CMR, 2015). As previously indicated, the production in the agroforestry is lower due to the shade generated by the trees and the competition for water and nutrients established between the trees and the maize, but also due to the lower available area of maize in the plots with trees (tree land was discounted; 10.5 m were considered out of 12). Similar results have been observed previously in poplar-maize systems (Ding and Su, 2010). Although the yield of maize obtained in the agroforestry plots was lower compared with the plots without trees, the economic and environmental benefits probably would be higher in the agroforestry plots, as reported previously by Palma *et al.* (2007)

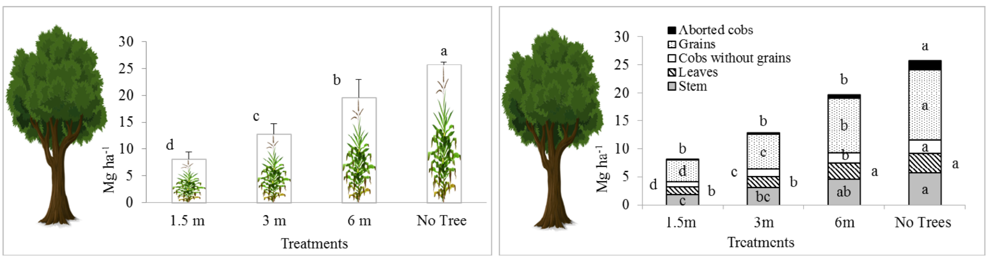

Figure 1. Total yield of maize (Mg dry matter ha^{-1}) and yield of the different components of maize (aborted cobs, cobs without grain, stems, grains and leaves) (Mg dry matter ha^{-1}) under the different treatments in 2015: a distance of 1.5 m, 3 m and 6 m between trees and maize rows. Different letters indicate significant differences between treatments. Vertical lines indicate mean standard error.

in silvoarable systems established in Mediterranean and Atlantic regions of Europe. Therefore, we can recommend employing silvoarable maize systems as long as the tree density is adequate to allow the growth of trees and maize without competition.

Conclusions

The maize yield per hectare increased with the distance from the trees, and the maize yield combined with trees was lower compared with the yield found in the plots without trees due to the surface occupied by trees, and eventually by competition for light, water and nutrients. It is necessary to extend our study further with consideration of economic and environmental factors in order to recommend an adequate tree density for the production of maize in this type of agroforestry system.

Acknowledgements

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Effects of wilting and *Lactobacillus buchneri* on the aerobic stability and kinetics of gas loss during fermentation of oat silage

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Abstract

Our objective was to evaluate the effects of wilting and a heterolactic inoculant on the aerobic stability and dynamics of gas formation during fermentation of whole-crop oat silages. A plot of white oat (*Avena sativa* cv. URS Charrua) at head-emergence stage was split equally. During the morning, half of the plot was cut and allowed to wilt for 5 h (30% dry matter (DM), Wilt), and the other half was directly harvested in the afternoon (21% DM, DC). Both forages were mechanically chopped and treated with *Lactobacillus buchneri* (4×10^5 cfu g^{-1}; LB) or distilled water (5 ml kg^{-1}; Ctrl). Treated forages were packed into 1.96 litre gas-tight silos (4 replicates per treatment). After 112 d of storage, silos were opened for measuring the aerobic stability. Treating oat silage with LB decreased the aerobic deterioration, even though the control silages had prolonged aerobic stability. Wilted silage yielded less gas (l kg^{-1} DM) and had lower fermentative losses than oat ensiled with original moisture. However, LB increased gas production and DM loss during fermentation in both wilted and direct-cut oat. Hence, LB might not be recommended for treating oat silage with up to 30% of DM.

Keywords: aerobic deterioration, *Avena sativa*, carbon dioxide, fermentative loss

Introduction

In silages, gases are the main sink of dry matter (DM) loss and are formed as by-products of microbial activity, especially in heterofermentative pathways. Our objective was to evaluate the effects of wilting and a heterolactic inoculant on the dynamics of gas formation during fermentation and aerobic stability of whole-crop oat silages.

Materials and methods

A plot of white oat (*Avena sativa* cv. URS Charrua) at head-emergence stage was split equally. During the morning, half the plot was cut and allowed to wilt for 5 h (Wilt), and the other half was directly harvested in the afternoon (DC). Both forages were mechanically chopped (8 mm), divided in two piles, and treated with *Lactobacillus buchneri* 40788 (4×10^5 cfu g^{-1}; LB) or distilled water (5 ml kg^{-1}; Ctrl). Afterwards, treated forages were packed (0.40 porosity) into 1.96 litre gas-tight silos (4 replicates per treatment). The internal pressure of the silos was measured by using a pressure transducer and converted to volume (Daniel and Nussio, 2015). Accumulated gas production per kg DM was fitted with an exponential 1-pool model ($G_t = G \times [1 - e^{-(k \times t)}]$ to estimate the fractional rate of gas production (k) and gas pool (G). After 112 d of storage, silages were exposed to air for 10 d. Silage pH was measured every other day and aerobic stability was denoted as the length of time that elapsed before silage and ambient temperatures differed by more than 2 °C.

Results and discussion

Wilting oat crop during 5 h raised the DM content from 21 to 30% (Table 1), due to good weather conditions. Therefore, wilted oat silage yielded less gas (Figure 1), had lower fermentative losses and tended to have higher pH than oat ensiled with original moisture, probably because a lower microbial activity. Inoculation with LB decreased the aerobic deterioration, even though the control silages had

Table 1. Fermentative losses and aerobic stability of oat silages untreated (Ctrl) or inoculated with *Lactobacillus buchneri* (LB).

Item	Direct-cut		Wilted		SEM[2]	*P*-value[1]		
	Ctrl	LB	Ctrl	LB		F	I	F × I
Dry matter (DM) (% as fed)	21.59	20.54	30.82	30.11	0.313	<0.01	0.02	0.61
pH	3.90[c]	4.61[a]	4.05[b]	4.59[a]	0.034	0.07	<0.01	0.03
DM loss (%)	3.96	9.69	3.89	7.15	1.192	0.30	<0.01	0.32
Gas loss (% DM)	2.35[c]	7.76[a]	2.04[c]	5.48[b]	0.196	<0.01	<0.01	<0.01
Gas emission (% DM)	2.81[c]	8.02[a]	2.45[d]	5.96[b]	0.111	<0.01	<0.01	<0.01
Gas pool[3] (l kg^{-1} DM)	13.38[c]	37.50[a]	10.88[d]	27.64[b]	0.401	<0.01	<0.01	<0.01
Fractional rate of gas production[3] (d^{-1})	0.443	0.088	0.438	0.118	0.0188	0.52	<0.01	0.37
Aerobic stability (h)	184	240	176	240	16.3	0.82	<0.01	0.82

[1] F = forage effect, I = inoculant effect.

[2] Pooled standard error of the mean.

[3] Data were fitted with exponential model. Means within a row with different superscripts differ by Tukey's test (α=0.05).

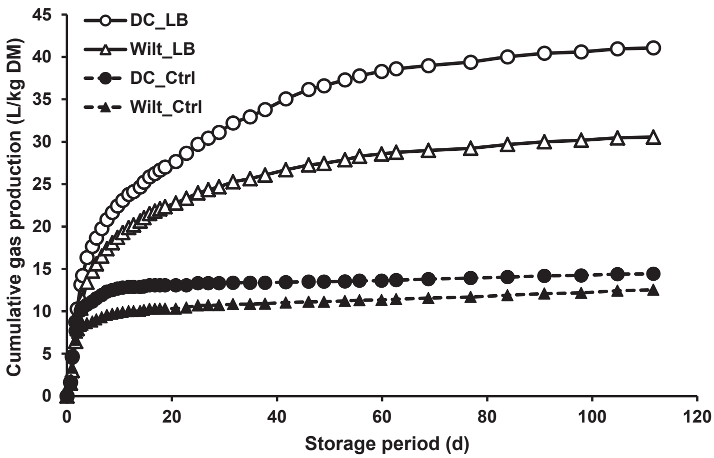

Figure 1. Cumulative gas production during fermentation of oat silages. DC = direct-cut; Wilt = wilted; Ctrl = without inoculant; LB = inoculated with *Lactobacillus buchneri*. See Table 1 for model parameters (kinetics).

prolonged aerobic stability and kept the pH stable for more than 8 d upon air exposure (Figure 2). On the other hand, LB increased gas production and DM loss in both wilted and direct-cut oat, particularly after 10 d of storage. As expected, the fractional rate of gas production was decreased in LB silages, due to the greater gas pool. The higher pH of inoculated silages indicates that lactic acid might have been converted to acetic acid and CO_2 by LB.

Conclusions

All silages had prolonged aerobic stability. Wilted silage had lower fermentative losses, whereas *L. buchneri* increased gas production during fermentation in both wilted and direct-cut silage. Hence, treating oat silage up to 30% of DM with *L. buchneri* might not be recommended.

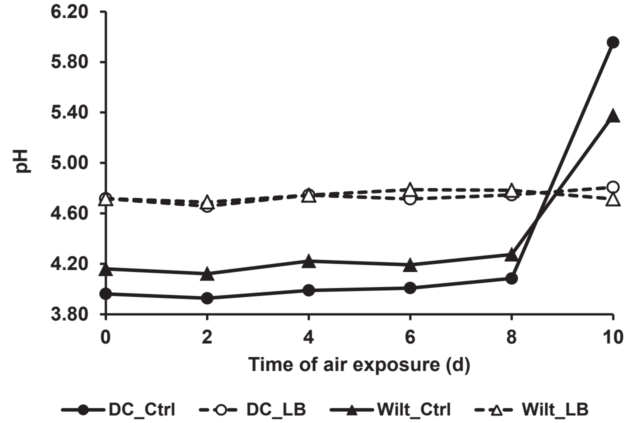

Figure 2. The pH during air exposure of oat silages. DC: direct-cut, Wilt: wilted, Ctrl: without inoculant, LB: inoculated with *Lactobacillus buchneri*. P=0.80 for forage effect, P=0.01 for inoculant effect, P=0.85 for forage × inoculant, P<0.01 for day effect, P=0.81 for forage × day, P<0.01 for inoculant × day, P=0.94 for forage × inoculant × day.

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Result-based and value-based payments – more efficient ways forward to promote grassland with multiple benefits

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Abstract

Result-based and value-based agri-environmental payments are potentially significantly more efficient than the existing management-based and cost-based payments. However, a core concern has been whether these payments are compatible with international regulations. This paper examines the obstacles with respect to the WTO and the CAP regulations, but also promising possibilities to introduce such payments for the supply of biodiversity and other public goods of permanent grassland.

Keywords: agri-environmental payments, multifunctional agriculture, permanent grassland, result-based payments, value-based payments

Higher agri-environmental payments to grassland with more environmental services

All agricultural land, and in particular permanent grassland, yields a spectrum of goods, not just market commodities such as meat, but also biodiversity, cultural heritage and landscape amenities that are non-market goods. These non-market goods all have the character of public goods, which means that they normally cannot be provided efficiently by the market mechanisms (Randall, 1972). The supply of public goods does not normally meet the social demand, at least not at the present technology and relative prices. This implies that grassland would be underpaid and the grassland area smaller than what is socially efficient.

The agri-environmental payments of CAP play a crucial role to offset this market failure, although considerable amounts of money from the Rural Development Programmes are also spent on schemes that are not for positive, public goods. EU expenditure on agri-environmental measures is budgeted to almost € 85 billion for the period 2014-2020, supplemented by co-financing from the member states. The agri-environmental payment schemes have, however, in many cases been assessed as inefficient with unnecessary high costs or small environmental effects. They have also been criticized by farmers for being complicated, with rigid control. The critique applies not least to the grassland schemes (see e.g. Kleijn *et al*, 2001; European Court of Auditors, 2011).

A promising alternative to the prevalent agri-environmental payments could be result-based payments (RBPs) or value-based payments (VBPs) (Table 1). *Result-based payments* are conditioned on the delivery of environmental services, in contrast to the prevalent payments which are based on complying with conditions about management practices. A typical scheme today may have a number of conditions to

Table 1. Typology of payment forms.

		Principle of pricing	
		cost-based agri-environmental payments	**value-based agri-environmental payments**
Type of conditions for receiving payment	**result-based agri-environmental payments**	a few, small scale schemes in some EU member states	socially efficient design
	management-based agri-environmental payments	the present agri-environmental payments	(technically possible, but in reality less relevant)

fulfil regarding what to do, when to do it and what not to do. With RBPs, a farmer would instead get the remuneration according to what the land provides in terms of biodiversity and other public goods as measured by indicators, mostly monitored annually. Pastures may, for instance, be rewarded for being free from brushwood or having a rich flora, measured by listed indicator species. The choice and design of the indicators are crucial to the efficiency of RBPs, since they differ with respect to policy relevance, precision, monitoring costs, temporal responsiveness, pedagogic qualities, etc.

Value-based payments are differentiated according to the quantity or quality of environmental services: the higher the (environmental) value, the higher the payment (Hasund, 2012). This contrasts to the present agri-environmental payments, which are *cost based*. Accordingly, they must not exceed the farmer's additional costs of producing the environmental service, including possible income forgone.

The distinction between RBPs and VBPs is thus that the latter are settled by a pricing of the environmental services according to their social value, independent of induced costs, while RBPs are settled by being independent of which management measures are carried out. The German and French RBP-schemes to grassland are using flora indicators for measuring the results, but they are not value-based as the payments are limited to calculated costs.

Many advantages but also minor risks

The motives to replace the present management-based and cost-based payments with result and value payments are higher efficiency, better flexibility, dynamic properties, more positive farmer attitudes with higher scheme participation, a potential to simplify the CAP and the schemes' control properties (Keenleyside *et al*, 2014; Matzdorf and Lorenz, 2010; Schroeder *et al.*, 2013). Larger environmental benefits may be achieved at lower costs because RBPs give farmers economic incentives directed straight at what is demanded by society. Farmers would then focus on delivering more of what they are paid for, such as more species, instead of just trying to comply with prescribed management practices (that may be more or less appropriate to give the environmental service). By paying according to the presence of environmental services the RBPs give a socially more efficient allocation of resources (Hasund, 2012). A great advantage of RBPs is that they enable more flexibility in production. Farmers are not constrained to technology that may be more expensive. Instead, they are in principle free to adapt the production to their own, the site's, the farm's and the weather's conditions. There are also stronger incentives to develop new production methods for the joint production of agricultural commodities and environmental services.

Farmers may, however, be exposed to larger economic risk if the results and payments depend upon factors that they cannot control, such as weather conditions. These risks may, on the other hand, be reduced if the indicators and payments are designed in steps or scaled, minimizing the risk of large, unexpected income losses. Interviewed farmers in Germany have also expressed that they are not discouraged by such risks (Matzdorf and Lorenz, 2010). The transaction costs of transforming from the present payment system to a system based on results must also not be neglected.

Result-based payments are compatible with WTO and EU regulations

Contrary to what has often been declared in policy discussions, RBPs are not restricted by the Agreement on Agriculture of the World Trade Organization (WTO). Nor are they restricted by the regulations of CAP, at least as long as they do not exceed verified costs. Actually, there are already a few, small-scale RBP-schemes operating in the EU, for example France and Germany. They are all developed for permanent grassland.

Value-based payments are partly restricted but may be introduced

The WTO rules do not restrict the use of VBPs. It is, however, not settled whether they will be classified into the WTO 'green box' or 'amber box'. If the green box criteria are not violated, there are no limits on the use as these payments then are exempt from the legally binding WTO ceiling on certain support to farmers (Hasund and Johansson, 2016). The WTO 'green box' applies to only minimally trade and production distorting support.

If, on the other hand, the VBPs will be within the WTO 'amber box', they are still permitted but their size is restricted by a legally binding ceiling level on trade-distorting support. The EU has, however, a very large margin (many billions of euros) to that ceiling. As long as the ceiling is respected, there are no restrictions in the WTO Agreement on Agriculture on how large the payments per hectare may be. Furthermore, if the support is less than 5% as calculated per value of favoured market products, the payments are not counted into the aggregate amount of restricted support. The CAP rules do, unlike the WTO agreement, not allow VBPs if these exceed the extra management costs, including income forgone to deliver the environmental service (Hasund and Johansson, 2016).

Conclusions

RBPs and VBPs have several appealing properties, not the least a potential for higher efficiency, simplification and larger flexibility for farmers. VBPs are not restricted to give just cost compensation, but do also imply an option to agriculture in making profit also on producing public goods, and not just on market commodities. However, if introduced at a larger scale, mechanisms have to be applied to verify that the payments do not exceed the social value of the addressed public goods. There are, in practice, no restrictions in the WTO Agreement against using RBPs or VBPs, even if the latter were classified into the 'amber box'. The CAP regulations have to be revised if they are to permit VBPs above additional costs, but they do not restrict the use of RBPs.

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Using 3-D laser measurements for biomass estimation in semi-natural grasslands invaded by *Lupinus polyphyllus*

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Abstract

Lupinus polyphyllus is an invasive neophyte that is threatening the biodiversity of semi-natural grasslands adapted to nutrient-poor conditions. 3-D laser measurement can be a tool to help conservation managers to develop the optimum management strategy for large areas, if it is possible to determine biomass and invasive plant biomass. Therefore, we evaluated the potential of a 3-D laser scanner to determine the biomass of 6 plots of semi-natural grassland. It was possible to determine dry matter yield with an accuracy of R^2 0.45 to 0.60 and fresh matter yield with an accuracy of R^2 0.72 and 0.81. Further research will concentrate on the improvement of coefficient of determination and identification of *L. polyphyllus* biomass by using the intensity of reflected laser pulse information which is also provided by the laser scanner and additional RGB information.

Keywords: *Lupinus polyphyllus*, invasive, 3-D laser, remote sensing

Introduction

The negative effect of invasive neophytes on natural ecosystems is a global problem because invasion often leads to structural changes in the natural vegetation, which can lead to drastic changes for the whole ecosystem including losses of biodiversity. The invasive species *Lupinus polyphyllus* Lindl. (hereafter named 'lupine') is one of the most important neophytes in Central Europe and endangers especially lower mountain semi-natural grasslands, for example in the German biosphere reserve 'Rhön'. Professional management and control of the invasive species requires a high investment in technical equipment, personnel and specific knowledge and causes high costs. Therefore, a tool is necessary to improve the knowledge about biomass yield of both natural and invaded semi-natural grassland areas over time to better plan cutting dates. A method is needed to easily estimate both semi-natural grassland vegetation and neophyte yield for large open grassland areas. The terrestrial 3-D laser seems to be a promising solution. As a first step towards the realisation of invasive and non-invasive biomass estimation on a large scale, we investigated the use of the 3-D laser scanner for overall biomass estimation in semi-natural grasslands invaded by lupine.

Materials and methods

To test and evaluate the potential of 3-D laser measurements for estimating the biomass yield of semi-natural grasslands invaded by lupine, six plots (each 8×8 m) were sampled with traditional field methods and a terrestrial laser scanner at three dates (25 May 2016, 14 June 2016 and 12 September). GPS coordinates were taken. Three plots were located in an area characterized by the occurrence of *Nardus stricta* and three plots were located in a mountain hay meadow characterized by *Trisetum flavescens*. The plots were subject to standard agricultural use by farmers, which included a harvest shortly after 15 June. The *N. stricta* plots were harvested a second time shortly before the September date; thus, no data on biomass could be collected for the three *Nardus* plots in September. The sampled plots were divided into 64 sub-plots and for each one the lupine cover was recorded. In addition, the biomass of three randomly chosen sub-plots (1 m^2) was harvested at 5 cm stubble height by hand and separated into lupine and non-lupine biomass. The biomass was weighed fresh and the dry matter content (DMC) was determined after drying in an oven for 48 h at 105 °C.

Each plot was measured with the 3-D laser (Leica ScanStadion P30) from four different sides around the sampling plot to minimize the effect of signal shadows. From the laser data information about ground structure, vegetation height and vegetation structure was extracted. The resolution of the sensor was 3 mm at a distance of 10 m, resulting in more than 4 Million points for each sampling plot.

In order to derive information about vegetation biomass from the point cloud, the point cloud space was divided in a 3-D grid of small regular cubes (voxel) with a resolution of 5 mm. The resulting number of voxels for each sampling date and plot were used as explanatory variables in a linear regression model to predict dry matter yield (DMY). As DMY showed a skewed distribution the values were logarithmised prior analysis.

Results and discussion

Table 1 shows the measured DMY estimated from 3 of 64 subplots per plot. It is obvious that the DMY increased significantly in the period from 25 May to 14 June to a DMY which can be considered typical for lower mountain semi-natural grasslands with no fertilisation input and with a one- or two-cut per year mowing regime (Hensgen *et al.,* 2014). The low DMY in September is due to the harvest of the areas in June by local farmers and the later grazing by sheep of the *Trisetum* areas. In the sampled subplots lupine DMC was between 7.8 and 21.2% for all cutting dates, partly caused by light rainfall before harvesting in June. For comparison, the DMC of the *Nardus* plots was between 15.5 and 23.1% and for the *Trisetum* plot between 7.8 and 36.2%. Nearly all plots were invaded by the lupine, which lead to estimated cover percentages of between 0 and 30%. Lupine dry matter contribution was between 0 and 39.1% of the measured DMY. A high percentage of lupine hinders the use of the harvested biomass as the lupine contains alkaloids which are toxic to animals (Veen *et al.*, 1992).

Prediction of total biomass with the 3-D laser data showed medium (*Trisetum* grasslands) to high (*Nardus* grassland) coefficients of determination (R^2_{adj} of 0.45 and 0.60, respectively (Figure 1)). As the voxels represent fresh biomass rather than DMY, the higher R^2 values for the *Nardus* grassland could be explained by the slightly higher DMC, which results in a smaller difference between fresh matter yield and DMY. Another hypothesis would be that the more homogeneous vegetation structure of the *Nardus* plots allow for a better accuracy of determination of biomass. The number of voxels was negatively correlated with the estimated biomass, which can be explained by the fact that a higher and thus denser biomass reduced the penetration depth of the laser impulse. Due to the reduction of potential hits for the laser beam the number of voxels is also decreasing with increasing biomass.

Table 1. Dry matter (DM) yield, lupine cover and lupine percentage of dry matter yield of 6 semi-natural grasslands at three cutting dates.

Area	Dry matter yield Mg DM ha^{-1}			Lupine cover % [0-100]			Lupine dry matter yield % of total DM yield		
	May	June	Sept.	May	June	Sept.	May	June	Sept.
Nardus 1	0.5	2.3	-	14.0	25.7	-	13.6	14.4	-
Nardus 2	0.8	2.6	-	13.0	7.0	-	11.9	5.6	-
Nardus 3	0.6	2.0	-	5.7	7.3	-	16.3	10.0	-
Trisetum 1	0.9	2.4	0.6	16.7	21.7	14.7	24.7	16.1	25.2
Trisetum 2	0.6	2.0	0.9	9.0	30.0	14.7	18.5	26.0	39.1
Trisetum 3	0.4	1.1	0.8	0.0	0.0	2.0	0.0	0.0	3.6

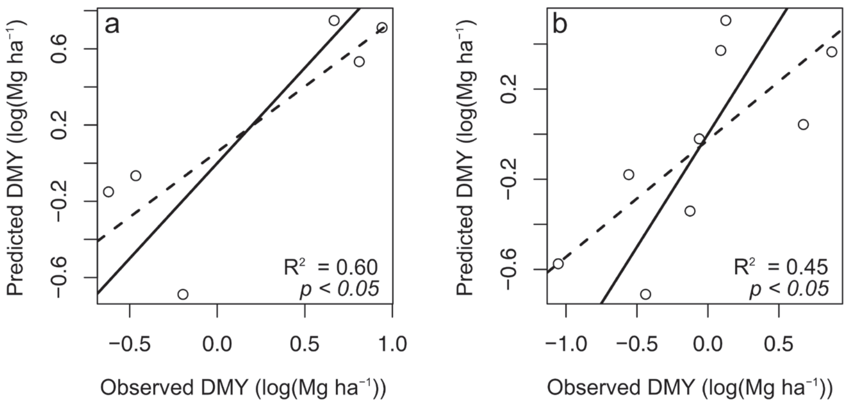

Figure 1 Plot of fit of observed dry matter yield (DMY) measured with traditional method in comparison to the predicted DMY calculated with data from the laser measurements. (A) Plot of fit for the *Nardus* grassland plots, (B) Plot of fit for the *Trisetum* grassland plots.

Conclusions

It can be concluded that the 3-D laser and the number of detected voxels are a feasible way to estimate semi-natural grassland biomass with a medium determination accuracy. In a next step of research, techniques will have to be developed to identify lupine biomass and estimate the amount of lupine and non-lupine biomass in order to install a fitting management regime to halt the spread of this invasive plant species and secure and conserve semi-natural grassland diversity.

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Innovative silage additives to reduce proteolysis in the silo

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Abstract

Fermentation during ensiling and rumination causes proteolysis and thereby a loss of nitrogen efficiency because produced ammonia is easily excreted by cows. However, some silage additives could improve this efficiency. Two experiments compared effects of seven additives on proteolysis in laboratory-scale silos. Firstly, chestnut and oak tannins, essential oils, zeolite and bentonite were compared to controls in vacuum packs filled with 850 g grass. Secondly, erythritol and lignosulfonates were added to grass ensiled and stored at 40°C. Additives were tested on grass fertilized up to 180 kg ha^{-1} of N. After 30 days of ensiling, dry matter content, pH and %NH_3 were analysed. Silage pH was not affected by additives. The first experiment showed significant effects of treatment, fertilization and interaction on %NH_3 (P<0.01). Tannins reduced ammonia production during ensiling compared with the negative control (-16 to -18%). The second experiment could not demonstrate a significant effect of reducing sugars. In both cases, additives were more effective on highly fertilized grass rich in crude proteins. However, it has yet to be proven that ammonia reduction is associated with more true proteins and that total N efficiency is improved. Tannins and reducing sugars are supposed also to have an effect during rumen fermentation.

Keywords: silage, nitrogen, efficiency, additives

Introduction

The high content of degradable nitrogen of grass silage often induces a low protein efficiency by ruminants. Quickly available to rumen microorganisms, this nitrogen is often taken in excess and largely excreted in the form of urine. This arises from the silage-making process itself, which causes a lysis of plant protein. The loss of NUE (Nitrogen Use Efficiency) leads to higher needs of proteins in cattle diets, higher feed costs and more N excretion causing environmental concerns. Scientists have been looking for ways to better conserve protein value of silage for years. Tannins have been identified as potential additives able to bind with proteins and prevent hydrolysis both in silage and in the rumen (Piluzza *et al.*, 2014). Reducing sugars which are prone to making complexes with proteins under heat (Maillard reaction), can thereby increase the proportion of by-pass proteins reaching the intestine (Wright *et al.*, 2005). It has been demonstrated that essentials oils have an impact on bacterial activity and could thereby reduce protein degradation during fermentations (Kung *et al.*, 2008). It has been shown that bentonite and zeolite reduce silage ammonia content (-43 and -34%, respectively) (Khorvash *et al.*, 2006). This article presents the first investigations about the effect of seven additives thought to have a potential impact on silage and rumen fermentation.

Materials and methods

Two experiments were conducted in spring 2016 in Gembloux, Belgium. Five field plots (100×18 m) of *Lolium multiflorum* were fertilized in March with different modalities assigned randomly. The control was unfertilized, three plots were fertilized with ammonium nitrate at respectively 30, 60 and 180 kg ha^{-1} of N, and the last one received Sulfammo (TimacAgro) at 60 kg ha^{-1} of N. All plots were mowed in May and pre-wilted for two days. On-field pre-wilted grass was harvested and chopped by a loader wagon. Plastic bags-in-boxes (10 l capacity, ref: 017.888.9, Brouwland, Beverlo, Belgium) were filled with 850 g of homogenized ryegrass. In the first experiment, 21 bags were made per plot (seven treatments repeated three times). Treatments were negative control (without additive), positive control (commercial preservative Sil70 at 3.5 g kg^{-1} fresh matter (FM), TimacAgro), chestnut tannin (0.8 g kg^{-1} DM), oak tannin (10 g kg^{-1} dry matter (DM)), blend of thymol and carvacrol (26 and 21 mg kg^{-1} FM), zeolite (20 g kg^{-1} FM) and bentonite (10 g kg^{-1} FM). The 105 bags were then sealed with a vacuum packaging machine (model UNICA GAS, Lavezzini, Fiorenzuola, Italy) and stocked in a room with monitored temperature (average = 22.7±0.69 °C). In the second experiment, nine bags were filled per plot: three without additive, three with erythritol (60 g kg^{-1} DM) and three with lignosulfonates (20 g kg^{-1} DM). These 45 bags were sealed and stored in a forced-air oven at 40 °C. After 30 days of ensiling, laboratory-scale silos were opened and homogenized. Each silage was analysed for pH with a conventional glass electrode. Oven-dried at 60 °C (≥72 h) and grounded in a Cyclotec mill (1 mm screen, FOSS, Hillerød, Denmark) silages were analysed for chemical composition by near infrared reflectance spectroscopy (XDS-system spectrometer, FOSS, Hillerød, Denmark). Predicted sugar content was confirmed by the Luff-Schoorl method on ten samples. Silage NH_3 content was determined according to the Kjeldahl method in the water extract (apparatus from FOSS, Hillerød, Denmark). We used ANOVA tests with Tukey post hoc test (IBM SPSS).

Results and discussion

These two experiments are a preliminary study to test additives selected for their potential effect on nitrogen both in silage and rumen with the aim of improving cattle NUE. This study assesses the effect of additives on proteolysis in the silo by comparing NH_3 content of silages. Before ensiling, wilted grass was poor in crude proteins (5.31% of DM without fertilization to 12.82% of DM for the highest fertilization) but rich in total soluble sugars (39.78 to 19.06% of DM, respectively). Dry matter content was lower for the highest fertilization (26.96%) than unfertilized (41.23%) because of higher yield and greater grass density during wilting. In both experiments, low nitrogen content could prevent us from detecting effects of some additives.

Experiment 1

pH ranged from 4.31 to 4.82 with a mean of 4.52 but showed no significant effect of additive (P=0.569). Table 1 presents NH_3 content (% of total nitrogen) of silages. NH_3 content ranged from 2.24 to 10.41%, the highest fertilization showing a higher NH_3 percentage than all other levels of fertilization (+162 to +276%) (P<0.001). Indeed ammonium nitrate increases crude proteins content of grasses and specifically the NH_3 part. Besides, additives had a significant effect on %NH_3 (P<0.001). Both tannins reduced ammoniacal content of silage compared to negative control (-16% for chestnut and -18% for oak tannins) suggesting a reduction of proteolysis during the ensiling process. Oak tannins resulted in even less NH_3 content than acid treatment (-9%). It seemed that tannins and proteins formed complexes effective at protecting proteins from plant and bacterial enzymes as described by other authors (Piluzza *et*

Table 1. %NH_3 in total nitrogen content of laboratory-scale silages at ambient temperature (means of 3 replications) in relation to fertilization level and additive.[1]

N rate	No additive	Sil70	Chestnut Tannin	Oak Tannin	Essential Oils	Zeolite	Bentonite	Mean
0	2.62	2.26	2.29	2.48	2.80	2.87	2.92	2.61^{ab}
30	3.12	2.29	2.24	2.25	2.44	2.41	2.63	2.48^{a}
60	3.46	3.01	3.31	2.72	3.83	4.71	3.89	3.56^{c}
60 Sulfammo	3.25	2.78	3.10	2.99	3.21	3.06	2.93	3.05^{bc}
180	10.41	10.14	8.27	8.24	10.32	9.39	8.56	9.33^{d}
Mean	4.57^{a}	4.09^{abc}	3.84^{bc}	3.74^{c}	4.52^{a}	4.49^{ab}	4.19^{abc}	

[1] Different letters in the same column or line represents a significant difference (P<0.05).

al., 2014). Other additives showed NH_3 levels similar to controls and were thereby considered inefficient at preventing proteolysis.

Furthermore, we observed a significant interaction between fertilization and additives ($P<0.01$). Additives are more effective at reducing NH_3 content when used on high nitrogen content grasses. These results are encouraging with a view to better use nitrogen-rich forages through actions on silage and rumen fermentations. Tannins are linked to proteins in the rumen but the complex is thought to dissociate in contact of abomasal acidic pH or duodenal alkaline pH (Piluzza *et al.*, 2014). These properties could allow a reduction of protein degradation in rumen and an increase in absorption in the intestine leading to improved NUE in addition to the effect on silage.

Experiment 2

Mean pH of these silages reached 4.92 (minimum of 4.70 and a maximum of 5.24). The ANOVA test showed no effect of additive on pH (P=0.378). Table 2 presents NH3 content (% of total nitrogen) of heated silages. In this experiment, we detected an effect of fertilization on NH_3 content ($P<0.001$) with the same observations as in the previous experiment. No significant difference between additives and control was observed (P=0.071). However, observed statistical power for the additive factor was low (0.524) which prevent us to draw any conclusions on additives.

Conclusions

Our results highlighted two promising additives to increase NUE in cattle through actions on silage and rumen fermentations. Chestnut and oak tannins were effective at reducing NH_3 content in silage suggesting a reduction of proteolysis during fermentation. This could be explained by the formation of tannin-protein complexes protecting proteins from enzymes but soluble in low pH. However, it has yet to be proven that reduced NH_3 content is linked with more true proteins and that total N efficiency is improved.

Table 2. %NH_3 in total nitrogen content of laboratory-scale silages at 40 °C (means of 3 replications) in relation to fertilization level and additive.[1]

N rate	No additive	Erythritol	Lignosulf.	Mean
0	3.23	3.22	3.21	3.22[a]
30	4.01	5.18	4.34	4.51[b]
60	4.36	4.71	4.07	4.38[b]
60 Sulfammo	4.24	4.68	5.01	4.64[b]
180	6.35	6.14	5.97	6.15[c]
Mean	4.44[a]	4.79[a]	4.52[a]	

[1] Different letters in the same column or line represents a significant difference ($P<0.05$).

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Multispecies pastures in Mediterranean zones: agro-ecological resilience of forage production subject to climatic variations

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Abstract

Since the end of the 20th century, the meteorological variability (frost, floods and drought) has increased in the Mediterranean area, reducing pasture productivity. Consequently, farmers are forced to buy forages and concentrates to secure their feeding system. These purchases threaten the financial balance of their farms. The main objective of this study is to find an alternative solution to secure the feeding system for Mediterranean livestock. In Europe and northern France, studies have shown a renewed interest in multispecies grasslands. In the southern European countries, Portugal has developed these grasslands in the Alentejo region. In the French Mediterranean zone, there was no study before 2012, when we started testing our mixtures in Montpellier (France). The results show the ability of multispecies grasslands to maintain satisfactory levels of biomass and crude protein despite contrasting weather conditions.

Keywords: grass-legume mixtures, Mediterranean grassland, adaptation to climate change

Introduction

Meteorological uncertainties are more frequent in Mediterranean regions where forage production from rangeland is irregular (Acar *et al.*, 2012). Some livestock farms cultivate temporary grassland, often mono-specific, to produce additional forage (Lemaire, 2008). However, this forage crop is also subjected to meteorological hazards. The impact of forage deficits tends to become widespread with the increase of the fodder cost. Several strategies are being explored to ensure greater forage self-sufficiency such as pastoral agro-forestry practices, adjustments in the conduct of animals (Guérin and Agreil, 2007) identification of ecotypes more drought resistant (e.g. *Festuca arundinacea*, *Dactylis glomerata*, *Medicago* spp.) or the introduction of multi-species grasslands.

Multi-species grasslands are being developed in Europe (Huyghe *et al.*, 2014). With these grasslands, farmers aim to: (1) reduce nitrogen inputs using legumes (Lüscher *et al.*, 2014); (2) have more resistant productivity to meteorological risks; (3) improve the nutritional value of fodder (Morel *et al.*, 2016); and (4) reduce competition from weeds. In Portugal, multi-species grasslands (dominated by leguminous plants) are spreading in the Alentejo region. This type of grassland does not exist in the French Mediterranean zone. Our research question is: can multi-specific grasslands (mainly composed of legumes) maintain a quite stable forage yield and nutrient quality in an uncertain meteorological context (spring frost, flooding, water deficit)? Our objective is to contribute to improving the food security of livestock farms.

Material and methods

The experimental site, located 10 km from the sea, is subject to sea breezes (impact on potential evapotranspiration). Rainfall surveys over 30 years show an annual average of 680 mm in just 85 days. The rains are more intense in autumn ('Episode cévenol' >200 mm/24 h, Fr.-météo). With the Fertiprado Lda R/D service (www.fertiprado.pt), we tested different mixtures with 7-8 species, fertilized with <50 P and K units and without irrigation. Each mixture contained 5 legume species (Table 1) and was sown on 8 plots of 12 m^2 in September 2012.

Table 1. Mixture composition.

	M1	M2	M3	M4
Avena strigose				X
Lolium multiflorum (2n)	X		X	X
Lolium multiflorum (4n)	X	X	X	X
Triticum secale		X		
Medicago scutellata	X			
Trifolium alexandrinum	X	X	X	X
Trifolium michelianum	X			
Trifolium resupinatum majus	X	X	X	X
Trifolium squarrosum	X	X	X	X
Vicia sativa		X	X	X
Vicia villosa		X	X	X

Half of the plots were mowed in early May (once); the remaining half was mowed twice (mid-April plus end of May). The evolution of the floristic composition, the yield and the quality of each mixture were measured. We used the linear quadrat method, a self-weighing mower (the dry matter was obtained with an oven (48 h, 60 °C) and a near-infrared spectrometer (NIRS) on fresh material after cutting in the field green and then dry.

Results and discussion

During these 3 years (2012/2013; 2013/2014; 2014/2015), the weather profiles were much contrasted. In September 2013, heavy rains ('Cévenol episode') damaged some seedlings. However, over the course of 2 years we have had satisfactory seeding and the development of vegetation cover under climatic constraints and very varied soil water capacity (Figure 1). The 2012/2013 season was characterized by available water (PAW) until the end of May. This situation is common in this area. The 2014/2015 season shows a deficient PAW from April and reaches wilting point at the beginning of May (Figure 1).

The 2014/2015 season saw a reduction in biomass production (Figure 2). However, for an early water deficit and a negative PAW of about 50%, this reduction represents only 10% compared to the 2012/2013 season. In addition, the crude protein content is higher in the year of water deficit, probably induced by a concentration phenomenon (Figure 2). Thus, the amount of protein produced by the forage remains

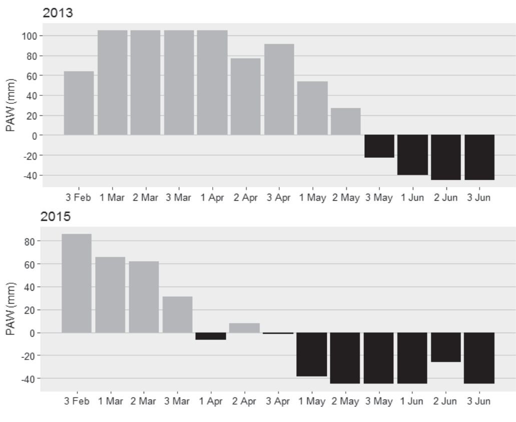

Figure 1. Excess or deficit of soil water per decade.

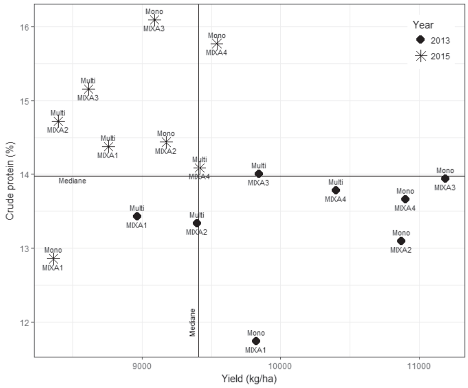

Figure 2. Forage yield and crude protein content according to years with different water deficit and number of mowing.

stable between two seasons. The different water availability affects the yield only slightly and does not affect the amount of protein.

The mixtures make it possible to complement species, in particular for legumes (Figure 3). This allows the vegetation cover to maintain its productivity and a satisfactory quality of biomass. Water stress does not affect the digestibility of crude protein and fodder. Some species are less adapted to the pedoclimatic conditions of the test plots, e.g. *Trifolium alexandrinum* is sensitive to cold; *Vicia* spp. do not grow after mowing. The most suitable species were: *Trifolium resupinatum majus, Trifolium squarrosum* and *Lolium multiforum.*

Conclusions

The mixtures produce a high biomass (9 to 11 t ha^{-1} $year^{-1}$) with a satisfactory crude protein content (about 15-16%) under water stress. The mixture composition (5-6 species, including 4 legumes) allows the herbaceous mixtures to adapt to the important meteorological variations. These mixed Mediterranean grasslands have shown a capacity of resilience adapted also to other French regions (Gastal *et al.*, 2012). Technical itineraries regarding autumn and spring sowing need more investigation.

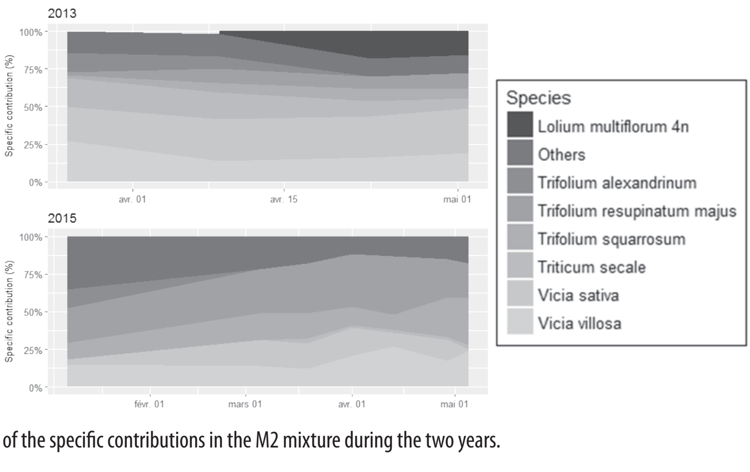

Figure 3. Evolution of the specific contributions in the M2 mixture during the two years.

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Novel grasslands for agricultural production and flood mitigation

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Abstract

As part of a five-year research programme 'SureRoot', novel *Festulolium* hybrid populations are being developed that, in addition to providing high forage yields, will also improve soil structure sufficiently to generate important ecosystem services such as flood mitigation. As an aid to their selection, representative genotypes from these populations are being monitored for their root growth and sequential changes over time by detailed root imaging. Significantly larger and deeper root systems were found in the *Festulolium* populations compared with the perennial ryegrass controls. For their agronomic performance, novel amphiploid *Festulolium* populations and ryegrass varieties are being assessed as monocultures and in binary mixtures with a wide range of white and red clover varieties in experimental field plots.

Keywords: *Festulolium*, legumes, root ontogeny, flood mitigation

Introduction

The strategy for grass and legume variety breeding at IBERS is climate-smart and holistic, providing resilience to climate change, and wherever possible, additional ecosystem services. Examples include genome introgression from species' wild relatives, which has been successful in *Festulolium* and in white clover (*Trifolium repens* L.), and has in both increased water-use-efficiency (Marshall *et al.*, 2016). For ruminant nutrition, *Festulolium* and red clover (*Trifolium pratense* L.) have properties that enhance protein use and have potential to lower the 'environmental footprint' of livestock agriculture (Humphreys *et al.*, 2014). Furthermore, in pilot studies, both *Festulolium* and white clover have, in direct comparisons with perennial ryegrass (*Lolium perenne* L.), demonstrated greater ability to enhance soil structure and increase porosity to improve water retention and reduce rainfall run-off (Macleod *et al.*, 2013; Marshall *et al.*, 2016). Previous research (Humphreys *et al.,* 2014) indicated that two novel amphiploid *Festulolium* hybrid populations, Bx511 (*L. perenne* × *Festuca mairei*) and Bx514 (*L. perenne* × *Festuca arundinacea* var *glaucescens*), have agronomic performance equivalent to current UK National List perennial ryegrass varieties, and in the case of Bx514, provide additional benefits to enhance ruminant nutrition by combating rumen-based plant-mediated proteolysis (Humphreys *et al.*, 2014). However, neither population has been previously assessed in agronomic mixtures with forage legumes, as is common practice on-farm, so nothing is known of their combined crop performance.

In order to assess the benefits for agriculture and as a strategy for future flood mitigation, *Festulolium* hybrid populations are currently being assessed, both with and without legumes, as part of the project 'SureRoot' (www.sureroot.uk) funded jointly by BBSRC and UK-based grass industries and the livestock sector. In the project, two UK National Capability facilities are used: the National Plant Phenomics Centre (NPPC) at IBERS, Aberystwyth and the Farm Platform at North Wyke, Devon. The former assists in selection of future grass and clover varieties with improved root systems, and the latter monitors their effects over time on soil hydrology and rainfall run-off at the field scale. In order to extend this assessment further, both in terms of location and the incorporation of alternative forms of livestock management, the efficacy of the *Festulolium* populations and legumes is also being assessed on eight commercial development farms dispersed across England and Wales. Reported here is a small field-plot trial based at IBERS, which has been set up to analyse the productivity of the two *Festulolium* hybrid populations Bx511 and Bx514 when grown either in monoculture or as binary mixtures with a range of forage legume germplasm. In addition, we present a summary of the first detailed results from the NPPC of the root ontogeny of the amphiploid *Festulolium* Bx511 and Bx514, and also two diploid drought-tolerant *Festulolium* introgression lines, Bx509 and Bx510, in which fescue-derived genes have been transferred onto diploid perennial ryegrass (cv. AberStar) chromosome 3 (as described in Marshall *et al.*, 2016).

Materials and methods

As part of the BBSRC-LINK Programme 'SureRoot', two selection procedures undertaken at IBERS are used to select the most suitable *Festulolium* populations and *Festulolium*-legume mixtures for inclusion in field trials on the Farm Platform and on the eight commercial development farms.

Field trial

A trial (5 cuts year^{-1}) was set up to measure the forage production of Bx511 and Bx514, another *Festulolium* cv. Prior (*L. perenne* × *F. pratensis* (meadow fescue) (4x), described in Macleod *et al.*, 2013), and *L. perenne* cv. AberMagic grown as monocultures and in binary mixtures with six white (*Trifolium repens*) and five red (*T. pratense*) clover varieties/lines. The experiment was set up using a randomised complete block design and results were analysed using ANOVA. Results are available for years 1 and 2 of this experiment.

Root ontogeny

For each plant population a representative group of 50 genotypes were selected and divided into groups of five plants of equal size established in 10 transparent perspex root columns (12×12×50 cm) filled with Levingtons F2 potting compost. Three clonal replicate sets were made of each plant population (i.e. 30 root columns per population). The populations were (1) *Festulolium* hybrid populations Bx511 and Bx514, (2) two drought-tolerant diploid *Festulolium* introgression lines, Bx509 and Bx510, (3) two perennial ryegrass controls, cvs. AberBite (4x) and AberStar (2x). Following establishment root columns were transferred over four consecutive months to the NPPC for detailed root image capture. Comparisons between plant populations were based on the means of merged images of replicates of the 50 selected genotypes. Detailed statistical analysis undertaken by the NPPC comprised pair-wise comparisons between the *Festulolium* populations and their controls. Analyses involved application of a single-step multiple comparison Tukey's Range Test, with the incorporation of the Bonferroni correction to the derived *P*-values.

Results and discussion

Field trial

The average legume-grass mixture significantly ($P<0.05$) out-yielded the average grass monoculture in both years (by 2.1 Mg ha^{-1}in year 1, and by 1.4 Mg ha^{-1} in year 2) i.e. the mixtures demonstrated 'overyielding'. The identity of the companion grass had no effect on the annual total yield of the mixtures in either year, nor did it have an effect on the annual yield contributions of the various legumes, demonstrating no differences in grass/legume compatibility between the grasses. The *Festulolium* populations Bx511 and Bx514 performed well both as monocultures and in mixtures with forage legumes and demonstrated the capacity to produce well-balanced grass-legume mixtures.

Root system ontogeny and flood mitigation

Four consecutive monthly measures of root growth of the *Festulolium* populations Bx511 and Bx514 compared with their control perennial ryegrass cv. AberBite showed the *Festulolium* roots increasing in number and diameter over time. By month four, roots of Bx511 were consistently more frequent compared with AberBite in the upper 20 cm (P<0.05), and also at greater depths in the root columns (P<0.01). Whilst overall the differences were not as great, by month four, root numbers of Bx514 were also significantly more frequent (P<0.01) at 25-40 cm depths when compared with cv. AberBite. The diploid, drought-tolerant *Festulolium* introgression lines Bx509 and Bx510 have an otherwise common genetic background to that of their control perennial ryegrass cv. AberStar (Humphreys, unpublished data). Throughout the soil profile in root columns, Bx509 produced significantly more roots than cv. AberStar (P<0.01) over the course of the analysis. By months three and four the distribution of roots in Bx509 near to the surface and at depth was not significantly different. By contrast, in cv. AberStar, roots were consistently more apparent near to the soil surface. Whilst the difference in overall root number of Bx510 and cv. AberStar was not as great, by months three and four, in contrast to the ryegrass variety, numbers of roots found at depth was greater than found near to the surface. The high water-use-efficiency reported for Bx509 and Bx510 (Marshall *et al.*, 2016) compared to cv. AberStar, is likely to derive, at least in part, from their more extensive root systems, particularly at depth. Extreme root growth followed by significant turnover at depth by the *Festulolium* cv. Prior was implicated previously in alterations to soil structure that generated reduced rainfall run-off (Macleod *et al.*, 2013). As part of the SureRoot project, cv. Prior was sown on hydrologically isolated fields at the Farm Platform at North Wyke. The outcomes (not shown) indicated that rainfall run-off, compared with permanent pastures, was significantly lower over a six-day period immediately following high rainfall. This extended to 20 days when cv. Prior was used in combination with white clover. A similar, or better, outcome on soil hydrology following use of the agronomically high-performing Bx511 and Bx514 would potentially result in multiple benefits. In preliminary field-plot studies at IBERS, water infiltration rates (measured as cm h^{-1}) assessed in soils under Bx511 and Bx514 was around twice that in soils under cv. AberBite (0.3 cm h^{-1}). If this response is replicated at the field scale then both these *Festulolium* populations should mitigate excessive rainfall run-off and thereby reduce flooding.

Conclusions

The field experiment showed that *Festulolium* populations Bx511 and Bx514 were productive both in monocultures and in mixtures with a wide range of forage legumes. Preliminary studies also indicate increases in water infiltration rate in soils under these populations. A detailed root system analysis of various novel *Festulolium* populations compared with that of their ryegrass controls demonstrated their improved root distribution, particularly at depth, which may be the reason for their previously reported greater water-use-efficiency.

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Reed canary grass and tall fescue for combustion in grassland ecosystems

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Abstract

The use of grasses for energy purposes, and the yield increase from the use of digestate, is an attractive combination. The aim of this study was to evaluate the yield of herbaceous biomass for combustion as influenced by the management of reed canary grass (*Phalaris arundinacea* L.) and tall fescue (*Festuca arundinacea* Schreb.). This experiment was conducted on an *Endocalcari-Epihypogleyic Cambisol* (*CMg-p-w-can*) and involved tall fescue and reed canary grass, which were assessed for biomass yield and quality as well as bioenergy potential. Management differed in N fertilization (90 kg N mineral or digestate ha^{-1}) and two different harvesting times were chosen to measure the biomass productivity and energy potential. The experimental findings suggest that the biomass yield and energy potential depended on the grass species and management. Both grass species were most productive when fertilized with mineral nitrogen. Of the two grass species and harvesting dates tested, the highest biomass and energy yields were produced by reed canary grass swards cut in July.

Keywords: digestate, nitrogen fertilization, biomass yield, energy yield

Introduction

Grass biomass in grassland ecosystems can be used for several purposes. Maximizing the multiple-uses of grassland resources is one of the pathways to use grassland biomass as an alternative feedstock to solid biofuel. With the expansion of biogas production, larger amounts of digestate are produced, which need urgent solutions for the proper disposal of this product. Research has found that digestate has potential to be used as an agricultural fertilizer (Sieling *et al.*, 2013). Perennial grasses are one of the plants that can derive benefit from the use of digestate, because it contains a variety of nutrients, including nitrogen, which is the most important element of plant nutrition (Möller and Müller, 2012).

The aim of this study was to evaluate the response of reed canary grass and tall fescue to digestate N, compared with using mineral fertilizer N, and to estimate their effects on dry matter and energy yield depending on management of the grass biomass for combustion.

Materials and methods

Field experiments were carried out in Central Lithuania (55° 40′N' 23° 86′E) on an *Endocalcari-Epihypogleyic Cambisol*, light loam. The swards of tall fescue (*Festuca arundinacea* Schreb., cv. Navas) and reed canary grass (*Phalaris arundinacea,* cv. Chiefton) were sown in the spring of 2012 on arable land. Soil was ploughed in autumn. The experiment was laid out in a randomized complete block design with three replicates. Fertilization treatments (0 and 90 kg N ha^{-1} yr^{-1}) were used and each experimental plot received the same N rate, supplied as a single application of mineral nitrogen (ammonium nitrate) or digestate nitrogen at the beginning of the vegetation season (April). The biomass yield measurements were performed in the first, second and third year of sward use during 2013-2015. The swards were cut once or twice per season. In the two-cut management, the first cut was made at the end of July; the second cut and the cut of the one-cut management were performed in second week of October.

The energy yield was calculated by multiplying the dry matter (DM) yield by the heating value. Heating value was measured with a colorimeter and expressed in MJ kg^{-1} DM. The experimental site is characterised by an average annual air temperature of 6.5 °C and average annual precipitation of 650 mm. Overall, the growing seasons were changeable in temperature and moisture conditions compared with the long-term annual average. The results were statistically processed by ANOVA and Fisher's LSD test ($P \leq 0.05$).

Results and discussion

The biomass yield in the first year of sward use was significantly affected by nitrogen fertilizers and cutting management (Table 1). In the second year of sward use, the nitrogen fertilizers and interaction between nitrogen and cutting management significantly influenced the biomass productivity of grasses.

Reed canary grass accumulated a higher biomass yield than tall fescue in both cut managements without N fertilization in the first year of use. In the swards fertilized with mineral nitrogen or digestate in the one-cut management tall fescue was more productive. In the second year, in almost all the cases of sward use, higher yields were obtained from reed canary grass, except in the one-cut management swards fertilized with mineral nitrogen. In the first two years differences between grass species were not significant. Management of swards and climatic conditions usually have substantial influence on the productivity of different grass species. As shown in previous studies, tall fescue exhibited better yield potential than reed canary grass (Tilvikiene *et al.*, 2016). Reed canary grass in northern countries is reported as a suitable grass for energy that can produce high yields of biomass (Heinsoo *et al.*, 2011; Kolodziej *et al.*, 2016). In all the three years of sward use, the highest annual biomass yield of both grass species was achieved in the mineral nitrogen fertilization treatments, compared with that receiving digestate, except for tall fescue fertilized with digestate and in one-cut management in the first year of sward use. There were no significant differences in one-cut management swards in the first and second

Table 1. Biomass productivity and energy yield as influenced by management of grasses.[1]

Grass species	Cutting management	Nitrogen application	Biomass yield dry matter (kg ha^{-1})			Biomass energy yield (GJ ha^{-1})		
		(kg N ha^{-1})	2013	2014	2015	2013	2014	2015
Reed canary grass	Two cuts	0	4,761	5,319	1,168	88.6	95.7	21.0
		$90_{\text{(mineral)}}$	8,807	5,290	4,821	163.8	96.9	88.3
		$90_{\text{(digestate)}}$	6,955	4,895	2,942	129.3	90.6	54.4
	One cut	0	3,618	2,742	1,600	67.1	48.4	28.3
		$90_{\text{(mineral)}}$	5,778	5,062	4,201	105.6	92.5	76.7
		$90_{\text{(digestate)}}$	5,265	4,692	3,164	94.6	85.5	57.7
Tall fescue	Two cuts	0	4,789	5,097	889	88.2	91.3	15.9
		$90_{\text{(mineral)}}$	7,505	4,375	4,020	137.3	78.7	72.4
		$90_{\text{(digestate)}}$	6,132	4,242	2,148	112.8	74.7	37.9
	One cut	0	3,509	2,172	2,140	65.0	38.3	37.8
		$90_{\text{(mineral)}}$	5,820	6,450	4,301	108.5	113.4	75.6
		$90_{\text{(digestate)}}$	6,020	4,129	3,439	111.6	73.1	60.9
A (Grass species)			ns	ns	*	ns	ns	ns
B (Nitrogen application)			*	*	*	*	*	*
C (Harvest management)			*	ns	*	*	ns	ns
A×B			ns	ns	*	ns	ns	ns
A×C			ns	ns	*	ns	ns	ns
B×C			ns	*	ns	ns	ns	ns
A×B×C			ns	ns	ns	ns	ns	ns

[1] * Effect is significant at $P<0.05$; ns = not significant.

year of use. However, in the third year both grasses significantly yielded more when fertilized with mineral nitrogen. In the mineral N fertilizer treatment, greater yield increase was recorded for both grasses in the two-cut management. Although fertilizing with digestate did not give higher biomass yield than with mineral nitrogen fertilization, it should be noted that the use of digestate was of significant benefit compared with the unfertilized swards. These findings are in accordance with other results on positive effects of fertilization on biomass yields in grasslands (Hensgen *et al.*, 2016), including findings that the digestate can have a positive impact on the soil properties (Möller, 2015).

Energy yield of both grasses was significantly affected by fertilization in all experimental years; and in the first year of use by cutting management also (Table 1). Both grasses produced higher energy yield in the first year of use in the two-cut management. Similar trends were observed in the second year of sward use; however, tall fescue produced the highest biomass energy yield in the treatments fertilized with mineral nitrogen in the one-cut management. Similar tendencies in energy values have been shown in previous results (Jasinskas *et al.*, 2008; Kolodziej *et al.*, 2016).

Conclusions

As expected, reed canary grass and tall fescue swards with nitrogen fertilization and harvested twice per season produced the highest biomass and energy yields. The use of digestate had a positive effect on both of the grass species tested; however, its use was not superior to the use of mineral nitrogen.

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Effect of mulching and mowing on species diversity of low-input lawns

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Abstract

Mulching becomes increasingly a standard method used for the maintenance of extensive grass stands. The reason can be seen in cost savings, as these maintenance systems do not include the loading and haulage of the mown material or the disposal-related costs. The effect of mulching and mowing on proportions of guilds (grasses, legumes, and other species = weeds) was studied at the Vatín Research Station of Fodder Crops. The experiment was established in 2006 with a grass-legumes mixture. The study was conducted through six growing seasons during the period 2007-2012. Results showed that mulching significantly affected the botanical composition of grass-legumes mixture, where a decrease in the legume proportion was observed. In contrast, mulching supported a significant increase of the grass component in the stand. Other species were not significantly influenced by mulching.

Keywords: sward structure, mowing, mulching

Introduction

Grass stands are an essential part of public greenery. They represent a growing component where lack of management, poor quality and neglected state are among the main aesthetic problems of the cultural environment of landscapes and settlements. Mulching becomes increasingly a standard method used for the maintenance of low-input grass stands (lawns). The reason can be seen in cost savings. However, exact results of a long-term research into the effect of mulching on the quality of low-input lawns are still not available. Much more data can be found about grassland and pasture swards in which the issue has been investigated for several tens of years. Gaisler *et al.* (2004) and Raus *et al.* (2012) point out the effect of the frequency of defoliation. A high frequency increased the species diversity of grassland swards. Kvítek *et al.* (1998) studied the effect of mulching on the increase of grass component with the decreasing representation of legumes. The aim of the work was to find out how leaving the cut grass material on the ground in the form of mulch affects the structure of the low-input lawns.

Materials and methods

This research project was conducted at the experimental station of the Department of Animal Nutrition and Forage Production, Faculty of Agriculture, Mendel University in Brno, the Czech Republic (49°31'6'N, 15°58'7'; altitude 560 m a.s.l.). The station lies in the potato-growing production region with average annual temperature of 6.1 °C and total annual precipitation amounting to 737 mm. The experiment was established in 2006 with a grass-legumes mixture that consisted of: *Lolium perenne* 25%, *Poa pratensis* 25%, *Festuca rubra* 30%, *Festuca ovina* 5%, *Anthoxanthum odoratum* 5%, *Cynosurus cristatus* 5%, *Trifolium repens* 3%, and *Lotus corniculatus* 2%. The study was conducted through six growing seasons during the period 2007-2012. Soil at the experimental area is cambisol modal mesobasic.

The experiment was carried out in a randomized complete block design with three replicates. Experimental plots were sized 2×2 m. Plots were mulched (clippings returned) or mown (clippings removed) five times a year at a 40 mm cutting height at the following terms: second decade of May, second decade of June, third decade of July, second decade of September and third decade of October. The plots were unfertilized. Sward structure was assessed using a subjective method for evaluating cover before the first mowing (May). The sward structure is expressed as the cover of grasses, legumes, other species and empty spots.

Statistical analyses were performed using repeated measures ANOVA with multiple post-hoc comparisons according to Tukey test ($P \leq 0.05$). The Statistica 10 software (StatSoft) was used for the analysis.

Results and discussion

Results presented in Figure 1 indicate that mulching had a statistically significant effect on the sward structure. On average over the 6-year monitoring period, the mulched variant exhibited 66.9% of grasses in the sward, which was a significantly higher value than in the mown variant (52.3%). On the other hand, the share of legumes was significantly higher in the mown variant (36.6%) than in the mulched variant (21.3%). With increasing age of the sward, the mulched variant exhibited an increasing share of grasses at the cost of clover (Figure 2). By contrast, the share of grasses in the mown variant stabilized as early as the second productive year with the exception of a decrease in 2011. Thus, it can be assumed that mulching encourages a greater representation of grass species capable of utilizing nitrogen from the decaying grass matter by which the occurrence of clover crops is suppressed. The presence of other species in the sward were not affected by the different method of lawn maintenance and their degree of coverage were recorded on the average of years was 6.6 and 6.4% in the mown and mulched variant, respectively. The assumption that mulching will lead to a higher representation of weeds in the sward was not corroborated. Heckman *et al.* (1999) described the positive influence of mulching on the infestation of a pure meadow grass stand weeds as well. 5.4 and 4.4% of empty spots were found on the average of years in the mulched and mown variant, respectively. However, the difference was not statistically significant.

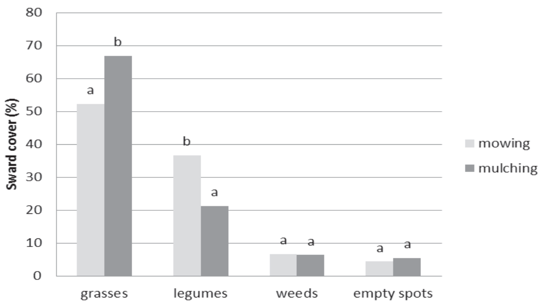

Figure 1. Effect of mowing and mulching on the structure of the low-input lawns (average of years 2007-2012). Different letters indicate significant differences within the same category ($P \leq 0.05$).

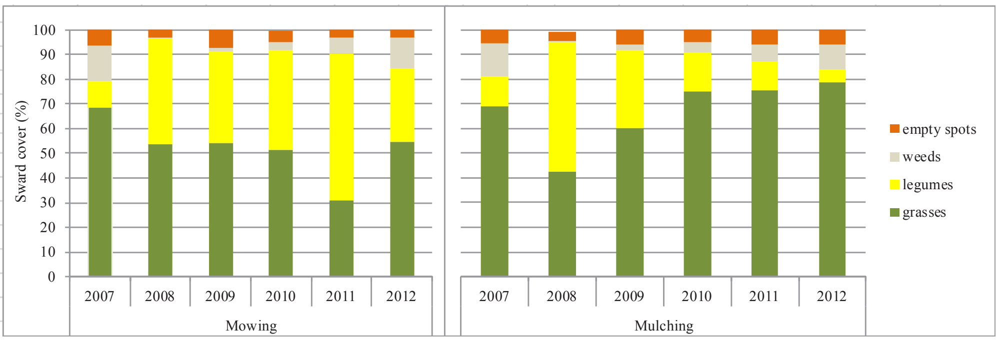

Figure 2. Effect of mowing and mulching on the structure of the low-input lawns in individual years.

Conclusions

The aim of our research was to study the effect of mulching on the structure and sward height of the low-input turf. Results gained from a clover-grass stand mulched 5-times a year in the period 2007-2012 show that, compared with mowing, mulching very profoundly encouraged the occurrence of sown grass species and reducing at the same time the occurrence of clover to an acceptable percentage. The effect of mulching on the infestation by other species was not demonstrated. The results of this show that returning the cut material on the ground can be used without decreasing the sward quality. Mulching significantly reduces the share of legumes in the green sward, which may contribute to better appearance of low-input lawns.

Acknowledgements

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Agrotechnological measures in lucerne and fodder galega seed production

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Abstract

Fodder galega (*Galega orientalis* Lam.) is a well-known new fodder crop in Estonia, and has a certain place among legume fodder crops. Different sowing rates, row spacing, optimal and mixtures of herbicides were investigated for seed production in Estonian hybrid lucerne (*Medicago varia* Mart.) cv. Juurlu and fodder galega cv. Gale. The control of dicotyledonous weeds with herbicides is required in the year of sowing. The control of weeds in the seed production years reduces the costs for seed harvesting, drying and cleaning. The control of couch grass (*Elytrigia repens)* is necessary and gives good results in the years of seed harvesting. The higher yield from lucerne cv. Juurlu was obtained with sowing rate of 2.0 kg ha^{-1}. The average seed yield of lucerne was 271 kg ha^{-1} and fodder galega 225 kg ha^{-1}. Desiccant Basta 150 SL (glufosinate-ammonium) at a rate of 1.0 l ha^{-1} was used before harvesting of seed from fodder galega to remove the green parts of plants, to favour uniform ripening and to diminish the losses in seed harvesting, which gave an extra yield of 35%.

Keywords: fodder galega, lucerne, seed production, herbicides, sowing rate

Introduction

The seed production of hybrid lucerne (*Medicago varia* Mart.) varieties has been unstable in Estonia because the yield varies by year, depending on weather conditions at the time of flowering and harvesting. The potential seed capability of lucerne varieties bred in Estonia is high, reaching up to 500-600 kg / ha in years of favourable weather (Bender, 2000). Hybrid lucerne cvs. Karlu and Juurlu had good winter hardiness up to 60°49/ N in Finland (Mela *et al.*, 1996; Jolkkonen, 2013). Fodder galega (*Galega orientalis* Lam.) is very persistent with a high yield potential. The herbage of fodder galega has a high nutritive value when the first cut is made at stem elongation, budding or at the beginning of flowering (Meripõld *et al.*, 2011). Fodder galega has given stable seed yield during five harvest years with an average seed yield of 253 to 357 kg ha^{-1} (Raig *et al.*, 2001). Under Estonian conditions the most important factor for legumes grown for seed is the weather and weed control. The objective of this research was to identify the agronomic techniques necessary for producing the hybrid lucerne varieties Juurlu and fodder galega cv. Gale.

Material and methods

The trials were established in 2012 and 2014 to study the impact of different seeding rates and suitable herbicides on seed yield and quality. The seed fields were established at Kuusiku on a typical soddy-calcareous soil having pH_{KCl} 7.2 (ISO 10390); P- and K-content were 124 and 165 mg kg^{-1} (Mehlich III methods) respectively. The hybrid lucerne Juurlu was sown at the beginning of July in a pure stand, with 60 cm row spacing. The seeding rates were 2.0 and 4.0 kg ha^{-1}. Fodder galega variety 'Gale' was sown in May in a pure stand in a wide row spacing of 40 cm. The sowing rates were 6.0 and 8.0 kg ha^{-1}. The seeds were treated just before seeding with nodule bacteria. The trials were established in a split-plot design with 4 replicates and the size of the harvested plot was 66 m^2. In the seeding year, weed control was carried out at the third true leaf stage of both lucerne and galega. In the harvest year it was carried out at the beginning of June. The PK fertilizers (P_{35} and K_{90} kg ha^{-1}) were applied in autumn before the seeding year.

In 2014 and 2015 the seed field was treated before harvesting with the desiccant Basta 150 SL (Glufosinate-ammonium) at a rate of 1.0 l ha^{-1} to dry the green material, to ensure uniform seed ripening and to decrease harvest losses. The weather conditions during the trial period were very different. The vegetation period of 2013 was relatively favourable for the seed production by legumes. During flowering and seed maturation the temperature was higher and the precipitation lower than the long-term average. In June the amount of rainfall was 40.2 mm and in July 41.6 mm. During flowering, the year 2014 was unfavourable and especially rainy in June (96 mm, 196% of the normal amount). Growing degree days (over 5 °C) were 1,277 °C up to harvest. The summer of 2015 was very favourable for grass seed production, with much sunshine and the growing degree days were 1,257 °C up to harvest. In the second half of June the amount of precipitation was 32 mm and in July 74 mm (82% of the normal amount). The vegetation period in 2016 was less favourable for legume seed production than in 2015. There were fewer pollinators because of the wet weather and lack of sunshine. May was dry (rainfall 10.8 mm). The time of flowering in June (rainfall 79 mm) and harvesting time in August (rainfall 113 mm) were rainy. Growing degree days were 1,615 °C up to harvest. Statistical analyses (ANOVA and Fisher´s LSD) were carried out by Agrobase 20™.

Results and discussion

In the year of sowing, the following annual weeds invaded the experimental plots in great numbers: European field pansy (*Viola arvensis*), drug fumitory (*Fumaria officinalis*), shepherd's purse *(Capsella bursa-pastoris),* wild mustard (*Sinapis arvensis*), corn spurry (*Spergula arvensis*), common chickweed (*Stellaria media*), cleavers (*Galium aparine*), field forget-me-not (*Myosotis arvensis*), hempnettle (*Galeopsis spp*.), scentless mayweed (*Matricaria indora),* and perennial thistle weeds (*Cirsium arvense, Sonchus arvensis)* etc. Chemical control of dicot weeds in the year of seeding was effective. The efficacy of both mixtures (Table 1) was good in controlling annual weeds, whereas the Basagran mixture removed scentless mayweed and wild camomile better, and Stomp was better for European field pansy. The effect of MCPB was better at controlling crucifers in the field of fodder galega. MCPB and Stomp and their mixtures gave satisfactory results in the control of chickweed, and Basagran mixtures were very good. These mixtures gave 70-80% control of the above-mentioned weeds. The effect of herbicides on perennial weeds remained low, at about 30% control. In the year of harvest graminaceous weeds occurred in both seed fields. To control grasses, Agil (applied at half of the recommended rate) was added to Stomp and Basagran, and Basagran and MCPB. The efficacy of spraying was satisfactory. Agil destroyed couch grass (*Elytrigia repens)* and increased the effect of other herbicides on dicot weeds due to good stickiness but at the same time it had a damaging effect on the crop (Meripõld, 2011). The control of couch grass avoids the seed contamination with sclerotia of ergots. In the years of high precipitation, the vegetative growth of lucerne continued at the time of flowering, pod formation and seed ripening, which decreased the seed yields. In 2015, the lucerne seed yield was between 306-361 kg ha^{-1}, in the following unfavourable year only 182-110 kg ha^{-1} of seed was produced. Throughout the two years, the seed yield of the lucerne 'Juurlu' was significantly higher with the low seeding rate (2.0 kg ha^{-1}) (Table 2).

In Estonia, seeds of fodder galega seeds ripen at the beginning of August, at a suitable time for combine harvesting. In 2014 the desiccant was used in this experiment before harvesting to remove fodder grass from the fodder galega seed field and to ensure uniform ripening of seeds to avoid a decrease in yield. The extra yield was 170-180 kg ha^{-1} (Table 2). The seed yields of the fodder galega 'Gale,' averaged over the three years, were significantly higher at the lower seeding rate (6.0 kg ha^{-1}). The three-year average seed yield was 225 kg ha^{-1}.

Table 1. The herbicide variants.

Species	Variant 1			Variant 2
	Basagran	Stomp	MCPB	Basagran
	(*Bentazone* 480 g l^{-1})	(*Pendimethalin* 330 g l^{-1})	(*MCPA* 400 g l-^{1})	(*Bentazone* 480 g l^{-1})
Seeding year				
Lucerne	1.0 l	1.5 l		2.0 l
Fodder galega		1.5 l	1.5 l	2.0 l
	Variant 1			**Variant 2**
	Basagran	**Stomp**	**MCPB**	**Agil**
	(*Bentazone* 480 g l^{-1})	(*Pendimethalin* 330 g l^{-1})	(*MCPA* 400 g l^{-1})	(*Propaquizafop* 100 g l^{-1})
Harvesting year				
Lucerne	2.0 l	1.5 l		0.8 l
Fodder galega	1.5 l	-	1.5 l	1.0 l

Table 2. The dependence of lucerne and fodder galega seed yield on sowing rate.[1]

Species and cultivar	Sowing rate	Seed yield by year (kg ha^{-1})				
	(kg ha$^{-1)}$	2013	2014	2015	2016	Average
Medicago varia Mart., cv. Juurlu. cv)	2.0			361^{a}	182^{a}	271^{a}
	4.0			306^{b}	110^{b}	208^{b}
LSD 95%				31	18	13.2
Galega orientalis cv. Gale	6.0	254^{a}		240^{a}		225^{a}
	8.0			220^{b}		174^{b}
LSD 5%		5.8	14.6	14.4		6.2

[1] Means within each column followed by the same letter are not significantly different according to Fisher's least significant difference test.

Conclusions

The effect of MCPB was better at controlling crucifers in the field of fodder galega. MCPB and Stomp and their mixtures gave a satisfactory result in the control of chickweed and Basagran mixtures were very good. The control of couch grass gave good results in the years of harvest. The significantly highest yield was achieved with a seed rate of 2 kg ha^{-1} of lucerne and 6 kg ha^{-1} of fodder galega.

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Performance of sainfoin-grasses mixtures

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Abstract

Recently, farmers have developed a new interest in sainfoin (*Onobrychis viciifolia* Scop.). Due to its anthelmintic properties, it is a natural way to control nematode parasitism in small ruminants. However, this legume is sensitive to competition by other plants, with consequent difficulty in maintaining clean pure stands. From 2013 to 2015, a trial was carried out in four places in Switzerland in a way to assess the prospects offered by various grasses sown in mixture with sainfoin. Eight mixtures were compared with sainfoin in pure stand, as well as with an alfalfa-grasses mixture. The management consisted of 3-4 cuts per year without nitrogen mineral fertilizers. The yield and the proportion of legumes fell sharply during the third year. However, the combination of sainfoin with cocksfoot (*Dactylis glomerata* L.), meadow fescue (*Festuca pratensis* Huds.) and tall oat-grass (*Arrhenatherum elatius*) (L.) P. Beav.) proved to be the most stable and productive. On this basis, a new mixture was recently recommended for the creation of low intensive hay meadows, especially for organic farming.

Keywords: *Onobrychis viciifolia*, grass-legume mixture, ley

Introduction

Sainfoin is a drought-resistant legume growing on calcareous soil and beneficial when fed to sick animals (Frame *et al.*, 1997). It prevents sheep from parasitic gastro-intestinal nematodes and from bloat (Aufrere *et al.*, 2013). In Switzerland, its cultivation in pure stand is, however, difficult to control due to its low competitive ability under wet conditions, leading to weed invasion. Before the intensification of forage production, sainfoin was mainly used in association with cocksfoot and tall oat-grass (Stations Fédérales, 1972). Recently, the demand for sainfoin-grasses mixtures has increased in different regions, especially by organic and small ruminant herders. For example, in France (Rhône-Alpes), the Multi-Sainfoin mixture composed with six different plant species enables prodution of high yields with four cuts per year, during two to three years (Manteau, 2011). In order to compare the productivity and botanical composition of various sainfoin-grass mixtures under Swiss conditions a series of field experiments was conducted.

Materials and methods

Field plots (6×1.5 m) of 10 seed mixture variants were sown at 3 sites in spring 2013. The comparison focussed on monoculture (mono; V1), four simple combinations (duo; V2-V5) and five complex mixtures (multi; V6-V10), one of which (V10) was based on lucerne (Table 1). The sainfoin *bifera* cultivar Perly originating from Switzerland was used (Azuhnwi *et al.*, 2011). No nitrogen fertilizers were applied. All plots were harvested three to four times in each of the two harvest years, 2014 and 2015. Fresh weights were measured and dry matter (DM) yields were calculated after drying samples. The experiment was a split-plot design with four replicates. Total DM yields in each of the two harvesting years were analysed by analysis of variance (ANOVA) using *post hoc* Tukey test to discern significant effects of the variants. In each site and at each growing cycle, visual assessment of all plots was made. A score for general impression scaling from 1 (best score) to 9 (worst score) was given, including criteria such as sward density, weed invasion, drought resistance and general assessment. Simultaneously, legume proportion was estimated in percentage of the biomass.

Table 1. Composition and seed rate (kg ha^{-1}) of the ten experimented variants.

Species	Seed variant									
	Mono	Duo				Multi				
	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
Sainfoin *Onobrichis viciifolia*	180	100	100	100	100	100	100	100	100	
Lucerne *Medicago sativa*										15
White clover *Trifolium repens*										3
Cocksfoot *Dactylis glomerata*		12				5	5	5		6
Tall oat-grass *Arrhenatherum elatius*			20			10	10			
Tall fescue *Festuca arundinacea*				18				15	15	12
Meadow fescue *Festuca pratensis*					18		10		12	
Timothy *Phleum pratense*						3		3	3	
Seed rate (kg ha^{-1})	180	112	120	118	118	118	125	123	130	36

Results and discussion

With large differences between sites and years, on average, all mixtures achieved higher annual yields than the pure sainfoin variant (Figure 1). However, all mixtures with sainfoin had lower yield than the mixture with lucerne. These effects were only statistically significant in the second harvest year. Among the variants with sainfoin, V7 performed best on the three sites and over two years (13.1 Mg ha^{-1} year^{-1} on average). In 2015, the decrease in production was mainly due to a severe summer drought. As a result, only 3 cuts were performed, compared to 4 cuts in 2014.

Simultaneously with the drop in yield, deterioration in the sward density and homogeneity was observed on all plots. By means of visual assessment, sainfoin pure stand (V1) obtained the worst score for general impression (Table 2) reflecting a progressive invasion by weeds (mainly *Poa trivialis* and *Taraxacum officinale*). Nevertheless, sainfoin proportion represented 86% of the harvested biomass in V1 in average in 2015, while it had considerably decreased in mixtures. V5 (with sainfoin) and V10 (with alfalfa) expressed a better stability in their botanical composition than the other mixtures. Among grasses associated in duo with sainfoin (V2 to V5), cocksfoot (V2) had the highest capacity for competition and meadow fescue (V5) the lowest. Multispecies mixtures (V6 to V9) showed similarities regarding their sainfoin proportion and their behaviour score (Table 2).

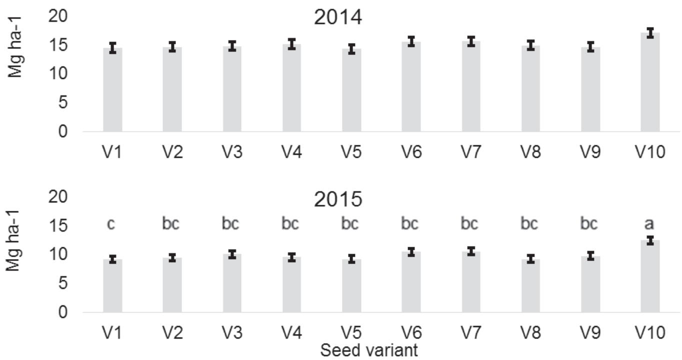

Figure 1. Annual dry matter (DM) yield (Mg ha^{-1}) of 10 seed mixture variants over two harvest years (n=12). Error bars indicate 95% confidence interval. In 2015, letters indicate significant differences at P<0.05.

Table 2. Score for general impression (1 = best; 9 = worst) and legumes proportion (%) of 10 seed variants over 2 harvest years.

	Seed variant									
	Mono	Duo				Multi				
	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
Score for general impression										
2014	4.91	3.10	3.42	3.41	3.50	3.16	3.38	3.34	3.47	3.01
2015	6.21	3.88	3.78	3.80	4.05	3.83	3.96	3.81	3.81	3.91
mean	5.56	3.49	3.60	3.61	3.77	3.50	3.67	3.58	3.64	3.46
Legumes proportion (%)										
2014	92.4	44.8	55.9	58.5	65.8	56.4	59.4	57.6	62.6	76.0
2015	86.2	35.9	44.8	50.6	65.6	43.8	45.5	49.0	48.4	67.2
mean	89.3	40.4	50.3	54.5	65.7	50.1	52.5	53.3	55.5	71.6

Conclusions

Evidence from these field experiments over two harvest years shows that mixtures provide higher DM yield than sainfoin grown in pure stand. The yield decline, as well as a decrease in density in the second year, can be attributed to a severe drought that occurred during summer. This effect was more pronounced for the sainfoin monoculture than for mixtures. Cocksfoot, tall oat-grass and meadow fescue were well suited to be associated with sainfoin. Compared to lucerne, sainfoin was less competitive with grasses and allowed a better balance in the botanical composition.

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Mulberry (*Morus* spp.) as a fodder source to overcome climate change

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Abstract

Agroforestry practices are excellent tools to mitigate and adapt to climate change. Climate change will increase the summer drought period in the south of Europe. Feed shortage can be overcome by the use of tree leaves and soft branches to feed to livestock. The leaves of mulberry are known to be of high quality (protein content and digestibility) for animals. This experiment aims to determine productivity and fodder quality of four *Morus* spp. clones: *M. alba criolla*; *M. alba tigrenda*, *M. alba illaverde* and *M. nigra*. A randomized block design (3 blocks × clone) was set up in the northwest Spain (Lugo, Galicia) in 2015. One year after establishment, field measurements were recorded (survival, tree height, base and crown diameters) and plant subsamples were taken to determine production and protein content. The four *Morus spp*. clones showed a high survival rate (>90%). *M. alba ill*averde produced the highest yields and growth compared with other mulberry clones. Despite differences in production, all four *Morus spp*. clones have shown high leaf protein content (100-180 g kg^{-1}) with no significant differences among them. Thus, our initial study showed promising results on the use of mulberry leaves as animal fodder in temperate agroforestry systems.

Keywords: mulberry varieties, high protein, livestock, agroforestry

Introduction

Agroforestry can be a useful tool to reduce farmers' vulnerability to climate change. An increasing risk of severe summer droughts has been predicted for most of Europe in the coming decades (Kovats *et al.*, 2014). The use of trees as a fodder crop to overcome pasture shortage during summer is a common practice reported in many Mediterranean countries. However, foliage of most fodder trees tends to have low protein contents, moderate digestibility and high fibre content which limits their potential as an alternative livestock forage (Papanastasis *et al.*, 2006). Mulberry trees are used as a livestock fodder in many countries around the world (e.g. India and Japan) but not much is known about their performance in European temperate countries. The leaves of the mulberry are known for their high protein and mineral content, good amino acid profile and high digestibility for both ruminants and monogastric animals (Sanchez, 2000). The objective of this experiment was to determine the adaptation, productivity and fodder quality of Cuban and Galician-sourced *Morus spp.* trees in the temperate region of northwest Spain.

Materials and methods

A randomized block design (three blocks × clone) comprising four clones of mulberry (CR: *Morus alba criolla*; TI: *M. alba tigrenda*, IL: *M. alba illaverde* and MN: *M. nigra*) was set up in Lugo (North-west Spain) in autumn 2015. *Morus* spp. clones were sourced from different origins: IL from Lugo and MN from Ourense (Northwest Spain), CR and TI from Cuba. Plant seedlings were produced by *in vitro* propagation methods (Fernandez-Lourenzo *et al.*, 2004). In each field plot (250×250 cm), 25 plants were planted at a planting distance of 50×50 cm. The tree height, crown and base diameter were measured using a metric tape and a calibre on each individual plant in September 2016. Plantation survival checks were also done at this stage. To determine dry matter yield and protein content of leaves and stems, in the nine central plants of each plot, one shoot from each plant was taken. Dry matter yields were calculated after oven drying plant shoot samples at 45 °C until constant weight. The crude protein (CP) content was determined by using the Kjeldahl method and estimated by multiplying Kjeldahl-nitrogen by a conversion factor of 6.25 (Whitehead, 1995). The experimental data obtained were statistically tested by performing an analysis of variance (ANOVA) and the Tukey test to discern significant differences among clones, using the software Minitab (17^{th} edition).

Results and discussion

The four clones of mulberry tested in this experiment showed a good survival rate ranging from 93 to 100% after one year of being planted, with no significant differences among cultivars (Table 1). Besides their good initial plant establishment, not all mulberry clones showed the same growth capacity. Growth variables (tree height, base and crown diameter) were significantly different (P<0.001) among clones (Table 1). IL was the cultivar that showed the highest height, followed by CR, TI and MN. In our experiment, IL also showed the highest base and crown diameter compared with the other mulberry clones (Table 1). There were also significant differences among yields for the four different clones (P<0.001). IL produced the highest leaf and stem yield and consequently the greatest total yield compared with the other three clones (Figure 1). The greatest growth and higher productivity shown by the Galician-sourced mulberry clone IL could be because the plant material was collected in the same area in which the experiment was established (this was necessary for the *in vitro* propagation) and therefore it was better adapted to the specific local conditions of the experimental site. Despite the differences in growth and yield among clones, no significant differences were found when leaf and stem CP content was tested (Figure 1), thus showing no clear differences in fodder quality among *Morus* spp. clones. The CP contents of leaf (100 to 180 g kg^{-1}) and stem (40 to 180 g kg^{-1}) are within the range of other studies reporting young leaf and stem CP values (50 to 270 g kg^{-1}) from several mulberry varieties (Sanchez, 2000). The leaf CP values from this study are similar to other CP contents from fodder trees grown (e.g. black alder, ash and hazel) in Europe (Emile *et al.*, 2016) and also to the values obtained (80 to 180 g kg^{-1}) during spring in pasture under silvopastoral systems in the same area (Rigueiro-Rodríguez *et al.*, 2007). Therefore, this study shows the promising potential of using mulberry trees as a forage resource to complement pasture under temperate agroforestry systems.

Table 1. Percentage of survival, tree height, crown and base diameter for *Morus alba criolla* (CR), *M. alba tigrenda* (TI), *M. alba illaverde* (IL) and *Morus nigra* (MN).

Clone	Survival (%)	Tree height (cm)	Base diameter (mm)	Crown diameter (cm)
CR	96	61.4±3.3 b	6.5±0.3 c	34.7±1.7 b
IL	96	90.9±4.3 a	12.2±0.3 a	51.3±3.1 a
MN	100	35.2±1.7 d	9.2±0.4 b	32.0±1.5 b
TI	93	49.0±3.4 c	5.9±0.3 c	33.2±2.0 b
ANOVA *P*-value[1]	n.s.	***	***	***

[1] Different letters indicate statistical significant differences among clones. n.s = non-significant, ***= P<0.001.

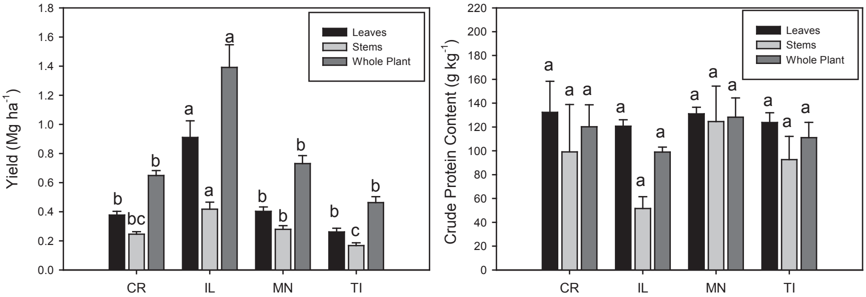

Figure 1. Yields (Mg ha^{-1}) and crude protein contents (g kg^{-1}) for *Morus alba criolla* (CR), *M. alba tigrenda* (TI), *M. alba illaverde* (IL) and *Morus nigra* (MN). Different letters indicate significant differences among clones.

Conclusions

The four mulberry clones tested showed a good initial plant establishment, this indicating their potential for being grown successfully under similar climatic conditions. According to our study, *M. alba illaverde* was the best-performing clone in terms of production and growth. Increasing the duration of the field trial and the addition of more experimental sites will provide further evidence of the best cultivar(s) for temperate climatic conditions.

Acknowledgements

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Vegetation dynamics of seed mixtures containing tall fescue (*Festuca arundinacea* Schreb.)

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Abstract

Tall fescue (*Festuca arundinacea*) is known to be a drought-tolerant grass. For this reason, there is interest to include it in seed mixtures for areas where forage production is endangered by recurrent drought. Two seed mixtures containing respectively 40 and 60% by seed weight of tall fescue, and containing both a rough-leafed and a soft-leafed cultivar, were investigated in a four-year field experiment at two drought-endangered sites (835 m a.s.l., low altitude and 1,205 m a.s.l., high altitude). Three cutting frequencies (two to four cuts year^{-1}) coupled with increasing fertilization rates (equivalent to 1 to 2 livestock units ha^{-1}) were applied. The yield proportion of all species was assessed each year before the first cut. The proportion of tall fescue exhibited year- and treatment-dependent changes over time, achieving towards the end of experiment a yield proportion of about 15 to 25%. At this time, both the soft-leafed and the rough-leafed cultivars showed similar yield proportions. *Arrhenatherum elatius* and *Poa pratensis* (mainly at low and at high management intensity respectively) increased over time, while *Dactylis glomerata*, *Lolium perenne* and non-sown forbs declined.

Keywords: *Festuca arundinacea*, seed mixtures, vegetation dynamics, mountain environment

Introduction

Festuca arundinacea is known to be a grass that is tolerant of drought conditions because of its deep root system and a its high water use efficiency (Cougnon *et al.*, 2013; Pardeller *et al.*, 2014). For this reason there is increasing interest to include it in seed mixtures for the establishment of permanent meadows in areas endangered by recurrent drought. First attempts to develop a seed mixture containing *F. arundinacea* for intensive use in a mountain environment showed that reasonable yield and forage quality can be achieved (Peratoner *et al.*, 2010). Also the roughness of the leaves of this species, which are detrimental to forage palatability, is reduced by forage conservation (Peratoner *et al.*, 2011). However, less is known about the vegetation dynamics to be expected when using such seed mixtures in a mountain environment. The present paper focuses on the changes over time of the botanical composition of meadows established with seed mixtures containing different proportions of *F. arundinacea* and managed with different intensities.

Materials and methods

A field trial was established after tillage of the former grassland at two experimental sites (San Genesio/Jenesien, 835 m a.s.l., 46°31/25''N 11°20'22''E, sowing date 15 September 2010; Falzes/Pfalzen, 1,205 m a.s.l., 46°49'18''N 11°53'42''E, sowing date 27 August 2010) and conducted over four years aiming at optimizing the composition of a seed mixture with tall fescue and at exploring the suitability of its use for permanent mountain meadows at drought-endangered, non-irrigated locations. Two factors were studied: seed mixture (Fa40 and Fa60, containing the same species, but 40 and 60% by seed weight of tall fescue respectively) and management intensity (low: 2 cuts year^{-1} with a fertilization level equal to 1 livestock unit (LU) ha^{-1}; medium: 3 cuts year^{-1} with a fertilization level equal to 1.4 LU ha^{-1}; high: 4 cuts year^{-1} with a fertilization level equal to 2 LU ha^{-1}). The plots were fertilised with the farm's own manure. The seed mixtures (Table 1), both sown at a seed rate of 30 kg ha^{-1}, contained in the same proportion a rough-leafed cultivar of tall fescue (Kora) and a soft-leafed one (Barolex). The experimental design was a Latin rectangle with 3 replications and a plot size of 4×4 m. The botanical composition was assessed

Table 1. Composition of the investigated seed mixtures in percent seed weight.[1]

Seed mixture	Ae	Dg	Fa [rl]	Fa [sl]	Fr	Lp	Php	Pop	Tr
Fa40	13	14	20	20	5	4	3	15	6
Fa60	9	8	30	30	4	3	2	10	4

[1] Ae = *Arrhenatherum elatius*; Dg = *Dactylis glomerata*; Fa = *Festuca arundinacea*; Fr = *Festuca rubra*; Lp = *Lolium perenne*; Php = *Phleum pratense*; Pop = *Poa pratensis*; Tr = *Trifolium repens*; [rl] rough-leafed; [sl] soft-leafed.

in each plot prior to the first cut by visual estimation of the yield proportion of each species occurring in the plot. Statistical analysis was performed for the most abundant species (or species group, in the case of non-sown forbs) by means of mixed models taking into account the seed mixture, the management intensity, the year and their interactions as fixed factors. The year was considered to be a repeated factor with the plot as a subject. The design factors and the experimental site were considered to be random. Post hoc comparisons were performed by Šidák test. Data transformation was performed as necessary to fulfil the prerequisites for ANOVA, and a probability of $P<0.05$ was regarded as significant.

Results and discussion

The plant stand showed marked changes over time. All analysed species except *Phleum pratense* exhibited significant interactions between management intensity and year (Table 2).

F. arundinacea increased over time and achieved a yield proportion of around 15% at low management intensity and 25% at the higher intensities (Figure 1). At the end of the experiment, both the rough-leafed and the soft-leafed varieties had similar yield proportions in the plant stand (on average over all treatments 11.9 and 10.6%, respectively). *Dactylis glomerata* and *Lolium perenne* decreased over time, with lower management intensities being favourable in the first years for *D. glomerata* and detrimental for *L. perenne*. The non-sown forbs exhibited a relatively large yield proportion at establishment, particularly at the higher management intensities, declining afterwards to values around 15%, irrespective of the management intensity. These high values at trial establishment provide indirect evidence of the slow establishment of *Festuca arundinacea* as observed elsewhere (Badoux, 1971). *Arrhenatherum elatius* at low management intensity and *Poa pratensis* at the medium and high intensity increased steadily over time, becoming together with *F. arundinacea* the most relevant species in the plant stand. The yield share of *Trifolium repens* exhibited some fluctuation, achieving over the whole observation period values between 5 and 10%.

The seed mixture had a significant effect only on *Phleum pratense* as a main effect and on *F. arundinacea* as an interaction with the year. The seed mixture affected the yield proportion of *F. arundinacea* only

Table 2. *P*-values of the investigated effects for the most abundant species.[1]

Effect	Fa	Ae#	Pop#	Dg	Lp#	Php#	Tr#	Nsf
Seed mixture (SM)	**<0.001**	0.425	0.151	0.107	0.052	0.968	0.674	0.270
Management intensity (MI)	0.162	**<0.001**	**0.008**	**0.002**	**0.002**	**<0.001**	0.549	**<0.001**
Year (Y)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
SM×MI	0.106	0.904	0.390	0.178	0.570	0.089	0.663	0.862
SM×Y	**0.001**	0.537	0.655	0.176	0.816	0.406	0.909	0.713
MI×Y	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.148	**<0.001**	**<0.001**
SM×MI×Y	0.704	0.645	0.181	0.275	0.741	0.129	0.461	0.127

[1] Significant effects are highlighted in bold. Nsf = non-sown forbs. # analysis with square root-transformed data.

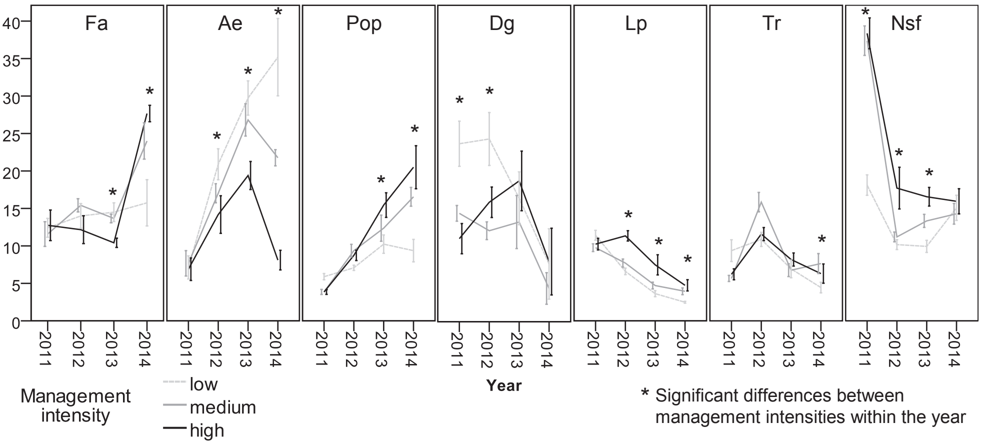

Figure 1. Changes in the yield proportion (%) of the most abundant species over time depending on the management intensity.

in the first two years, with a higher proportion in the seed mixture leading to a higher yield proportion (15.8 vs 8.7% in 2011 and 17.4 vs 10.3% in 2012). Afterwards, differences due to the seed mixture were no longer observed.

Conclusions

Marked changes in the botanical composition of the plant stands over time were mainly dependent on the management intensities, with higher management intensities being more favourable to *F. arundinacea*. Higher proportion of *F. arundinacea* in the seed mixture seems to increase the yield share of this species only in the initial phase, but may be beneficial to enhance the establishment chances of this species.

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Seasonal variation of phenolic content and antioxidant capacity in edible biomass of tagasaste

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Abstract

Tagasaste (*Chamaecytisus proliferus*) is a perennial leguminous fodder shrub naturalized in a range of environments for its valuable traits. This research was aimed at evaluating the seasonal variation of bioactive compounds and antioxidant properties in edible biomass components (i.e. leaves, twigs, flowers, and pods) of tagasaste grown in a Mediterranean environment of Sardinia (Italy). Season influenced the polyphenolic content and antioxidant capacity of leaves and twigs. Total phenolics of leaves ranged from 48 to 112 g gallic acid-equivalent kg^{-1} dry weight. Antioxidant capacity of leaves peaked in late summer, reaching 44 mmol/100 g dry weight of Trolox equivalent. The levels of total phenolics and flavonoids were highly correlated with antioxidant capacity. The research provides new insights into antioxidant activity of edible biomass components of tagasaste. Results also indicate that this leguminous shrub has remarkable potential for improving animal nutrition and welfare during the most critical forage shortage for rainfed farming systems of Mediterranean climatic areas.

Keywords: antioxidant capacity, edible biomass, forage quality, polyphenols, tree lucerne

Introduction

Chamaecytisus proliferus (L. fit.) Link var. *palmensis* (Christ) Hansen and Sunding, commonly known as tagasaste, is cultivated as a fodder tree and has achieved agricultural importance around the world, particularly in areas of Australia and New Zealand (Chinea *et al.*, 2013). A marked feature of this shrub species is its ability to retain evergreen leaves and hence maintain a relatively high nutritive value during the dry season. Increasing attention is being paid to forage secondary compounds such as polyphenols and condensed tannins, which can reduce intestinal infections in grazing animals (Piluzza *et al.*, 2013). Based on its favourable chemical composition, tagasaste may have a role as a high quality feed source and/or supplement for ruminants, which could be used to correct the critical seasonal shortage of forage caused by prolonged dry periods and harsh environmental conditions. Our specific objectives were to investigate the seasonal variation in bioactive compounds and antioxidant properties in edible biomass components (i.e. leaves, twigs, flowers, and pods) of tagasaste grown in a Mediterranean environment of Sardinia (Italy).

Materials and methods

Tagasaste used in the study was grown on experimental plots of the Council for Agricultural Research and Economics (Sanluri, Italy). At bi-monthly intervals, starting from November 2013 and ending in September 2014, undisturbed plants were pruned at a cut height of 50 cm. Sampling months corresponded to late autumn (November), winter (January), early spring (March), late spring (May), summer (July) and late summer (September), respectively. Edible biomass was then subdivided into leaflets (hereafter referred to as leaves), young twigs (thin stems <3 mm of diameter), flowers and pods. Biomass subsamples were kept on ice, freeze dried and ground to a fine powder for chemical analysis. The powdered material was then used for extract preparation following Piluzza *et al.* (2014). Antioxidant capacity was determined by means of the improved ABTS ((2,2'-azinobis (3-ethylbenzothiazoline-6-

sulphonic acid) diammonium salt)) and by DPPH (1,1-diphenyl-2-picrylhydrazyl) (Piluzza *et al.*, 2014) assays. Total phenolics (TotP), non-tannic phenolics (NTP) and tannic phenolics (TP) were determined using the Folin Ciocalteau colorimetric assay according to procedures previously described by Sulas *et al.* (2016). Total flavonoids (TotF) were quantified by colorimetric assay using aluminium trichloride, following procedures previously reported (Piluzza *et al.*, 2014). The data were subjected to analysis of variance, using Statgraphics Centurion XVI version (StatPoint Technologies Inc., 2009). Differences between means were assessed by the Fisher's least significant difference (LSD) procedure for means separation.

Results and discussion

The content of phenolics and the antioxidant activities detected by means of the two *in vitro* assays (ABTS, DPPH) on the tagasaste leaves and twigs for each cutting date are shown in Table 1 and 2. The highest antioxidant capacities were observed in late summer, and the values (34.2 and 44.3 mmol TEAC 100 g^{-1} dry weight (DW), respectively) were five- to six-fold higher than those recorded in late spring. TotP ranged from 48.5 (winter) to 112.1 (late summer) g gallic acid equivalent (GAE) kg^{-1} DW. High TEAC values corresponded to high TotP contents, and low TEAC values to lower TotP contents. In twigs, both ABTS and DPPH assays exhibited wide seasonal variations of antioxidant capacities from early spring/late spring to late summer, when again the highest values (6.9 and 5.9 mmol TEAC 100 g^{-1} DW, respectively) were recorded. Both ABTS and DPPH assays evidenced high TEAC values in flowers compared to mature pods (6.1, 2.2 vs 3.6, 2.2, respectively). The concentrations of TotP, NTP and TP

Table 1. Bi-monthly trend of Trolox Equivalent Antioxidant capacity (TEAC) by ABTS and DPPH methods, total phenolics (TotP), non-tannic phenolics (NTP), tannic phenolics (TP), total flavonoids (TotF) of tagasaste leaves.[1,2]

Cutting	TEAC (mmol 100 g^{-1} DW)		TotP	NTP	TP	TotF
	ABTS	DPPH	(g GAE kg^{-1} DW)	(g GAE kg^{-1} DW)	(g GAE kg^{-1} DW)	(g CE kg^{-1} DW)
Late autumn	14.4^{b}	22.2^{b}	76.8^{b}	65.4^{b}	11.3^{b}	43.5^{b}
Winter	8.6^{c}	9.5^{d}	48.5^{d}	45.2^{d}	3.3^{c}	21.3^{d}
Early spring	11.5^{bc}	10.2^{d}	65.6^{c}	54.1^{c}	11.5^{b}	36.6^{bc}
Late spring	7.0^{c}	7.1^{d}	49.7^{d}	40.3^{d}	9.4^{bc}	20.5^{d}
Summer	9.1^{bc}	15.2^{c}	75.4^{b}	56.4^{c}	19.6^{a}	30.2^{c}
Late summer	34.2^{a}	44.3^{a}	112.1^{a}	95.6^{a}	16.5^{ab}	67.7^{a}

[1] DW = dry weight; GAE = gallic acid equivalent.

[2] Values in columns having different superscript letters are significantly different at $P<0.05$.

Table 2. Bi-monthly trend of Trolox Equivalent Antioxidant capacity (TEAC) by ABTS and DPPH methods, total phenolics (TotP), non-tannic phenolics (NTP), tannic phenolics (TP), total flavonoids (TotF) of tagasaste twigs.

Cutting	TEAC (mmol 100 g^{-1} DW)		TotP	NTP	TP	TotF
	ABTS	DPPH	(g GAE kg^{-1} DW)	(g GAE kg^{-1} DW)	(g GAE kg^{-1} DW)	(g CE kg^{-1} DW)
Late autumn	5.0^{b}	3.5^{bc}	23^{ab}	17.3^{bcd}	5.8^{a}	5.7^{d}
Winter	3.9^{bc}	3.2^{c}	18.2^{cd}	16.4^{cd}	1.8^{b}	7.7^{c}
Early spring	2.8^{c}	2.7^{c}	16.6^{d}	14.0^{d}	2.6^{b}	5.4^{d}
Late spring	3.9^{bc}	2.6^{c}	21.2^{bc}	18.4^{abc}	2.8^{b}	9.0^{b}
Summer	4.8^{b}	4.4^{b}	23.3^{ab}	21.4^{a}	1.9^{b}	7.7^{bc}
Late summer	6.9^{a}	5.9^{a}	25.8^{a}	21^{ab}	4.8^{a}	11.3^{a}

[1] DW = dry weight; GAE = gallic acid equivalent.

[2] Values in columns having different superscript letters are significantly different at $P<0.05$.

in flowers were high. The highest antioxidant capacity found in tagasaste leaves was twice as high as in leaves of forage chicory (Piluzza *et al.*, 2014). Kumara Mahipala *et al.* (2009) found a content of total polyphenols of 44 g kg^{-1} and total tannin of 8.9 g kg^{-1} in tagasaste grown in Western Australia. Our study reported similar results for total polyphenols in the cutting of winter (48.5) and late spring (49.7) in leaves; for tannic phenolics similar results were at the late spring cutting (9.4) in the leaves. Karimi *et al.* (2013) reported TotP and TotF contents of 37 and 12.6 mg g^{-1} DW, respectively in leaves of *Medicago sativa* L., investigated as medicinal plant; these values were markedly lower than our results, but they were obtained with a methanol extraction.

Conclusions

Season affected phenolics and antioxidant capacity of leaves and twigs. The highest antioxidant capacities of both leaves and twigs were detected in late summer. Results highlight that tagasaste biomass, as a source of natural antioxidants, has remarkable potential for improving animal nutrition and welfare during the most critical forage shortage for rainfed farming systems of Mediterranean climatic areas.

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Antioxidant properties and phenolic content of plant species occurring in Mediterranean grasslands

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Abstract

The assessment of antioxidant properties and phenolic content of grassland species is particularly useful for finding new sources for natural antioxidants, functional foods and nutraceuticals. Knowledge about antioxidant properties and phenolic content of many plant species used as traditional plant remedies is limited. We determined Trolox Equivalent Antioxidant Capacity (TEAC) by the two assays 1,1-diphenyl-2-picrylhydrazyl (DPPH) and the 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) and the phenolic content by the Folin Ciocalteau method on 16 wild plant species, chosen among those traditionally used for ethnoveterinary practices. TEAC values exhibited extremely large variation, from 1.08 (*Opuntia ficus indica*) to 131.14 (*Pistacia lentiscus*) mmol TEAC 100 g^{-1} DW in the ABTS assay. Total phenolics, calculated as gallic acid equivalent (GAE), showed variation ranging from 12.7 (*Hedera helix*) to 147.68 (*Pistacia lentiscus*) g GAE kg^{-1} (DW). High TEAC values corresponded to high phenolic content, while plants with low antioxidant activity exhibited low total phenolic content. These results suggest that phenolic content might be useful as an indicator of antioxidant properties and encourage investigations on Mediterranean grassland species as sources of antioxidants.

Keywords: ABTS, antioxidant capacity, grassland species, total phenolics

Introduction

The isolation of natural extracts from plant species is a preliminary step in view of the utilisation for the production of new phytotherapic substances for animals and humans. Plant secondary metabolites such as polyphenols play an important role in the defence against free radicals. The antioxidant properties of polyphenols are due to their redox properties, which allow them to act as reducing agents, hydrogen donators, metal chelators and single oxygen quenchers. Polyphenolics exhibit a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, hepato-protective, antithrombotic, antiviral, anticarcinogenic and vasodilatory actions; many of these biological functions have been attributed to their free-radical scavenging and antioxidant activity (Zhang and Tsao, 2016). Mediterranean flora is remarkable for its diversity and it is a rich source of medicinal plants (Bullitta *et al.*, 2007; Gonzáles-Tejero *et al.*, 2008). Further knowledge is needed about content of polyphenolics, flavonoids, condensed tannins and antioxidant properties of Mediterranean plant species. The aim of this study is to determine the antioxidant capacity, total phenolics (TotP), extractable condensed tannins (CT), total flavonoids (TotF) and their relationship with antioxidant capacities in some plant species occurring in Mediterranean grasslands.

Materials and methods

A total of 16 plant species recorded in a previous ethno-veterinary study as traditionally used for ethno-veterinary practices (Bullitta *et al.*, 2007), were collected in North Sardinia. Harvested plant samples were kept on ice, freeze dried and ground to a fine powder for chemical analysis. The powdered material was then used for extract preparation as reported by Piluzza *et al.* (2014). Antioxidant capacity was determined by means of the improved ABTS [(2,20-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt)] and by DPPH (1,1-diphenyl-2-picrylhydrazyl) assays with some modifications (Piluzza and Bullitta, 2011; Surveswaran *et al.*, 2007). Total phenolics (TotP) were determined using the Folin-Ciocalteu colorimetric assay according to procedures previously described by Piluzza and Bullitta (2011). The butanol assay was used for quantification of the extractable condensed tannin content from samples according to Piluzza and Bullitta (2011). Total flavonoids (TotF) were quantified by colorimetric assay using aluminium trichloride, following procedures previously reported by Piluzza and Bullitta (2011).

Results and discussion

The amount of phenolics and antioxidant activities detected by means of two in-vitro assays (ABTS, DPPH) on the 16 plant species traditionally used as remedies in ethno-veterinary practices are shown in Table 1. ABTS assay exhibited an extremely large variation of antioxidant capacities among species, from 1.08 (*Opuntia ficus indica*) to 131.14 (*Pistacia lentiscus*) mmol TEAC per 100 g dry weight (DW). The total antioxidant capacity determined through the DPPH assay also showed a wide variation from 2.4 (*O. ficus indica*) to 164.4 (*P. lentiscus*) mmol TEAC per 100 g DW. High antioxidant capacities were found in the same set of species in both assays. The species *Cistus creticus*, *P. lentiscus*, *Euphorbia characias*, *Smilax aspera*, *Umbilicus rupestris* and *Pteridium aquilinum*, showing high antioxidant properties and high total phenolics content, were traditionally used to treat animal skin diseases and gastrointestinal disorders (Bullitta *et al.*, 2007; Piluzza *et al.*, 2015).

The total antioxidant capacity determined through ABTS assay and TotP were significantly correlated (R^2=0.97). Significant correlations were also found between the total antioxidant capacity assayed by DPPH method and TotP (R^2=0.98). Previous studies showed that phenolic compounds are major antioxidant constituent in selected herbs, vegetables and fruits and there are direct relationships between their antioxidant activity and total phenolic content (Surveswaran *et al.*, 2007). Values of TotF (Table 1)

Table 1. Total antioxidant capacity (TEAC) by ABTS and DPPH methods, total phenolics (TotP), total flavonoids (TotF) and condensed tannins (CT) of the examined plant species (mean ± standard deviation).

Species	Plant organ used	TEAC (mmol per 100 g DW)		TotP	TotF	CT
		ABTS	DPPH	(g GAE kg^{-1} DW)	(g CE kg^{-1} DW)	(g DE kg^{-1} DW)
Artemisia arborescens L.	whole plant	5.3±0.2	8.5±1.5	10.9±0.8	16.4±0.9	-
Arundo donax L.	leaves	7.0±0.6	6.6±0.1	21.0±1.3	10.1±1.0	-
Charybdis maritima L. Speta	leaves	13.3±0.6	17.5±0.9	34.5±1.1	12.7±0.3	15.4±0.3
Cistus creticus L. subsp. *eriocephalus* (Viv) Greuter & Burdet	leaves	110.8±0.6	102.1±4.3	114.5±9.3	19.8±1.4	13.1±2.3
Dipsacus fullonum L.	whole plant	3.6±0.3	4.0±0.6	19.5±0.7	16.3±0.5	-
Euphorbia characias L.	whole plant	67.4±0.6	79.6±2.7	95.1±15.9	26.4±0.5	10.4±1.3
Hedera helix L.	leaves	3.7±0.1	3.8±0.4	12.7±1.6	9.8±0.8	-
Malva sylvestris L.	whole plant	6.1±0.7	8.8±0.8	15.4±1.1	10.4±0.5	-
Matricaria chamomilla L.	whole plant	5.4±0.6	11.6±1.8	20.9±0.61	12.9±0.7	-
Opuntia ficus-indica L. Miller	cladophylls	1.1±0.2	2.4±0.5	12.8±1.1	2.6±0.2	-
Parietaria officinalis L.	whole plant	9.7±0.7	13.5±1.6	20.7±0.1	16.6±1.4	10.1±0.4
Pistacia lentiscus L.	leaves	131.1±2.2	164.4±18.4	147.7±5.4	34.4±0.9	29.9±0.7
Pteridium aquilinum L. Kum	whole plant	29.5±0.4	36.6±3.3	67.1±2.7	40.2±1.1	23.0±1.2
Pteridium aquilinum L. Kum	root	8.2±0.3	9.3±0.7	17.3±0.5	7.2±0.5	3.9±0.7
Smilax aspera L.	leaves	27.7±1.9	38.8±2.3	61.8±4.4	47.3±3.0	11.4±1.1
Umbilicus rupestris S. D.	whole plant	26.4±1.4	36.2±2.6	38.7±4.9	12.6±0.7	5.5±0.4
Urtica dioica L.	whole plant	3.9±0.1	3.8±0.1	9.2±0.3	8.01±0.6	-

[1] GAE = gallic acid equivalent; CE = catechin equivalent; DE = delphinidin equivalent; DW = dry weight.

showed variation ranging from 2.6 (*O. ficus indica*) up to 47.3 g catechin equivalent (CE) kg^{-1} DW (*S. aspera*).

The CT (Table 1) were found only in *Charybdis maritima, C. creticus* subsp. *eriocephalus*, *E. characias*, *Parietaria officinalis*, *P. lentiscus*, *P. aquilinum*, *S. aspera*, *U. rupestris*; all of them were used to treat skin diseases and gastrointestinal disorders in all domestic animals (Bullitta *et al.*, 2007; Piluzza *et al.*, 2015). Among these species, *P. lentiscus* (29.9 g delphinidin equivalent (DE) kg^{-1} DW), revealed a CT content within the range considered by Min *et al.* (2003) to be beneficial for protein metabolism in ruminants, favouring a decreasing in the rumen degradation of dietary proteins and increasing absorption of aminoacids in the small intestine of cattle and sheep.

Conclusions

The good linear correlations obtained between phenolic concentration and antioxidant capacity determined by the DPPH and ABTS assays suggest that phenolic content could be used as an indicator of antioxidant properties of the examined plant species. The results may support the rediscovery of traditional plant species from Mediterranean grassland and their uses as sources of antioxidant.

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Effects of different cover crops on grapevine properties and development of the vine mealybug in Mediterranean environment

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Abstract

The influence of complete cover cropping (inter- and intra-row) on grapevine growth, yield and must quality was evaluated during a three-year field trial in a commercial vineyard located in north-western Sardinia (Italy). Effects on developmental and reproductive parameters of the vine mealybug (*Planococcus ficus*) were also investigated. The experimental treatments were: natural covering, legume mixture, grass mixture, and conventional soil tillage, which served as the reference treatment. Sown cover crops reduce the grape production on average by 30%. However, different cover crops modify yield components in different ways. In addition, the total ground cover also influenced the must quality parameters in a positive way according to enological target intended. The grass cover increased the content of sugar, anthocyanins and polyphenols, thus improving the general quality of the must. The legume mixture and natural cover both reduced the total polyphenols and anthocyanins detected in the must at harvest. All the *P. ficus* biological parameters examined were affected by the ground management practices. Mealybugs reared on grapevines subjected to soil tillage and legume cover showed a faster development time and higher survival, fecundity and fertility than those developed on natural cover and grass plots. Utilizing competitive cover crops, while reducing yields, would improve must quality and reduce pest development.

Keywords: cover crops, *Vitis vinifera* L., grape quality, mealybug development

Introduction

Cover crops, an important ecological vineyard management tool, are widely used in vineyard inter-rows combined with herbicide strips under the vines. The overall results of this soil management strategy have showed no influence on crop yield, while changes in the must composition were observed after 2-3 years (Mercenaro *et al.*, 2014). Conversely, very few studies have investigated the influence of complete ground (intra and inter-row) cover crops on grapevine, especially when cultivated in semi-arid conditions. Cover cropping the entire vineyard soil may provide increased control of excessive vine vigour, improving wine quality and reducing herbicide use. Moreover, cover crops can also alter vineyard insect pest dynamics and may play a role in integrated pest management programmes. The aim of the present work was to study various complete ground cover crops, i.e. (1) a cultural practice to reduce excessive grape vigour and productivity, (2) a tool to control the development and reproduction of the vine mealybug (*Planococcus ficus*) in a commercial vineyard under Mediterranean climatic conditions.

Materials and methods

The experiment was carried out during 2013-2015 in a vineyard (*Vitis vinifera* L., cv. Carignano) located in NW Sardinia. The following ground management systems were compared using a randomized complete block design with four replications, each plot of 200 m^2 in size: (1) natural covering (NC) with a dominance of annual grasses (*Bromus hordeaceus* L., *Avena sterilis* L. and *Vulpia myuros* L.); (2) cover crop of an annual self-reseeding legume mixture (LM) with *Medicago polymorpha* L. cv. Anglona (50%)

and *Trifolium yanninicum* Katzn. and Morley cv. Gosse (50%); (3) grass mixture (GM) cover consisting of a summer semi-dormant perennial grass, *Dactylis glomerata* L. cv. Currie (80%) and an annual self-reseeding grass, *Lolium rigidum* Gaud. cv. Nurra (20%); and (4) soil tillage (ST) as the reference treatment. The following data on the cover crop were collected: establishment and re-establishment in four sampling areas (25×50 cm), soil covering rate (%) and presence of unsown species (visual score), dry matter yield (DMY) and its botanical composition in four sampling areas of 100×50 cm in each plot. On the vine, grape production and characteristics of the cluster, berries and must quality were collected. The content of nitrogen in leaves was estimated with the SPAD 502 chlorophyll meter. The response of *P. ficus* to different ground management systems was investigated in artificial cohorts established on grapevines. Experimental vines were inspected before the study to ensure the absence of wild populations of mealybugs in the canopy and under the bark. Biological parameters of *P. ficus*, as development time, survive, fecundity and fertility, were recorded. All data were compared using a generalized linear mixed model with cover crops as fixed and blocks as random effects. In order to compare parameters among years, the treatment factor 'year' was included as a fixed effect. Treatments were compared by Tukey's post hoc test at the significance level of $P<0.05$.

Results and discussion

Growth of cover crops varied over the years due to weather conditions. The production of DMY differed significantly among mowing dates and years. Plots were mowed once in 2013 and 2014 and three times in 2015. Natural covering (NC) produced significantly less DMY than legume mixture (LM) in 2013 and less than either LM or grass mixture (GM) in 2014. In the last year of the study, LM and NC were, in general, more productive than GM. Sown species dominated the stands of LM and GM with >61% and >85% of DMY (Figure 1), respectively. The most common weeds were: *Plantago lanceolata* L., *Conyza canadensis* (L.) Cronq., *Senecio vulgaris* L., *Avena sterilis* L., *Poa annua* L., and *Sonchus oleraceus* L. The ground cover management system significantly affected the leaf nitrogen content of grapevines. Relative to the grapevine vigour, the GM treatment showed statistically lower pruning weights than all the other treatments. Soil tillage promoted more yield than cover crops (about 30%) in all experimental years, except in 2013. The must quality changed significantly from vintage to vintage. Overall, effects of cover crop treatments on the sugar content were not consistent among years, while the anthocyanin content of grapes in NC was consistently the lowest. In 2015, anthocyanins in GM were higher than LM and ST. All the vine mealybug-related parameters were significantly affected by ground cover, especially in 2014 and 2015. In 2013, the management systems did not affect the fecundity of *P. ficus* females, while the fecundity in 2014 was higher in mealybugs developed in ST and LM grapevines compared with those reared in NC. In 2015, the number of eggs oviposited by mealybugs in LM was higher than that observed

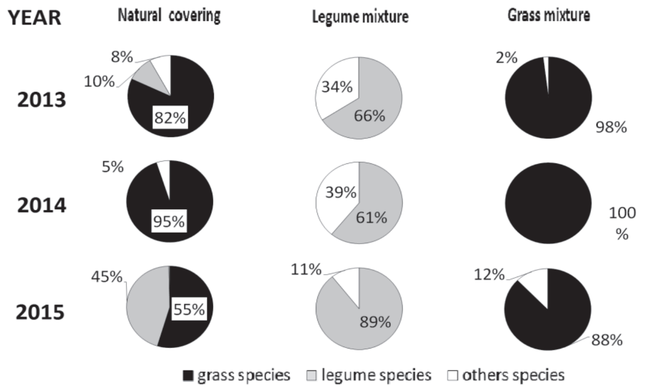

Figure 1. Percentage species contribution to dry matter production for each year of the study.

in ST treatment, which in turn was higher than that recorded in NC and GM plots. The fertility was statistically higher in LM (2014 and 2015) and in ST plots (2015) compared to NC and GM.

In our experiment, all the cover crops promoted lower yields compared with conventional soil tillage from the second year of the study, most likely due to the competition for water and nutrients. However, not all cover crops competed in the same manner with vines, as only grass cover crop (GM) had a negative impact on the following year's grape production. Ground management may also contribute to improve the must quality by influencing the concentrations of anthocyanins relative to standard tillage. Although further aspects need to be considered in order to fully understand the influence of cover crops in regulating mealybug populations, cover crops should additionally be considered in integrated pest management programs. In fact, ground management systems affected all the investigated biological parameters of *P. ficus*, in particular development time, fecundity and fertility. In particular, GM and NC reduced grape growth and nitrogen content relative to ST, resulting in a negative effect on mealybug performance.

Conclusions

Complete vineyard ground cover significantly influences grapevine growth, yield and must composition and, if properly chosen, may represent a sustainable tool to improve the quality of wines. In our experiment, complete grass cover can be used to limit excessive vegetative growth and improve must quality; however, generalized recommendations on suitable ground management systems in vineyards are difficult as the response to cover crop is site-specific and variety-dependent. Although total ground cover does not reduce *P. ficus* populations effectively, complete grass and natural cover cropping negatively influenced the vine mealybug development, creating unfavourable conditions for pest development.

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The effect of fermentation residue and wood ash fertilisers on the productivity of grasses

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Abstract

Energy-efficient farming systems that include bioenergy production from biomass can provide high agricultural output and a use of resources that is in accordance with environmental requirements. Plant nutrient recycling by using bioenergy waste products as fertilisers is a significant step in promoting it. The aim of our studies was to evaluate the effectiveness of two bioenergy by-products (wood ash (WA) and anaerobic digestion fermentation residue (FR)) when used as fertilisers for *Phalaris arundinacea* and *Festulolium*. A field experiment was conducted in the central part of Latvia (56°42' N and 25°08' E) from 2013 to 2015. In all treatments (WA, FR, MF – mineral fertilisers) the same amount of main plant nutrients was applied annually: N 100; P_2O_5 80; K_2O 160 kg ha^{-1}. Dry matter yield (DMY) was assessed in single-cut and two-cut regimes. During the three ley years DMY varied considerably depending on the fertiliser, harvest regime and species, ranging up to 10.0 Mg ha^{-1} for *P. arundinacea* and 7.7 Mg ha^{-1} for *Festulolium*. The best results were provided by the WA and MF treatments. Surface application of FR provided a significant DMY increase compared to the control; however the yield was considerably lower than that of the WA and MF treatments, suggesting partial nitrogen losses from surface application.

Keywords: *Phalaris arundinacea*, *Festulolium*, yield, fertilisers, fermentation residue

Introduction

The growth of bioenergy production increases the quantities of waste that are rich in nutrients and that can be used in agriculture to supply and recycle nutrients (Cordell *et al.*, 2009) and to reduce waste discharge and the use of chemical fertilisers (Rancane *et al.*, 2015; Voca *et al.*, 2005). Anaerobic digestion converts a major part of organic nitrogen to ammonium, which is then directly available to plants as a nitrogen source (Koszel and Lorencowicz, 2015). Wood ash has been shown to contain reasonably high concentrations of plant nutrients, e.g. P, K and Mg (Burvall, 1997). Despite the fact that fermentation residue (FR) and wood ash (WA) contain many important nutrients for plants, they may cause some problems, and there has been insufficient research on their fertiliser efficiency. The aim of our study was to compare the effectiveness of supplying grasses with bioenergy waste products and mineral fertilisers, with equal amount of NPK.

Materials and methods

The experiment was conducted from 2013 to 2015 in the central part of Latvia (56°41′ N, 25°08′ E). The soil of the experimental field is classified as *Endoluvic Epistagnic Phaeozem (Loamic)/Stagnic Retisol (Cutanic, Drainic, Loamic)*. It is fine sandy loam (WRB 2014) with pH_{KCl} 5.7, total carbon 24.3 g kg^{-1}, plant available phosphorus (P_2O_5) 95 mg kg^{-1} and potassium (K_2O) 130 mg kg^{-1}. Five fertilisation treatments of reed canary grass (RCG) (*Phalaris arundinacea* L.) and festulolium (FL) (×*Festulolium pabulare*), both sown in pure stands, were compared. Treatments were: (1) C – control – not fertilised; (2) MF – mineral fertilisers (ammonium nitrate, potassium sulphate and superphosphate); (3) WA – wood ash; (4) FR1 – fermentation residue used once per season; and (5) FR2 – fermentation residue split into two applications. In all treatments the same amounts of nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) were applied annually (100, 80, 160 kg ha^{-1}, respectively). Before the establishment of the experiment all fertilisers were incorporated into the soil and in the following years they were applied by surface application. The liquid fraction of separated fermentation residues (FR) with DM content from 4.4 to 5.4% and average OM content 3.7% was used during the period of three years. The chemical content of FR ranged between 2.7 and 5.1 g l^{-1} N_{total} (on average 58% was in the form of NH_4-N), 0.4 and 0.77 g l^{-1} P_2O_5 and 3.3 and 3.7 g l^{-1} K_2O. Two methods of use of FR (treatments 4 and 5) were compared: FR1 application of the entire dose once, at the beginning of vegetation, and application of half at the beginning of vegetation and the other half at the end of vegetation after harvest. Treatment 3 was stabilized WA from the boiler house; its average main plant nutrient content of NPK g kg^{-1} was 0.5:11:32. Every time before treatment FR and WA were analysed for NPK content and the missing quantities of elements on each plot, if necessary, were equalised by using mineral fertilisers.

The experiment was designed as a randomized complete block with four replicates. Two harvest regimes were used: (1) single-cut in autumn at the stage of crop senescence (end of September – beginning of October) and (2) two-cuts per season: first cut was taken at the full panicle emergence, the second simultaneously with the single cut.

The experimental data were analysed by three-way analysis of variance with species, harvest regime and fertiliser as factors. Multiple comparisons were performed by means of least significant difference test (LSD) at the 0.05 probability level (Excel for Windows 2003).

Results and discussion

The dry matter yield (DMY) varied significantly (P<0.05) depending on the fertiliser (55.9%), harvest regime (3.2%), and species (26.9%). DMY of reed canary grass, on average in three years, ranged from 3.9 to 4.6 Mg ha^{-1} (without fertiliser) to 8.0/8.1 Mg ha^{-1} (MF) and 7.9/8.1 Mg ha^{-1} (WA) in two-harvest/one-harvest regimes, respectively (Figure 1).

The DM yields of festulolium were slightly lower: on average in three years DM ranged from 2.1 Mg ha^{-1} (without fertiliser) to 7.0 Mg ha^{-1} (MF and WA) (Figure 2). Decline in yield in the second ley year was recorded due to the long-term black frost period in the previous winter. Festulolium is not so winter-hardy in such condition (Berzins *et al.*, 2015). There were slightly higher DMY in all treatments of festulolium for the one-harvest regime.

During the three years, both grass species gave higher DMY in response to MF and WA applications; in both treatments almost all the N was provided in the form of ammonium nitrate. Although applications

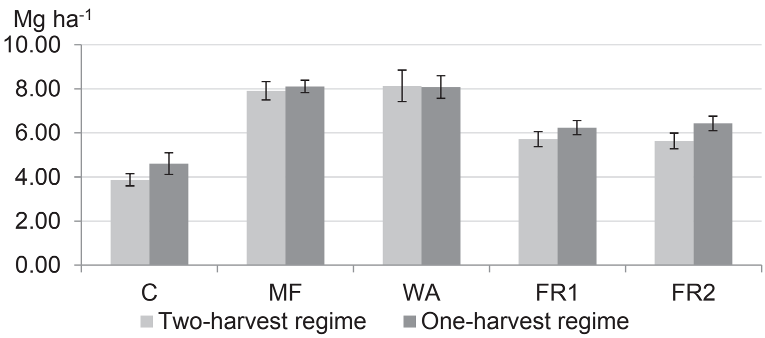

Figure 1. The average dry matter yield of reed canary grass in three ley years for different fertilisation treatments and harvest regimes. C = control; MF = mineral fertiliser; WA = wood ash; FR = fermentation residue.

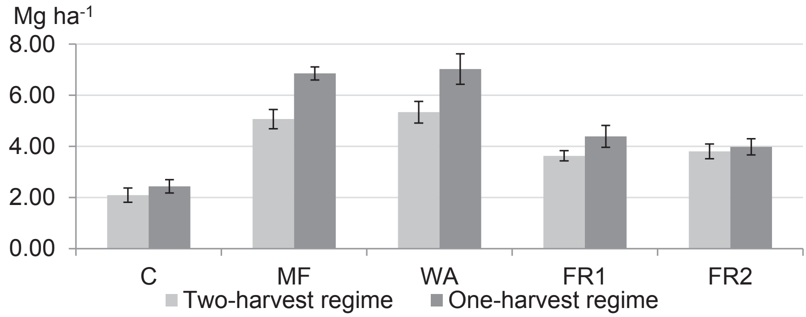

Figure 2. The average dry matter yield of *Festulolium* in three ley years in different fertilisation treatments and harvest regimes. C = control; MF = mineral fertiliser; WA = wood ash; FR = fermentation residue.

of FR ensured a significant increase of DMY for both species compared with the unfertilised control, the yield obtained was not as high as for the WA and MF treatments, which suggests partial nitrogen losses from FR applied on the surface. Ammonia (NH_3) volatilisation following the ammoniacal fertilisers and liquid manure to agricultural land may have resulted in a loss of plant available nitrogen (Nyord *et al.*, 2007). The DMY of both grass species did not vary significantly between the FR1 and FR2 treatments.

Conclusions

All types of fertilisers provided significant increase of DMY compared to the control; however better results for both grass species were ensured by the use of MF and WA. There were no significant differences using FR once or twice. Higher biomass yield was provided by RCG than by FL. Slightly higher yields of FL were obtained in the one-harvest regime.

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Producing milk in mountainous areas using little concentrate feed: four contrasted strategies

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Abstract

Dairy farmers in mountainous areas tend to use high amounts of bought concentrate feed, whereas their milk production is low. Furthermore, the recent European milk crisis has shaken up farmers and farm management strategies, particularly for feeding cattle. We surveyed 18 farmers in the mountains of the French Massif Central who manage to use little concentrate-feed. We analysed the broad range of their reported technical practices on cattle, grassland, fodder, crops, equipment and label management, and classified the farmers according to their sets of practices. There emerged four contrasted strategies: (1) betting on stored fodder and farm feed; (2) maximizing grazing period and quality; (3) betting on hardier breeds, (4) designing farmland to optimize its use. Using low amounts of bought concentrate appears achievable in different types of mountain dairy systems, provided that farmers consistently 'synergize' their herd-, grass- and land-management practices.

Keywords: dairy system, low input, mountains, management

Introduction

In France's mountainous regions, grasslands comprise 89% of the farmland area (Dobremez and Borg, 2015). Dairy farmers in such areas tend to buy high amounts of concentrate feed, while their milk production remains low compared to other dairy systems (Chassaing-Journal *et al.*, 2008). The current situation with increased natural, economic and sanitary hazards is forcing farmers to rethink their farm management strategies, particularly for inputs like concentrate feed (Devienne *et al.*, 2016). At the downstream sector, the 'grass and mountain origin' is increasingly used to differentiate products on competitive markets. For the mountainous farms, one important challenge is to identify practical conditions to be successful in minimizing bought concentrate feed (Lebacq *et al.*, 2015). In the French Massif Central, where concentrate feed use is higher than in other mountain areas, the issue is crucial for dairy farmers.

Materials and methods

We focussed on three different mountain areas in the temperate Massif Central. They share similar altitudinal range (1000-1,800 m. a.s.l.) but differ in morphology (valley/plateau) and annual precipitation (less/more than 1400 mm). Based on interviews with livestock experts, we identified and surveyed 18 farmers who used only small quantities of bought concentrate feed (i.e. significantly under the regional average of 1,700 kg cow^{-1} year^{-1}) in these areas. Then, based on the literature and expert opinion, we pre-identified a broad range of feasibly implementable technical practices on cattle, grass, fodder, crops, equipment, and label management to limit the use of concentrate feed. We built a questionnaire on the technical practices implemented by the farmers surveyed and their motivations. Finally, clustering methods (combining multivariate components analysis and Ward's method; Escoffier and Pagès, 2008) were used to classify and compare the 18 farms according to the set of technical practices.

Results and discussion

Despite sharing mountainous conditions and a will to minimize the use of bought concentrate feed, the 18 farmers implemented contrasted sets of technical practices to achieve this goal (Table 1). The clustering methods singled out four sets of combined practices, each combination interpreted as a strategy for using low amounts of bought concentrate feed (Figure 1).

Betting on stored fodder and farm feed (4 farms, ①). These farmers limit grazing and increase stored fodder and farm cereals production. They diversify their land-use by seeding cereals or legumes. Many fields are specifically dedicated to hay or crop production while others are specifically dedicated to grazing. They use chemical fertilizers (TP1) and concentrate their manure spreading onto some plots (TP2). These farms are relatively large (more than 110 ha, 54 dairy cows (DC) on average), with high-potential breeds (Montbeliarde and/or Holstein) and high productivity per DC (6,100 l $year^{-1}$). Their concentrate-feed purchase is below 750 kg DC^{-1} $year^{-1}$.

Maximizing grazing period and quality (4 farms, ②). These farmers make intensive use of their grasslands. They try to expand the grazing period as much as possible (TP4) and adapt their grazing patterns each year (TP3). They also try to maximize stored fodder and therefore they fertilize hay and grass silage fields with both manure and chemical fertilizers (TP1, TP2). Their farms are relatively small (80 ha, 38 DC on average) and unploughable. They are the least-performing farms in terms of minimizing the use of bought concentrate feed (more than 1,200 kg DC^{-1} $year^{-1}$).

Table 1. Technical practices implemented by farmers to buy low amounts of concentrate-feed.

Management lever	Technical Practice Code	Number of farmers adopting the practice (/18)
Crops and grass	*Concentrating manures on hayfields and cereals (if any) TP1*	12
	Applying synthetic fertilizers TP2	9
Herd	*Adapting mowing and grazing patterns to the year's weather TP3*	9
	Maximizing the grazing period TP4	10
	Using hardier breeds TP5	6
	Using the animals' reserves TP6	6
Farmland tenure	*Arranging fields for pasture use TP7*	10
	Expanding the field pattern TP8	7
	Consolidating land holdings TP9	9

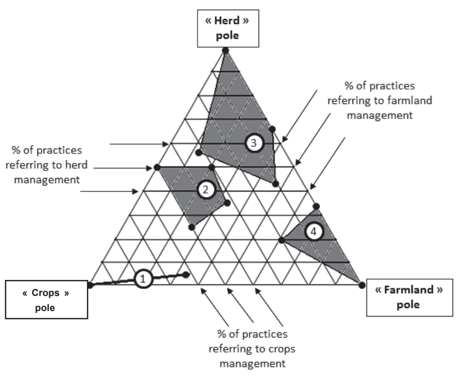

Figure 1. Representation of the four sets of technical practices on crops and grass, herd and farmland tenure (①, ②, ③, ④ are the barycentres of clusters 1, 2, 3, 4, respectively).

Betting on hardier breeds (5 farms, ③). These farmers choose hardy breeds and breeding schemes looking for hardiness and behaviour criteria (TP5). These choices enable them to cope with forage shortages in bad years (TP6). They optimize the use of pastureland through arrangements (TP7) and time allocated to grazing DC (TP3, TP4). Their farms are relatively small (about 80 ha, 31 DC on average) and their productivity per cow is quite low (4,000 l year^{-1} on average). They are the best-performing farms in terms of minimizing the use of bought concentrate feed (less than 500 kg DC^{-1} year^{-1}).

Designing farmland to optimize its use (5 farms, ④). These farmers rely on land arrangements (TP7), land expansion (TP8) and land consolidation (TP9). They have large farms (184 ha, 72 DC on average). Even if dairy production per cow is not maximized (5,850 l year^{-1} on average, with Montbeliarde and/or Holstein breeds), their yearly total production is high (430,000 l on average). Most of them are cheese-makers, with greater value-added but also greater need for workforce. Their concentrate feed purchases are under 1000 kg DC^{-1} year^{-1}.

Globally, farm-size and intensification are significantly different between these four strategies in spite of their common mountains conditions and goal about concentrate-feed.

Conclusions

Farm-structures, practices and strategies for purchasing low amounts of concentrate feed in mountain dairy systems are significantly contrasted. The four strategies identified in the studied areas enlighten the various sets of conditions needed to succeed in limiting the amounts of bought concentrate feed, and they indicate the leading role of having high consistency among herd-, grass- and land-management practices.

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Legume benefits in Mediterranean cropping systems

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Abstract

The potential benefits from legumes in Mediterranean cropping systems are important and annual legumes also represent a strong point in the European Communities Common Agricultural Policy, Europe 2020. Indeed coupled subsidies are expected for its cultivation in Ecological Focus Areas, as imposed by Greening rules. Currently, there is a renewed interest in leguminous species overall, both as a valuable biological N source for Mediterranean farming systems and representing additional crop options for longer-term rotations. The main objective of this paper is to evaluate the benefits of field bean and burr medic for supporting organic wheat cropping, as part of existing forage-livestock farming systems. After harvesting of legume seeds, a remarkable amount of crop residues and fixed N remained in fields. This represents a relevant N input for the wheat crop in the rotation. Wheat grain yield ranged from 1,560 to 1,750 kg ha^{-1} matching average regional organic yields. Integrated crop-livestock systems represent an opportunity to improve farming sustainability due to the benefits that legumes have on soil nutrients and carbon stocks.

Keywords: cropping systems, N-fixation, forage legumes

Introduction

Forage and grain legume cultivation provide several productive and environmental benefits. Nevertheless, from 1961 to 2013, legume production has progressed towards intensive cropping, especially cereals (Preissel *et al.*, 2015). Castellazzi *et al.* (2008) define a crop rotation as sequence of crops that is fixed (each crop follows a pre-defined order), cyclical (in that it repeats itself) and has a fixed length. Annual legumes have replaced fallow in cereal agroecosystems, and ley-farming systems have developed rapidly in Mediterranean and temperate regions of Australia and are recognised as some of the most advanced, extensive and low-input cropping systems of the world (Loi *et al.*, 2005). Interactions between crops are an important component of how changes in cropping systems impact on their agro-economic and environmental performance (Reckling *et al.*, 2016). Cropping system management and agronomic practices can also affect carbon levels in agricultural soils because of their effect on the quality and type of crop residues returned to the soil (Mancinelli *et al.*, 2010). Nitrogen fixed by legume crops directly influences agricultural productivity and represents an important renewable source of N for agricultural soils. It is well documented that yields of non-leguminous crops can be improved by including a legume in the cropping sequence (People *et al.*, 2009). Farmers with more than 15 hectares of arable land must follow the crop diversification and the Ecological Focus Area (EFA) Greening rules established by the European Union. In this context, legume crops play an important role. The present project aimed at supporting organic wheat cropping, within existing forage-livestock farming systems, to produce high added value products (such as innovative ready meals and/or typical Sardinian fregola pasta). The main objective of this study was to evaluate legume benefits for the farm production systems.

Materials and methods

The study was conducted on two extensive farms (Zunchini and Tergu) in northern Sardinia, Italy, representative of many sites in the Mediterranean region (Cocco *et al.*, 2014). At Zunchini (40°44'N, 8°25'E; 28 m a.s.l; soil pH 8.2) soils were classified as Eutric, Calcaric and Mollic Fluvisols. At Tergu (40°51'N, 8°41'E; 350 m a.s.l; soil pH 6.5) the soils were classified as Eutric, Leptic, Vertic Cambisols and Eutric Leptosols. Livestock were represented by Sarda dairy ewes at Zunchini and by Charolais cattle at Tergu. At Zunchini durum wheat (*Triticum durum* L.) cv. Saragolla was grown after burr medic (*Medicago polymorpha* L.) and at Tergu durum wheat cv. Karalis was grown after *Vicia faba minor*. There was no fertilizer application or weeding. The amount of N fixed by legumes was assessed by the isotope dilution method (Sulas *et al.,* 2013). A rate of 4 kg N ha^{-1} of enriched ^{15}N fertilizer (10 atom % ^{15}N enriched ammonium sulphate) was applied to 3 m^2 sampling areas of legumes and wheat within each experimental unit. The dry biomass material was subjected to elemental analyser isotope ratio mass spectrometry (Cheshire, UK) to determine both N content (%N) and the atom% ^{15}N. Wheat quality traits, such as protein and moisture percentage, gluten, colour and basis weight of grain, were evaluated by NIR grain analyser.

Results and discussion

Absolute value of atom% ^{15}Nexcess and of N derived from atmosphere (%Ndfa) varied from 0.0304 to 0.0920 and from 45% to 74%, at Tergu for field bean and at Zunchini for burr medic, respectively. Values were comparable to previous results obtained in Sardinia (Sulas *et al.*, 2013). As only field bean and burr medic seeds were harvested, remarkable amounts of crop residues and fixed N remained on the fields, representing a relevant N source for the wheat crop in the rotation (Table 1). Our estimates are conservative because belowground N was not considered in this study. Crop residues can represent an additional fodder source for livestock reared at the same farm. As an alternative, crop residues left in the field provide an important tool for C sequestration (Lal, 2015).

Wheat grain yield (Table 2) ranged from 1,560 to 1,750 kg ha^{-1}, which is lower than that in conventional cultivation, matching regional organic yields. Wheat quality traits, such as moisture, gluten, colour and basis weight, both at Tergu and Zunchini (Table 2), were comparable to values obtained in conventional systems, representing, in meantime, suitable material for the production of ready meals and Sardinian Fregola pasta (niche market products). The use of legumes guarantees benefits for the cereal in the rotation, and for animal feeding and environment. According to the EU Greening rules, the proposed cropping system could also benefits wildlife species, providing food and cover.

Conclusions

Results provided evidence for the key role of legumes into existing forage-livestock farming systems for supporting organic wheat cropping to produce high added value products.

Table 1. Preceding legume, crops residues (t DM ha^{-1}) and available fixed N (kg ha^{-1}). Standard deviations are given in brackets.

Farms	Preceding legume	Crop residues[1] (t ha^{-1})	Available fixed N (kg ha^{-1})
Tergu	Field bean	5.1 (0.5)	47.3 (2.3)
Zunchini	Burr medic	6.5 (1.1)	101.0 (3.5)

[1] Leaves, stems, pod valves and pods after seed harvesting.

Table 2. Wheat production and quality traits. Standard deviations are given in brackets.

Farms	Grain yield kg ha^{-1}	Moisture %	Protein %	Gluten	Colour	Basis weight
Tergu	1,750 (276)	11.2 (0.2)	11.2 (1.2)	7.1 (1.8)	11.1 (0.5)	79.1 (0.7)
Zunchini	1,560 (268)	12.3 (0.6)	11.0 (1.1)	6.6 (1.1)	14.8 (0.3)	79.8 (0.2)

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Usefulness of nitrogen application in heavy soils compared to more favourable land in Ireland – utilisation of the Moorepark Grass Growth model

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Abstract

Ireland's temperate climate is favourable for grass growth, and efficiently grazed grass is the cheapest feed available on most dairy farms. In heavy soils, grass growth conditions and utilisation of grazed grass are less favourable than in free draining soils and will affect the dairy farm performance. The Moorepark Grass Growth model was used to compare the impact of weather, soil type and nitrogen (N) application on grass growth, number of grazing events and N leaching. Weather data (from the year 2015) and soil from two locations, Moorepark (free draining soil) and Athea (heavy soil), were used. Overall, the soil type and weather conditions for the heavy soil farm are limitations to grass growth.

Keywords: model, heavy soil, grass growth, nitrogen fertilisation

Introduction

Since the removal of the EU milk quotas, in Ireland new dairy enterprises are developing outside of the existing traditional milk production regions to more marginal soil types. In Ireland, the economic performance of dairy farms depends, to a large extent, on the efficient conversion of grazed grass to milk. Heavy soils can retain three to five times more water than well drained soils (Shalloo *et al.*, 2004). However, heavy soils have a low capacity to absorb rainfall due to poor soil structure and low porosity. In this study the Moorepark Grass Growth model (MGGm) (Ruelle and Delaby, 2016) was parameterised using weather and soil type data from a highly productive area (Moorepark (site M)) and from a heavy soil area (Athea (site A)) at different nitrogen (N) fertiliser application levels.

Materials and methods

The MGGm is a dynamic model developed in C++ describing the grass growth and the N fluxes of a paddock at a 2 m^2 level. The model is run with a daily time step simulating soil N mineralisation/ immobilisation and water fluxes, grass growth, N uptake and grass N content. The core model functions are described in Ruelle and Delaby (2016). The MGGm was used to compare the impact of the climate and soil type at two contrasting sites in Ireland: a free draining soil type – Moorepark (site M) (52.17N; 8.27W) and a heavy soil type – Athea (site A) (52.461N; 9.29W). Nitrogen fertiliser application was 0, 120, 240 or 360 kg N ha^{-1}. The 2015 weather data and the soil types of each site are described in Table 1.

The initial soil mineral N content for the first day of the simulation was set at 80 kg N ha^{-1} for both sites. A grazing event occurred as soon as the grass height reached 9 cm. The post-grazing sward height

Table 1. Description of the soil types and weather conditions for the Moorepark (M) and Athea (A) sites.

Site	Clay (%)	Sand (%)	Organic matter (%)	Rain (mm)	Average temperature (°C)	Average solar radiation (J cm^{-2})
Moorepark	26	22	7.0	1,209	9.9	947
Athea	47	13	2.3	1,653	9.1	975

was set at 3.5 cm for the first grazing event of the year and 4 cm thereafter. Residency time for each grazing event was 2 days. The number of animals grazing was calculated by the model using the biomass disappearing each day of the grazing event and assuming an intake of 16 kg dry matter (DM) cow^{-1} day^{-1}. The simulation was run for 10 years with repetitive use of the 2015 weather data to provide model stability. To further understand the impact of weather and soil type the site M weather data were applied to the site M soil and the site A soil type, and the site A weather data were also applied to both soil types. The result of the last year of the simulation are presented.

Results and discussion

Results are presented in Table 2 and Figure 1. Average grass growth across all N fertiliser application rates was of 11,120 kg DM ha^{-1} for the site M soil with the site M weather; 9,730 kg DM ha^{-1} for the site M soil with the site A weather; 8,151 kg DM ha^{-1} for the site A soil with the site M weather and 7,340 kg DM ha^{-1} for the site A soil with the site A weather. On average for the four N-fertiliser rates and the two sets of weather data, the site A soil type had less grass growth (-2,680 kg DM ha^{-1}) than the site M soil type. Similarly, on average for the four N-fertiliser rates and the two soils the weather from site A resulted in less grass growth (-1,100 kg DM ha^{-1}) compared to the site M weather.

The response to N fertiliser was variable. On the site M soil type, the response decreased as N fertiliser application increased (average response between 0 and 120 was 26 kg DM ha^{-1} per kg N; average response between 240 and 360 was 13 kg DM ha^{-1} per kg N). On the site A soil type the highest response to fertiliser was always the response between 120 and 240 kg N ha^{-1}, average 20 kg DM ha^{-1} per kg N, while average response between 0 and 120 kg N ha^{-1} was 11 kg DM ha^{-1} per kg N. This was due to the low soil mineral N content in site A over the years leading to a poor grass growth at the first level of fertilisation. The N-fertiliser responses were comparable to results from previously published research (e.g. Hennessy *et al.*, 2008).The site M weather permitted a greater number of grazing events (average of 0.75 events

Table 2. Impact of soil type, weather and N fertiliser application on the number of grazing days, number of grazing events, grass growth, final soil N mineral content and annual N leaching.

Soil type	Weather source	N fertiliser application rate (kg N ha^{-1})	Grazing days (days ha^{-1})	Grazing events	Grass growth (kg DM ha^{-1})	N min end year (kg N ha^{-1})	Leaching (kg N ha^{-1})
M	M	0	486	5	7,364	72	87
		120	687	7	10,479	86	104
		240	790	8	12,635	104	131
		360	881	9	14,001	133	168
	A	0	389	4	5,884	56	94
		120	579	6	8,960	67	119
		240	680	7	11,207	75	143
		360	793	8	12,870	90	175
A	M	0	296	3	4,440	24	33
		120	393	4	6,367	43	63
		240	585	6	9,694	56	84
		360	787	8	12,101	76	109
	A	0	298	3	4,764	17	32
		120	390	4	5,443	33	70
		240	492	5	8,402	41	96
		360	681	7	10,749	54	124

DM = dry matter; M = free draining soil type (Moorepark); A = heavy soil type (Althea).

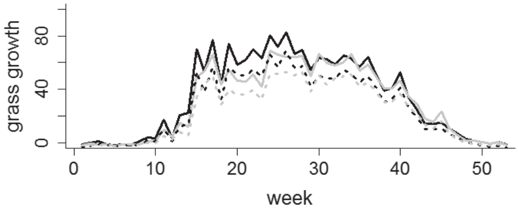

Figure 1. Average grass growth (kg dry matter ha^{-1}) using the Moorepark (black) and the Athea (grey) weather on either the Moorepark (plain line) or the Athea (dotted line) soil type.

more) and grazing days (average of +75 days). N leaching was greater on the site M soil type (+51 kg N on average) and with the site A weather (+9.25 kg N on average).

Conclusions

The heavy soil site was less favourable for grass growth and grazing due to its high clay content and low soil organic matter. Even if less important, the weather in that area was also a limitation to the grass growth. N leaching was lower on the heavy soil area, leading to a good response to fertiliser with less environmental impact.

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Land rehabilitation of a limestone quarry with native forage species

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Abstract

The use of forage species in rehabilitation activities, for example in abandoned areas previously exploited for extraction of raw materials, could perform the dual function of rehabilitation and production. Our research concerned the rehabilitation of a limestone quarry in a Site of Community Importance (SCI). The aim of the present study was to evaluate the agronomic performance of a native-based pasture mixture in terms of seedling establishment rate, soil cover, pastoral value and carrying capacity. Two sowing rates, 12 and 21 g m^{-2} seeds were compared on a steep slope. The results highlighted the good performance of the seed mixture based on native species belonging to different functional groups.

Keywords: rehabilitation, native species, mixtures, pastoral value

Introduction

The main objectives in rehabilitation activities are to preserve the soil from erosion and integrate the rehabilitated areas into the surrounding landscape (Gisotti, 1985). Areas exploited for raw material extraction, when abandoned, are usually recovered from decay with the application of environmental engineering practices and, if rehabilitation involves re-vegetation, seed mixtures already available in the market are usually used for interventions. These commercial mixtures are based on seeds often belonging to species and varieties of non-local provenance that show, in most cases, poor agronomic performance, i.e. low establishment rate and inadequate soil cover. The use of native species could offer a greater opportunity of success, due to their better adaptation, and in the case of native forage species, they could perform the dual function of rehabilitation and production (Porqueddu *et al.*, 2013). The aim of the present study was to evaluate a native seed-based pasture mixture, including species of different functional groups, for the rehabilitation of a limestone quarry.

Materials and methods

The experimental site (quarry 'Sas Funtanas') was located in Siniscola, NE Sardinia, Italy (40° 33' N, 9° 39' E) at an altitude of 560 m a.s.l. The study lasted four years (2011-2015). The area is characterized by a typical Mediterranean climate, with an average annual rainfall of 900 mm concentrated in autumn and spring, mild winters and severe drought from late May to early October. The vegetation surrounding the quarry consisted of a degraded Mediterranean garrigue under free mixed grazing (cattle, goats and sheep). The quarry was included in the perimeter of a Site of Community Importance (SCI – ITB021107 – Monte Albo) from 1995 and severe restrictions were imposed for the species and seeds to be used for re-vegetation. For these reasons, eight native species belonging to different botanical families and functional groups (low and fast establishing, N-fixing or not N-fixing species) were chosen to compose the seed mixture for quarry rehabilitation. Six mixture components were collected and selected by CNR-ISPAAM: *Medicago polymorpha* L. (40%), *Bituminaria bituminosa* (L.) C.H. Stirt. (2%), *Bituminaria morisiana* (Pignatti & Metlesics) Greuter (0.5%), *Melilotus indicus* (L.) All. (0.6%), *Lolium rigidum* Gaudin (26.4%) and *Plantago lanceolata* L. (4.7%). The remaining part of the mixture was represented by *Trifolium subterraneum* L. (13.4%) and *Dactylis glomerata* L. (12.4%) of Mediterranean origin.

The trial was carried out on a steep slope (average slope = 85%). The layer of quarry soil (12 cm), distributed on the slope surface with added compost (14%), was sandy-clay-loam (53.6% sand, 22.8% silt and 23.6% clay), sub-alkaline with pH 7.8 (in water), containing 3.5‰ N (Kjeldhal method), 9.77 ppm P_2O_5 (Olsen) and 5% organic matter. The slope area was divided into two plots: A1 = 900 m^2 and A2 = 1,800 m^2. Two different sowing rates of the mixture were used in the plots: 12 and 21 g m^{-2} viable seeds in A1 and A2, respectively. Mixtures were sown in November 2011 by hydro-seeding. In the first year, forty days from sowing, plant establishment was assessed by counting the seedlings on eight and twenty-two sample areas of 1/16 m^{-2} in A1 and A2, respectively. Sampling areas were chosen randomly. In spring, vegetation data were collected by applying a point intercept method on 50 m line transects to evaluate the species contribution to soil cover (Daget and Poissonet, 1969). The same measurements were conducted in three 'monitoring natural areas' (M1, M2 and M3) surrounding the quarry. For each plot and area, the Pastoral Value (PV) and the potential carrying capacity (livestock units (LU) ha^{-1} $year^{-1}$) were determined according to Daget and Godron (1995).

Results and discussion

A satisfactory establishment of seedlings was found in either A1 or A2 (Sanna *et al.*, 2016). No statistical differences were observed in the seedling establishment between the two seeding rates (2,810±568 standard deviation (SD) and 3,593±1,027 SD seedlings m^{-2} in A1 and A2, respectively).

During the period of observation, no remarkable statistical variations were recorded among soil cover rate. Its level always exceeded 65% (Sanna *et al.*, 2016). This soil cover rate is considered the minimum cover level, and below this there is increased risk of erosion (Thornes, 1988). As expected, the annual species in the mixture provided the higher contribution to soil covering in the first two years, especially in A2. The contribution of perennial species was initially negligible, but tended to increase in importance gradually, reaching rates of 30-40% and more in the last years. In spring 2015, *Bituminaria* spp. contributed to soil covering (14% in A2 and 28% in A1), while the contribution of *P. lanceolata* and *D. glomerata* was similar in both plots (about 10%). Starting from the second year, the spontaneous species also showed an increasing contribution, ensuring a higher plant biodiversity and a better inclusion of these areas in the surrounding landscape. A high PV and LU were detected in both sown plots A1 and A2 (Table 1).

Pastoral Value ranged from 88.0 (2012) to 33.5 (2014) in A1 and from 17.2 in M3 (2013) to 11.7 in M2 (2015). A maximum level of LU value ha^{-1} of 1.76 was recorded in the rehabilitation areas, whereas in the surrounding natural areas it was only 0.34 LU ha^{-1}. In the first two years, the high PV observed for the sown plots allowed the maintenance of an elevated livestock rate, while in the last two years, this value was lower. The observed decline of PV can be explained by the drop in the contribution of the sown species with higher specific index, such as *L. rigidum*. This shows that the introduced species are not too competitive. In the early years after establishment they reach a state of equilibrium with the surrounding spontaneous flora. Nonetheless, in the last two years, the PV is still more than double that

Table 1. Pastoral Value (PV) and potential carrying capacity (livestock unit (LU) ha^{-1} $year^{-1}$) of the two plots (A1 and A2) and the three surrounding natural areas (M1, M2 and M3).

	Spring 2012		Spring 2013		Spring 2014		Spring 2015	
	PV	LU ha^{-1}	PV	LU ha^{-1}	PV	LU ha^{-1}	PV	LU ha^{-1}
A1	88.0	1.7	85.8	1.7	33.0	0.6	36.0	0.7
A2	71.8	1.4	60.9	1.2	46.5	0.9	35.0	0.7
M1	13.0	0.2	12.0	0.2	12.2	0.2	12.9	0.2
M2	15.7	0.3	13.5	0.2	14.2	0.2	11.7	0.2
M3	17.1	0.3	17.2	0.3	15.0	0.3	12.7	0.2

of the external natural areas. Especially in areas of high natural value (HNV), the establishment of swards that have higher forage production could reduce the pressure from grazing areas that are more susceptible to degradation. Over the years of the study, no remarkable variations were recorded in terms of PV in the three natural areas.

Conclusions

The rehabilitation of abandoned areas by extraction processes generates environmental and landscape benefits, playing an important role for the socio-economic subsistence of rural populations. Moreover, the introduction of high quality pasture species could reduce the grazing pressure on HNV areas susceptible to degradation. Our study highlighted the good performance of the native seed-based pasture mixture, even at low sowing rate, encouraging research aimed at promoting the use of native species for land rehabilitation, especially when the seed market does not offer site-specific and well-adapted plant materials.

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Effect of temperature, acid substrate and heavy metals on lucerne

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Abstract

Lucerne (*Medicago sativa* L.) has a powerful root system and can be used for soil conservation and for the prevention soil erosion. The aim of the present work was to study the integrated effects of substratum acidity and heavy metals (cadmium and copper) on the adaptation of lucerne at different ambient temperatures when grown in the phytotrone complex. Changes in lucerne height and in the shoot biomass dry matter were indicators of plant adaptation to unfavourable environmental factors. Changes in nitrogen and sulphur contents in lucerne stems and leaves were also estimated. Lucerne grown at high temperature (27-20 °C) accumulated less aboveground biomass and was more sensitive to the effects of contaminants compared with lucerne grown at normal temperature (21-17 °C). With substrate acidification, lucerne stems and leaves accumulated markedly more sulphur, and the ratio of nitrogen to sulphur declined. Copper tended to increase the aboveground lucerne biomass at normal temperature and acted as a fertiliser. At high temperature both copper and cadmium tended to reduce, or reduced significantly, the lucerne biomass.

Keywords: *Medicago sativa,* cadmium, copper, substrate acidity, high temperature

Introduction

Lucerne (*Medicago sativa* L.) is generally considered to be a drought resistant species (Frame *et al.,* 2008). Lucerne has a powerful root system and can be used for soil conservation and for the prevention soil erosion. It can produce a high yield without nitrogen fertilization. One of the most valuable characteristics of lucerne is its longevity. Environmental pollution with heavy metals or acid rain has become an important factor that determines plant growth, development and productivity (Hoffmann and Persons, 1997). Such stress causes changes in physiological processes and the consequences of the effect depends on the species. The outcome of the exposure to stress is determined by the plant species, variety, exposure time or severity (Alexieva *et al.,* 2003). Most heavy metals are essential for plants; however, in excess they can inhibit growth and development, suppress photosynthesis, synthesis of photosynthetic pigments, metabolism and other processes. Acidification of field and forest soils is an undesirable phenomenon, since agricultural crop yields decline in acid soils (Tong GuanHe, 2005). The objective of the present study was to investigate physiological responses of lucerne of the exposure to differentiated and complex acid substrate and cadmium (Cd) and copper (Cu) ions and to explore plant adaptive capacity to adjust to these stressors at optimal and high temperatures.

Materials and methods

Experiments were done at the *LCARF* Institute phytotrone complex where we investigated the effects of substrate acidity and heavy metals on lucerne cv. Birute at different ambient temperatures. For this experiment we chose two of the most toxic heavy metals cadmium and copper. The plants were grown in peat substrate in pots of 5 l capacity, in three replications. Each pot was sown with 30 seeds of lucerne. The emerged seedlings were thinned out leaving 10 seedlings per pot, which were grown in a greenhouse for 45 days at 20 °C. At 4-5 leaf stage the plants were transferred to phytochambers to induce flowering and were kept for 35 days at +4 °C at 8 h photoperiod. After flowering induction the plants were transferred for 40 days to phytotron chambers: in one chamber the temperature during the daytime was 21 °C, at night 17 °C (optimal temperature), in the other chamber 27 °C during the daytime and 20 °C at night (high temperature), photoperiod 16 h. The source of light was SON-T Agro (PHILIPS) lamps. In period

16-40 days the experiment with heavy metals and acidity substrate was done in two stages – the adaptation stage and the main stage at optimal and at high temperatures. For adaptation tests acid concentration was 6 ml H_2SO_4 l^{-1} water, the concentration of cadmium ions was 0.16 m*M* $3CdSO_4{\cdot}8H_2O$ (0.123 g l^{-1}), and the concentration of copper ions was 2 m*M* $CuSO_4{\cdot}5H_2O$ (0.499 g l^{-1}). Before sowing, peat substrate of slightly acid reaction (pH 6-6.5) was irrigated with these solutions. Each pot received 0.5 l of solution. The pH level of the acid-irrigated substrate was 4.0. Adaptation stage lasted for 10 days. The acid concentration for the main exposure was 6 ml H_2SO_4 l^{-1} water, the concentration of cadmium was 0.16 m*M* $CdSO_4{\cdot}8H_2O$ (0.123 g l^{-1}), the concentration of copper was 4 m*M* $CuSO_4{\cdot}5H_2O$ (0.998 g l^{-1}). Each pot received 0.5 l of the solution. The ions of cadmium and copper did not change substrate acidity, whereas the pH of the substrate irrigated with acid solution decreased (pH 3.3-3.8). The control plants were irrigated with tap water. After the main exposure which lasted for 14 days, we measured plant height and aboveground biomass and carried out analysis. The data (two factors, temperature – factor A, contaminants – factor B) were processed using ANOVA.

Results and discussion

The height of plants exposed to cadmium ions or acid at optimal or high temperatures changed inappreciably during the adaptation period. After the main stage, which lasted for 14 days, the lucerne plants that were grown at optimal temperature were taller than those grown at high temperature.

Lucerne, like other plants and living organisms, when exposed to a complex of environmental factors, responds and adjusts. Leaf development is slower at day/night temperatures of 32/26 °C than at 22/16 °C (Wilson *et al.*, 1991). Temperatures above optimal and those above 30 °C have a greater negative effect on root growth, rather than on stem growth (Frame *et al.,* 2008). In our experiments high temperatures exerted a negative impact on lucerne growth and adaptation. At optimal temperature the greatest increase in plant height was obtained when copper sulphate had been used during the adaptation and the main stage, or during the first stage the plants had been exposed to copper ions and during the second stage to cadmium ions (Table 1). This is not confirmed by the dry matter data of the lucerne aboveground biomass. In these treatments biomass was not significantly higher than in the control treatment where lucerne was irrigated by water. At higher temperature, there was no significantly higher of accumulated biomass. Biomass significantly declined, unlike at lower temperature, when in the adaptation or main stages lucerne was irrigated with acid solution. Our findings suggest that lucerne that was grown at higher temperature produced less aboveground biomass than that grown at optimal temperature, and contaminants reduced the aboveground biomass of lucerne (Table 2). The contents of sulphur and nitrogen accumulated in the stems and leaves of lucerne plants grown at higher temperature were similar to those in the plants grown at optimal temperature. The ratio of nitrogen to sulphur in the biomass of lucerne was lower when the substrate was irrigated with acid solution.

Conclusions

Lucerne grown at higher temperature (27-20 °C) accumulated less aboveground biomass and was more sensitive to the effect of contaminants and adapted less well than lucerne grown at normal temperature (21-17 °C). At normal temperature copper tended to increase the aboveground biomass of lucerne and it acted as a fertiliser. At high temperature both copper and cadmium tended to reduce, or reduced significantly, the biomass of lucerne.

Table 1. Biomass, content of nitrogen, sulphur in dry matter (DM) of lucerne as affected by exposure to contaminants and temperature.[1]

Adaptation phase	Main phase	Optimal temperature (21-17 °C)					High temperature (27-20 °C)				
		Nitrogen (N) %, in DM	Sulphur (S) %, in DM	Ratio N:S	DM biomass g pot^{-1}	Increase in height in final test (cm)	Nitro-gen (N) %, in DM	Sulphur (S) %, in DM	Ratio N:S	DM bio-mass g pot^{-1}	Increase in height in final test (cm)
W	W	3.03	0.30	10.1	7.8	8.5	3.32	0.34	9.7	6.1	5.1
W	A	3.42	0.48	7.1	5.9	5.0	3.30	0.44	7.4	5.2	4.1
W	Cu	2.87	0.32	9.1	8.0	10.2	3.37	0.32	10.6	4.1	4.0
W	Cd	3.41	0.32	10.7	7.8	6.3	3.24	0.30	10.9	4.5	3.8
A	W	3.77	0.46	8.2	4.3	4.0	3.53	0.58	6.0	2.9	1.8
A	A	3.26	1.19	2.7	5.6	2.3	2.96	1.46	2.0	4.0	3.2
A	Cu	3.18	0.45	7.0	5.4	4.9	3.51	0.68	5.1	5.1	4.8
A	Cd	3.57	0.48	7.5	4.5	3.8	3.08	0.52	5.9	5.0	4.3
Cu	W	3.84	0.42	9.1	7.2	6.4	3.33	0.39	8.6	5.2	4.1
Cu	A	2.90	0.54	5.3	8.3	11.3	3.08	0.46	6.6	7.1	8.3
Cu	Cu	3.32	0.34	9.7	8.1	10.1	3.23	0.41	7.9	5.5	6.0
Cu	Cd	3.28	0.34	9.6	7.1	10.4	3.01	0.31	9.5	6.0	7.1
Cd	W	3.36	0.37	9.2	4.2	3.6	3.10	0.39	7.9	4.7	2.8
Cd	A	3.13	0.49	6.4	7.7	6.6	3.43	0.46	7.4	5.9	6.5
Cd	Cu	3.06	0.32	9.6	7.9	8.7	3.32	0.40	8.3	4.7	2.6
Cd	Cd	3.39	0.32	10.6	6.0	4.8	3.28	0.48	6.8	5.5	5.8
$LSD_{0.05}$		0.31	0.06		1.4	2.5	0.29	0.05		1.2	2.1

[1] W = plants irrigated by water; A = exposed to 6 ml H_2SO_4 up to one litre diluted water solution; Cd = exposed to 0.16 mM $CdSO_4 \cdot 8\ H_2O$ solution; Cu = during adaptation stage irrigated with 2 mM $CuSO_4 \cdot 5\ H_2O$ solution – during the main stage irrigated with 4 mM $CuSO_4 \cdot 5\ H_2O$ solution.

Table 2. Biomass, content of N and S and increase in height of lucerne as affected by exposure to temperature (Temperature as factor A).

Temperature	Dry matter (DM) biomass (g pot^{-1})	Increase in height in final test (cm)	Nitrogen (% DM)	Sulphur (% DM)
Optimal (21-17 °C)	6.61	6.7	3.30	0.45
High (27-20 °C)	5.09	4.5	3.26	0.50
$LSD_{0.05}$	1.3	2.1	0.30	0.06

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Analysis of feeding preferences of wild animals in sown grasslands

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Abstract

Grassland restoration in many rural areas is often promoted to address problems of excessive density of wild animals. In order to monitor the real preference of wild animals on artificial grasslands, an experiment was established in a hilly area of Tuscany using different seed mixtures and pure stands of forage species that were utilized only by free ranging wild animals. Animal behaviour on different plots was determined by two methods: (1) a vegetation survey, in order to obtain an Electivity index (E) to evaluate browsing preferences of animals; (2) camera traps to detect the presence and frequency of different animal species in the area and on the plots. The results showed that sown species contributed highly to animal browsing, with differences according to the tested species and mixtures and to the date of feed intake, even if some spontaneous species were highly browsed by wild animals in given periods. Camera trapping showed the almost exclusive presence of roe deer, this species accounted for >97% of recorded videos. This confirmed the intake data obtained from vegetation analysis.

Keywords: animal browsing, feeding preference, forage mixtures, camera traps

Introduction

In many rural areas of Europe habitat improvements are often carried out to address the problems of excessive density of wild animals (Cervasio *et al.*, 2016). One of the most common methods of intervention is grassland restoration, to recover open areas encroached by shrubs and to reproduce forage areas for animal utilization, and thereby avoid or reduce damage to other crops or forest (McAllister *et al.*, 2014). For a proper assessment of these interventions, it is important to monitor the evolution of the restored areas. Moreover, the real appreciation of wild animals on recovered grasslands is to evaluate their use of sown species, because of the possibly different feeding behaviour of wild animals compared with domestic species. For these reasons, an experimental trial was conducted in a hilly area in Central Italy with sown plots left to utilization by wild animals and monitored by different methods.

Materials and methods

The experiment was established inside the Parco Mediceo di Pratolino (Tuscany, Italy), a 155 ha area, mainly covered by forests with about 30 ha of grassland. Six different treatments were compared in 5×3.5 m plots with three replications arranged in a completely randomised block design (seeding date 16/4/2015). The trial was established in a semi-natural grassland, cut once a year. Comparisons were made between two pure stands (*Onobrychis viciifolia* and *Medicago sativa*, treatment 1 and 2 respectively) and four complex forage mixtures with numbers of species varying from 6 to 12 (treatments 3 to 6). Plots were left to browsing activity of wild animals free ranging in the area where no domestic stocks are present. Botanical composition was determined by vertical point-quadrat transect according to Daget and Poissonet (1971) from which the percentage presence (P_i) of each species was obtained and used for next elaboration. Moreover, a visual estimation of animal intake on each species occurring along the transect was performed by scores ranging from 0 (no utilization) to 3 (plant highly browsed) in order to obtain the percentage defoliation activity caused by animals (D_i) of each species. Therefore, it was possible to calculate an Electivity index (E_i) for each plant species found along transects adapting the formula proposed by Nagaike (2012): $E_i=(D_i - P_i)/(D_i + P_i)$, where E varies from -1 (avoided species) to +1 (species highly searched), whereas 0 indicates that species were used in proportion to their availability.

Moreover, to evaluate the presence of animals browsing in sown plots, six camera traps (Innocenti *et al.*, 2015) were located on the perimeter of the experimental area able to record automatically 30-s videos, in order to obtain number and species of animals and also, with comparative analysis of recorded videos, the plots frequented during each event of browsing.

Results and discussion

Analysis of camera traps shows clearly that the most represented animal recorded in the experimental area along the trial was the roe deer (*Capreolus capreolus*), with roughly 97% of recorded videos (Table 1). Thus, we can state that the following results concerning browsing selection can be almost completely attributed to this species. Hares (*Lepus europeus*) were the second-most present species (2%) especially in the initial phase of sward establishment or after cutting of plots, while other species were poorly represented. Animal presence was higher in the plots sown with sainfoin, in according with data obtained by vegetation analysis (see hereafter), even if the effect of plant composition on this parameter was not significant.

In Table 2 values of Electivity index are reported for the main species encountered along botanical relevés, as average of plots along time. The two sown legumes present values higher than 0 at almost all sampling dates, demonstrating a great preference by wild animals. *Brassica napus* also scored in a remarkable way, after the period of establishment, while the most represented grass (*Dactylis glomerata*) reached a score higher than 0 only at the final time of data collection. Among the native species occurring in the plots, only *Cichorium intybus* presented values that were always positive, as the other most widespread forb (*Plantago lanceolata*) reported an extreme variability along time, with only two periods characterized by an Electivity index greater than 0.

Table 1. Recorded videos during experimental period for animal species and treatments.

Pure stand/mixture	Roe deer	Hare	Wild boar	Pheasant	Crested porcupine	Total
1	629	6	0	0	0	635
2	312	5	0	2	0	319
3	471	18	2	4	0	495
4	272	5	1	0	0	278
5	212	4	0	0	2	218
6	437	6	1	1	2	447
Total	2,333	44	4	7	4	2,392

Table 2. Values of Electivity index for main species in different date of sampling (average of all treatments ± standard error when applicable).

Species	Origin	02/09/2015	24/02/2016	15/04/2016	25/05/2016	27/07/2016	31/10/2016
Onobrychis viciifolia	Sown	0.45±0.05	0.35±0.10	-0.05±0.31	0.54±0.03	0.47±0.04	0.47±0.09
Medicago sativa	Sown	0.06±0.15	0.32±0.11	0.60±0.06	0.31±0.11	0.14±0.12	0.51±0.02
Dactylis glomerata	Sown	-0.16±0.06	-1.00±0.00	-1.00±0.00	-1.00±0.00	-0.27±0.44	0.37±0.12
Brassica napus	Sown	-0.50±0.34	0.48±0.49	0.14±0.37	0.34±0.11	-	-
Cichorium intyibus	Native	0.01±0.00	-	-	-	0.25±0.02	0.24±0.20
Plantago lanceolata	Native	-0.59±0.17	0.08±0.14	-0.64±0.19	-0.14±0.06	-0.58±0.10	0.16±0.10

Results concerning overall animal intake preferences are reported in Figure 1, where evolution along time of values of Electivity index grouped for sown (A) and spontaneous (B) species are reported for all treatments. Grouped results confirm the important role played by sown species (especially sainfoin, n.1) that always present positive values and in this way a general appreciation by animals, whereas native species are always characterised by negative values of Electivity index, even if a great variability among seasons exists (higher than that presented by sown species). This demonstrates the great ability of wild animals to utilize also species considered of low forage interest, especially in periods of reduced biomass production and with low forage availability (such as summer) when the E values is closer to zero.

Conclusions

The experiment permitted the evaluation of the potential of camera traps and vegetation survey to detect wild animal browsing in grassland. Even if sown species were generally preferred by animals (mainly roe deer) grazing in the plot trial, some native species also presented an interesting role for these animals and they showed opportunistic feeding behaviour in critical conditions.

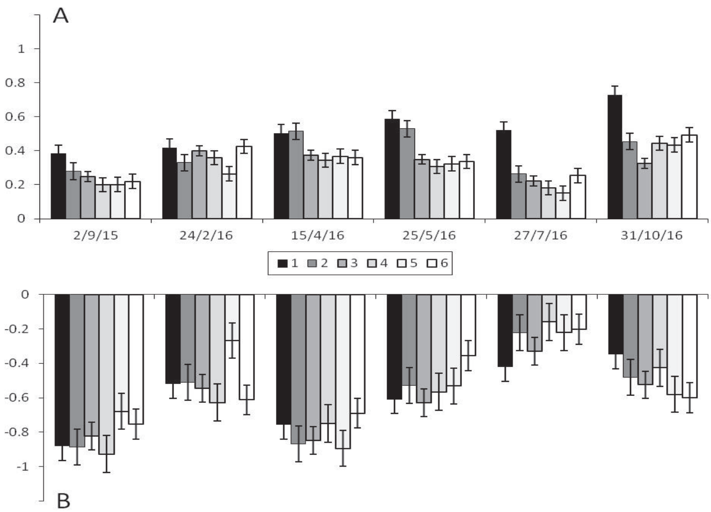

Figure 1. Average values of Electivity index grouped for sown (A) and spontaneous (B) species for different treatments in six dates of sampling.

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Effects of lamb production systems on carcass and meat quality

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Abstract

The aim of this study was to evaluate effects of different production models in Swedish lamb production on live weight gain (LWG), carcass quality and meat quality. The experiment included four production models for weaned male lambs: (group 1) indoor feeding; (group 2) grazing on cultivated pasture with or (group 3) without 0.3 kg concentrate supplementation daily per lamb; and (group 4) grazing on semi-natural pasture. Indoor lambs were fed silage (timothy, red clover and white clover) *ad libitum* and 0.8 kg concentrate daily per lamb. There were 20 lambs per group. Feed rations and LWG of the lambs were registered. As expected, the rearing system had a significant effect on LWG, with indoor lambs having the highest LWG (377 g day^{-1}), followed by cultivated pasture + concentrate (287 g day^{-1}), cultivated pasture (244 g day^{-1}) and semi-natural pasture (211 g day^{-1}). Furthermore, lambs on semi-natural pasture had lower conformation score and fat score than the other groups. There were, however, no differences in carcass pH or temperature 24 h after slaughter.

Keywords: pasture, muscle pH, muscle temperature, carcass quality, production system

Introduction

The Swedish sheep and lamb meat production accounted for 30.5% of the total Swedish consumption in 2015, which means that two-thirds of the total consumption imported (Jordbruksverket, 2016; Lannhard Öberg, 2016). To satisfy the consumers' demand of lamb meat, the import of sheep and lamb meat increased a lot, both in 2014 and 2015 (Lannhard Öberg, 2016). An increasing demand for high quality lamb meat produced in Sweden results in a need to know how lambs should be reared under Swedish conditions in order to reach a high and consistent quality of the meat. Swedish lamb meat can today be of varying quality, both sensory and technological, which could possibly be due to the different production systems, different breeds, ages at slaughter and other factors. The aim of this study was to investigate if different production systems affect the carcass characteristics in Swedish lambs. Three typical production systems (indoor feeding, grazing on cultivated pasture or on semi-natural pasture) were investigated and the carcass characteristics were compared. Further, it was also investigated whether supplementary feeding of concentrate affects the meat quality of lambs grazing on cultivated pastures.

Materials and methods

The experiment was conducted in 2016 at Götala Beef and Lamb Research Centre, Swedish University of Agricultural Sciences, Skara, Sweden. In total, 80 crossbred intact ram lambs (Dorset × Fine Wool) were included in the study. The lambs were either 50:50 (36 lambs) or 75:25 (44 lambs) Dorset and Fine Wool, respectively. Groups of 20 animals of each were assigned to one of four production models for weaned male lambs: group 1 on indoor feeding; group 2 and 3 on cultivated pasture with or without supplemented concentrate, respectively; and group 4 on semi-natural pasture. The liveweight at the start of the experiment was equal between groups (26.4, 26.8, 26.4 and 26.0 for group 1, 2, 3 and 4, respectively). Group 1 were housed in an enclosed pen made of metal gates. The animals had access to water, salt and minerals *ad libitum* in the pen. Group 1 was fed a total mixed ration consisting of silage *ad libitum* and a constant amount of 0.8 kg concentrate per lamb and day. Silage used consisted of approximately 75% timothy (*Phleum pratense* L.), 20% red clover (*Trifolium pratense* L.) and 5% white clover (*T. repens* L.). Group 2 and 3 grazed two different enclosed pastures of total 1.0 ha, divided into 3 paddocks each. The seed mix for the cultivated pastures consisted of 50% timothy, 20% meadow fescue (*Festuca pratensis* Huds.), 15% perennial ryegrass (*Lolium perenne* L.) 10% red clover and 5% white clover. In connection with the weighing, each group was moved once a week to a new paddock (0.3 ha). In addition to grass, Group 2 got 0.3 kg of concentrate per lamb and day given in feed troughs out on the pasture once a day. Group 4 grazed a semi-natural pasture and all lambs had free access to water and daily access to salt and minerals. For chemical composition of silage and pastures, see Table 1. Further, all lambs were weighed each week and hull assessment was used as a compliment to weight to determine when each individual lamb was mature enough to go to slaughter. At slaughter, parameters of lactate value (at slaughter), slaughter weight, carcass conformation and fatness and pH and temperature decline over 24 hours were recorded. Lactate was measured in blood from the debleeding procedure. The pH and temperature meters were inserted in the topside in all carcasses. The GLM procedure in SAS was used for statistical evaluation with treatment as fixed effect. Differences were considered significant if $P<0.05$.

Results and discussion

The rearing system had a significant effect on LWG, with group 1 having the highest LWG (Table 2). Further, group 4 had lower conformation and fat scores as well as dressing percentage than the other groups. Weight at slaughter showed a significant difference between groups. Group 1 had the highest weight at slaughter, significantly higher than group 3 and 4, whereas group 2 had a significantly higher slaughter weight than group 3 (Table 2). There were no significant differences between groups for lactate values, pH after 24 hours or the temperature after 24 hours (Table 3). The relationship between LWG and pH has been studied by others with varying results. In line with this study, some did not find any difference (e.g. Majdoub-Mathouthi *et al.,* 2013) while others have seen an effect (e.g. Hopkins *et al.,* 2005).

These results show that intact lamb rams can be reared under both intensive and extensive conditions and that would not negatively affect the final carcass temperature and pH. However, when it comes to conformation and fatness, a more intensive production such as group 1, 2 and 3, resulted in carcasses with

Table 1. Dry matter, crude protein, digestible protein, NDF and ash, and metabolisable energy (ME) in silage and pastures.[1,2]

Feed analysis	Group 1	Group 2	Group 3	Group 4
Dry matter (g kg^{-1})	29.7	23.3	22.8	22.3
Crude protein (g kg^{-1} DM)	167.7	182.5	167.8	198.0
Digestible protein (g kg^{-1} DM)	126.3	140.0	126.5	179.8
ME (MJ kg^{-1} DM)	11.4	11.5	10.4	11.6
NDF (g kg^{-1} DM)	507.0	421.0	452.8	396.0
Ash (g kg^{-1} DM)	70.3	90.3	83.0	86.3

[1] Group 1 = indoor feeding with silage, group 2 = cultivated pasture with supplemented concentrate, group 3 = cultivated pasture only and group 4 = semi-natural pasture.
[2] DM = dry matter; ME = metabolisable energy; NDF = neutral detergent fibre.

Table 2. Liveweight and age of the lambs reared in the different production models at start of the experiment and at slaughter.

Parameters	Group 1	Group 2	Group 3	Group 4	SEM	Significance
Weight at slaughter (kg)	50.6^{a}	50.3^{ab}	48.3^{c}	48.9^{bc}	0.54	0.0112
Days in experiment	64.7^{a}	82.4^{b}	91.3^{c}	109.1^{d}	2.56	<0.0001
Growth (g day^{-1})	377^{a}	287^{b}	244^{c}	211^{d}	7.92	<0.0001

[1] Group 1 on indoor feeding, group 2 on cultivated pasture with 0.3 kg supplemented concentrate per lamb daily, group 3 on only cultivated pasture and group 4 on only semi natural pasture; SEM = standard error of the mean.
[2] Rows with different superscript letters are significantly different at $P<0.05$.

Table 3. Carcass characteristics from lambs reared in the different production models.

Parameters	Group 1	Group 2	Group 3	Group 4	SEM	Significance
Conformation[c]	9.2[a]	8.7[a]	8.7[a]	7.9[b]	0.24	0.0024
Fatness[3]	7.4[a]	7.7[a]	7.4[a]	6.5[b]	0.17	<0.0001
Dressing (%)	42[a]	42[a]	41[a]	37[b]	0.40	<0.0001
Lactate (mmol l^{-1})	3.2	3.7	3.2	2.9	0.55	NS
pH after 24h	5.83	5.66	5.77	5.59	0.10	NS
Temperature after 24h (°C)	3.1	3.5	3.2	3.0	0.28	NS

[1] Group 1 on indoor feeding; group 2 on cultivated pasture with 0.3 kg supplemented concentrate per lamb daily; group 3 on only cultivated pasture; and group 4 on only semi natural pasture; SEM = standard error of the mean;

[2] Rows with different superscript letters are significantly different at P<0.05. NS = not signifucant.

[3] According the EUROP-system ranging from 1 (very bad conformation/very low fat) to 15 (very good conformation/very high fat).

a significantly higher conformation and fatness score compared to group 4, which could be profitable for the producer when animals are slaughtered. The results suggest that although group 3 was reared at lower intensity than group 1 and 2, this group could finish up for slaughter on only cultivated pasture, as there were no significant differences between group 1, 2 and 3 when comparing conformation and fatness of carcasses. Group 4, however, did not finish up for slaughter on only semi-natural pasture, according to conformation and fatness of carcasses. To get this type of lambs (group 4) ready for slaughter in the autumn, supplemented feeding or combining the utilization of semi-natural and cultivated pasture could be beneficial for carcass conformation and fatness. Alternatively, it may be beneficial to advance the lambing season to occur earlier in the spring to promote an earlier release on pasture.

Conclusions

The preliminary results from this study indicate that intact ram lambs reared according to four different production models (indoor, cultivated pasture with or without supplemented concentrate or semi-natural pasture), does not affect meat quality in terms of final pH and temperature in carcass. On the other hand, weight at slaughter, days in experiment, growth, conformation and fatness were affected by the rearing system and could be of importance in the actual production.

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Naturally occurring medicinal plants as ecosystem service of extensively used grassland

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Abstract

The economic importance of nature is based on the numerous ecosystem services and biodiversity it provides. The TEEB-Initiative (The Economics of Ecosystem and Biodiversity) intends to show and calculate the economic importance of ecosystem services and biodiversity. TEEB falls within the PES concept (Payments of Ecosystem Services). Various legally enforceable and also some voluntary agreements have been developed to protect different ecosystem services. Plants have an important role as an ecosystem service in supplying feed. Wild medicinal plants, however, have been neglected as an ecosystem service despite of their worldwide and growing economic importance. Plants that occur naturally in extensively used grasslands are of particular interest. In contrast to many western European countries, in states like Albania, Bulgaria, Romania and Poland extensive land use and, linked to this, extensive grassland is still present at a large scale. Such species-rich grasslands often harbour medicinal plants. The paper presents the first results of a research project that investigates mechanisms of connected value chains and the application and efficacy of certification and trading systems in the sector of medicinal plants from grasslands.

Keywords: extensively used grassland, medical plants, ecosystem services, certification

Introduction

Nature (ecosystems) provides numerous ecosystem services and biodiversity is also of economic importance. An economic view helps to reveal the hidden worth of ecosystem services and biodiversity and thus give arguments for the integration in private and public decisions for sustainable use and for the care of nature. Often this economic importance is only seen individually and from an economic point of view. This happens purposely, and also unintentionally, because decision-makers are unaware of the free and unpriced services of nature. The TEEB-Initiative (The Economics of Ecosystem and Biodiversity) has the aim of showing the economic importance of ecosystem services and biodiversity and to provide knowledge and understanding to decision-makers (TEEB, 2007). Numerous legally enforceable and binding agreements have been developed. Under international law the UN Convention on Biological Diversity (CBD, 2010) provides a legal basis. Since 2010 the conservation of ecosystem services is also the primary target of the CBD (CBD, 2010). On a European level, there is the EU-Biodiversity strategy (1998; revised in 2011) resulting in more precise resolutions binding EU member states (EU, 2011) or the European sustainability strategy from 2001 (EU, 2001). The most worldwide useful, complex and developed certification system for collection of wild plants is the Fairwild Standard (FWS) (MPSG, 2007).

Regarding plants, the supply of feed is counted as an ecosystem service (e.g. pastures provide feed for grazing animals and hay as winter feed for herbivores). But there are also naturally occurring medicinal plants that may provide an ecosystem service separate from that of feed plants. The worldwide interest in naturally occurring medicinal plants is of growing importance (Schippmann *et al.*, 2005). In Europe, a significant share of plants used for medicinal purposes or in the natural cosmetics sector occur most frequently in extensively used grassland. In contrast to many western European countries, in states like

Albania, Bulgaria, Romania and Poland there are still widespread extensively used grasslands which support medicinal plants at high numbers. Such countries have considerable potential to investigate the state and mechanisms of value chains and also to examine if and how systems and methods of certificate trading are implemented (Kathe *et al.*, 2003). The field study reported here, based on the assessment of often-complex value chains, allows for (1) detecting specific problems; (2) improving the quality of products and avoiding adulteration; and (3) giving recommendations for improvements and adjustments of certification systems.

Material and methods

To gain qualitative and quantitative data available literature and semi-structured interviews were applied. Preliminary interviews in November 2016 with two main stakeholders of herbal companies in Poland focussed on the following issues: list of harvested medicinal plants, collection regions, organization of work, adopted system of certification and control and character of difficulties that companies have to deal with. Additional sources of information were the nationwide institutions of the Polish herbal industry.

Results and discussion

First results from Poland show that around 140 species of medicinal plants are collected from the wild. Some of them are typical and distinctive for extensively used grassland, like *Hypericum perforatum*, *Filipendula ulmaria*, *Potentilla erecta*, *Euphrasia rostcoviana*, *Helichrysum arenarium*, *Alchemilla* spp., *Primula veris* and *Thymus pulegioides*. The regions with the most important potential for providing high quality plants are in eastern and south-eastern Poland, more precisely Warmia-Masurian, Podlaskie and Podkarpackie (Figure 1). Here, the tradition of harvesting wild plants is still part of the heritage of local communities. Local collectors are the first level in the value chains of producing natural medicine and cosmetic products (Booker *et al.*, 2012), as exemplified for Poland (Figure 2; Table 1).

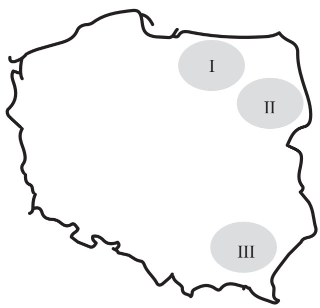

Figure 1. Main collection regions in Poland. I = Warmia-Masurian; II = Podlaskie; III = Podkarpackie.

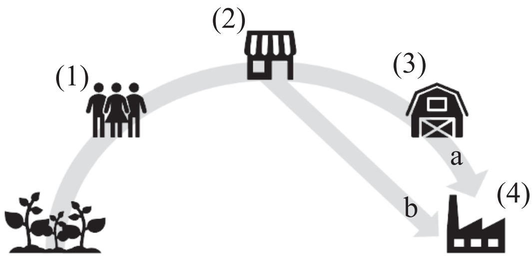

Figure 2. Two versions (a, b) of value chains in the trading of medicinal plants in Poland. (1) Collectors, (2) Herbal storage points, (3) 'Raw material companies', (4) 'Producing companies'

Table 1. Value chains of producing natural medicine and cosmetic products.

Industrial chain 'a'	Industrial chain 'b'
(1) Collectors are collecting the plants from the wild and sell them to:	(1) Collectors are collecting the plants from the wild side and sell them to
(2) Herbal storage points, these are part of bigger	(2) Herbal storage points, which are part of the
(3) 'Raw material companies' that sell all the herbs to the	(3) 'Producing companies' which manufacture medicinal and cosmetic products
(4) 'Producing companies' which manufacture medicine and cosmetic products	

There are at least two well-known raw material companies (RMC) active in the trading of medicinal plants in Poland. One of these companies has agreed to consider and comply with respect to FWS. Therefore, the traders at an early stage of the value chains offer educational training for collectors about good harvesting practices. As another consequence of implementing FWS, harvesting is undertaken according sustainability standards and the livelihood of local collectors is supported. More than one hundred harvested species are sold with the ecological certification that directly correlates with high quality habitats free from contamination. Harvesting standards have not yet been implemented under FWS since actors in this sector of the value chain still refuse to join. Only protected species according to Polish national or EU law, e.g. *Helichrysum arenarium* and *Primula veris* are under control and receive (in theory) special management. Consequently, sustainable harvest of medicinal plants is only guaranteed when standards are accepted and obeyed from each partner in a value chain.

Conclusions

The supply of medicinal plants derived from extensively used grasslands is an important aspect of ecosystem services. Most important are central and eastern European countries like Poland or Romania. Only traditional and extensive land use systems maintain the biodiversity of medicinal plants in grasslands. Clear quality standards for medicinal plants can be enforced by the relevant pharmaceutical and natural cosmetic industry. On the other hand, collecting medicinal plants from the wild supports livelihoods of the local collectors. In theory, this is best start for a win-win strategy. However, this requires that rules for sustainability are set and implemented and respected at all steps of often rather complex value chains.

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Soil respiration of permanent grassland under different management in central Apennines

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Abstract

The conservation of secondary grasslands enhances biodiversity and the provisioning of different types of Ecosystem Services. Soil contribution to greenhouse gases (GHG) emissions has been much studied but there are still uncertainties on how different land-use and management affect global warming. This study aims to assess the effect of different management on soil CO_2 emissions in a *Bromus erectus*-dominated grassland (central Apennines, 900 m a.s.l.). A randomized block design experiment was established to test three different treatments: (1) customary management (mowing in early July followed by a simulated grazing in November), (2) customary management and N-fertilization (100 kg ha^{-1} yr^{-1}), and (3) control (abandonment). This paper reports and discusses the CO_2 emission dynamics of the grassland under the different management from May to November 2016. The results showed that mowing significantly reduced soil respiration (Rs) between the end of July and the first half of August, while fertilization did not significantly affect Rs. The control showed a significant exponential relationship between Rs and soil temperature, which explained 77% of the seasonal variation. In contrast, both treatments (1) and (2) showed very low relationship between Rs and soil temperature due to the disturbances of the management practices.

Keywords: grassland, *Bromus erectus,* management, soil respiration

Introduction

Climate regulation, as an ecosystem service, is strongly influenced by management (Zhang *et al.*, 2016) which plays a fundamental role in greenhouse gases (GHG) emissions especially with regard to soil CO_2. Soil respiration (Rs) is defined as the return of CO_2 to the atmosphere (Schlesinger and Andrews, 2000) and includes the actions of microbes, soil fauna and root respiration (Hanson *et al.*, 2000). Grasslands cover approximately 40% of the earth's land area (Wang and Fang, 2009) and they have the potential to mitigate global warming (Oertel *et al.*, 2016). Under a perspective of climate change, the study of CO_2 emissions from grasslands appears to be of first and foremost importance. The paper aims to assess: (1) the differences in temporal dynamics of Rs, and (2) the influence of soil temperature on Rs rates in a *Bromus erectus*-dominated grassland under different types of management.

Materials and methods

The study area, characterized by a temperate oceanic climate, is located in central Apennines at 900 m a.s.l. (N 43°21'13.4"; E 13°03'31.6"). In 2016, cumulative precipitation was 1,152 mm and monthly mean air temperature ranged from 5.96 to 23.58 °C. In November 2015, a semi-natural *Bromus erectus*-dominated grassland area homogenous for soil and vegetation conditions was identified. The experimental design was a complete randomized block with three replicates and 4 m^2 (2×2 m) plots. Three treatments were applied: (1) the customary management with mowing performed in the first decade of July and simulated grazing in November (M-N0), (2) customary management with mowing performed in the first decade of July, simulated grazing in November and N-fertilization (100 kg ha^{-1} yr^{-1}) carried out early in April (M-N100), (3) abandonment as the control (C). The experimental area was fenced to prevent disturbance from livestock or wild animals. In each plot, two PVC collars (10 cm inner diameter and 10 cm height with perforated walls) were installed and inserted approximately 9 cm into the soil to assess soil respiration (Rs). The Rs measurements were performed *in situ* using a portable CO_2 infrared gas analyser (EGM-4 with SRC-1) and a soil respiration closed chamber system (PP-Systems, Hitchin, UK) equipped with a thermometer probe. At each measurement, soil temperature was measured at 10 cm soil depth. Soil CO_2 efflux and soil temperature were monitored every two weeks between 9:00 and 12:00 am (standard time) in order to avoid effluxes fluctuation (Almagro *et al.*, 2009; Fan *et al.*, 2015). Repeated-measures ANOVA was performed to analyse Rs to respect the time-dependency of the experiment (Peri *et al.*, 2015) while nonlinear regressions were used to analyse the effect of soil temperature on Rs.

Results and discussion

As observed by many authors in different ecosystems (i.e. Almagro *et al.*, 2009; Fan *et al.*, 2015; Peri *et al.*, 2015), temporal variations of the Rs were observed during the growing season. CO_2 effluxes of all the treatments reached their minimum in November, showing values of 0.25, 0.20 and 0.23 g CO_2 m^2 h^{-1} in C, for M-N0 and M-N100, respectively. Repeated-measures ANOVA within the date showed that Rs was affected by M-N100 and M-N0 treatments only on 26 July 26 and 10 August (Figure 1) when C showed almost the double of Rs rates compared to M-N0.

The control (C) showed two peaks of Rs on 26 July and 14 September when soil temperature was at 21.5 and 19 °C, respectively. In M-N0 the highest value was observed on June 28th when soil temperature was at 20.5 °C. After the mowing (performed in early July), Rs in M-N0 showed a decreasing trend despite a simultaneous increment of the soil temperature. After the mowing, M-N100 showed Rs and soil temperature trends similar to M-N0 (Figure 1). C showed a significant exponential relationship (P<0.01) between soil temperature and Rs, explaining 77% of the seasonal variability. Soil temperature explained only 30 and 23% of Rs seasonal variation in M-N0 and M-N100, respectively. This suggests that treatments perturbations (i.e. mowing) affected soil CO_2 effluxes (Figure 2).

Conclusions

These preliminary results highlight that different managements affected Rs dynamics in the studied grassland only in high summer, when Rs in the abandonment (Control) area was higher than in the mowing treatments. The absence of differences between the mowing treatments suggests that fertilization did not affect Rs rates but, at the applied N doses and cut frequency, the effects produced on the forage yield and quality should be taken into account. In the studied grassland, Rs proved to be affected mainly by soil temperature in the abandonment area and also by other factors (i.e. mowing or grazing) in the mowing treatments. This suggests the need for further studies to clarify which other environmental

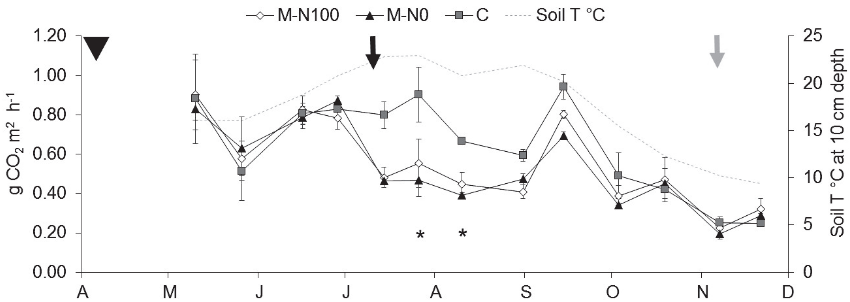

Figure 1. Soil temperature at 10 cm depth and seasonal variation of the Rs observed from 10 May to 21 November 2016. Dates labelled with * denote statistical differences for P<0.05. The dates of treatments applications are represented as follows: a black arrowhead for the fertilization (M-N100), a black arrow for the mowing (M-N0; M-N100) and a grey arrow for the simulated grazing (M-N0; M-N100).

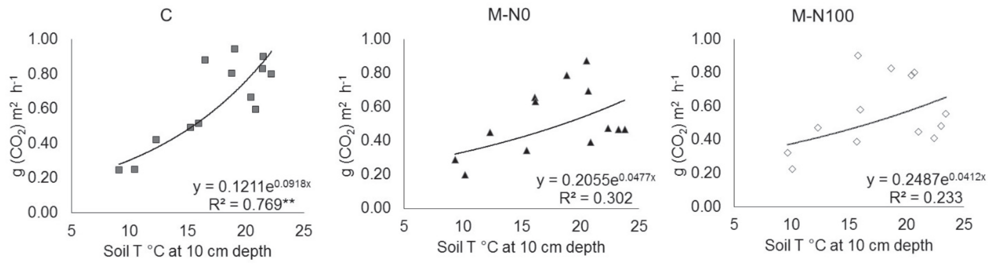

Figure 2. Exponential relationships (Rs = a e^{bT}) between soil T °C and Rs. ** indicate significance at P<0.01.

factors can explain soil CO_2 efflux variation, as for example to what extent soil temperature together with soil moisture affect Rs rates.

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Secondary compounds in the Sardinian endemic *Bituminaria morisiana,* a multipurpose forage legume

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Abstract

The legume genus *Bituminaria* L. includes some species with an interest both for forage and pharmaceutical use. Nonetheless, their secondary compounds may influence the palatability of forage. Little information is available on the endemic Sardinian species *Bituminaria morisiana* (Pignatti & Metlesics) Greuter, and to our knowledge, its essential oils and furocoumarins have not been studied. We identified 59 constituents by GC/MS analysis of the essential oils of five *B. morisiana* populations (Punta Giglio, Siliqua, Bitti, Burcei, Monte Gonareddu). In particular, 'Siliqua' showed six low molecular weight hydrocarbons and Z-β-farnesene. This latter was the main constituent in all oil samples. Other main constituents were 9,12-octadecadien-1-ol-(*Z, Z*), and 9,12,15-octadecatrien-1-ol-(*Z, Z, Z*). The GC/MS analysis of methanol extracts showed a low content of psoralens in *B. morisiana* accessions, especially in 'Burcei'. An exception was 'Monte Gonareddu', characterized by a high content of psoralen. These outcomes revealed the existence of variability among populations for some secondary compounds that will allow plant selection for specific uses and forage production.

Keywords: *Bituminaria morisiana*, psoralen, forage palatability, essential oils

Introduction

The legume *Bituminaria morisiana* (Pignatti & Metlesics) Greuter is a species endemic to Sardinia Island, where it is sympatric to the native widespread *Bituminaria bituminosa* (L.) C.H. Stirt. The species can be easily distinguished on the basis of some morphological traits and the presence of a typical bitumen smell in *B. bituminosa* only. *B. morisiana* is palatable for small ruminants in natural environments, but some secondary compounds contained in its leaves and sprouts may influence its acceptability to animals, similarly to what occurs for *B. bituminosa*. To our knowledge, few scientific works have focused on secondary compounds of *B. morisiana* (Cottiglia *et al.*, 2005; Iannuzzi *et al.*, 2016), and especially on its essential oils and furocoumarins (Porqueddu *et al.*, 2012). In order to characterize the composition of secondary compounds of *B. morisiana*, we collected five Sardinian populations and analysed the essential oils and the furocoumarins contents of their leaves. The main objective of our work was to assess the variability of secondary compounds in each population to discriminate between populations with low and high furocoumarin content.

Materials and methods

Plant material

Seeds of 5 native populations of *B. morisiana* were collected in Sardinia in sites characterized by different environmental conditions (microclimate, soil, altitude). Seeds were sown in plastic trays in October 2011. Plantlets were transplanted into plots in the experimental field of the Interdepartmental Centre for Plant Biodiversity Conservation and Enhancement (CBV) (40° 35' N, 8° 22' E) in April 2012. Plots were arranged in a completely randomized design with three replicates. Each plot was 20.50 m^2 and contained 12 seedlings of one accession, arranged in a row spacing of 1.80 m and a plant spacing of 0.80 m. Three samples of leaves per accession (one per plot) were collected in June 2014 for laboratory analyses, when all accessions were in the pre-flowering stage.

Essential oil extraction and analyses

Essential oils were extracted by water and steam distillation from 100 g of fresh leaves per replicate. Three replicates of each oil sample were analysed by a GC device (Hewlett-Packard, Model 5890A GC) equipped with a flame ionization detector (FID) and fitted with a ZB-5 fused silica capillary column (Phenomenex, 60 m × 0.25 mm, thickness 0.25 µm). Temperatures of injection port and detector were 280 °C. Column temperature was programmed from 50 to 135 °C at 5 °C min^{-1} (1 min), 5 °C min^{-1} up 225 °C (5 min), 5 °C min^{-1} up 260 °C and held for 10 min. Samples (0.1 µl each) were generally analysed without dilution using 2,6-dimethylphenol as internal standard, and injected using a split/splitless automatic injector HP 7673. Helium was the carrier gas used to propel oils. The GC-ms analyses were carried out with a GC-MS device (Agilent Technologies, model 7820A connected with a MS detector 5977E MSD), using the same conditions and column described for GC-FID. The column was connected with the ion source of the mass spectrometer. Mass units were monitored from 10 to 900 at 70 eV.

Furocoumarins extraction and analyses

Furocoumarins were cold-extracted from fresh leaves using a solution of MeOH/HCl 2M, under stirring at room temperature. The extract was filtered and taken to dryness. The residue, dissolved in water, was extracted 3 times with $CHCl_3$. The solvent was taken to dryness and the products analysed. The GC-ms analyses were carried out as described above. The quantification of furocoumarins was made using the addition method (Morris, 1980).

Statistical analysis

One way ANOVA was performed with Statgraphics Centurion XV (Statpoint Technologies, Inc. Warrenton, Virginia). When means were statistically different, Tukey test ($P \leq 0.05$) was applied to discriminate among means.

Results and discussion

Populations differed in their essential oil composition both for the type of compounds and their amounts present in the essence. We identified 59 constituents of the essential oils *in toto*, representing a rate ranging from 93.5 to 97.4% of the total. Z-β-farnesene was the main constituent in all oil samples (Table 1). Its content was remarkable in all populations, with a minimum of 27% in 'Bitti' and a maximum of 42% in 'Punta Giglio'. Other constituents that were present in appreciable concentrations were 9,12-octadecadien-1-ol-(*Z, Z*), with percentage ranging from 9.7 to 18%, and 9,12,15-octadecatrien-1-ol-(*Z, Z, Z*), with concentrations varying between 3 and 7.5%. The population 'Siliqua' presented six low molecular weight hydrocarbon (tryciclene, α-pynene, camphene, β-myrcene, D-limonene, β-fellandrene). This peculiarity was not found in the other populations.

Concerning furocoumarins, in the leaf extracts were also found the psoralens, as expected. Psoralens are constituted from a furan ring that can be fused to a coumarinic molecule in two arrangements giving the linear furocoumarins (ancestor psoralene) or the angular furanocoumarins (ancestor angelicin). In general terms, *B. morisiana* showed a lower content of psoralen compared to angelicin. In particular, 'Burcei' population showed no more than 55 mg kg^{-1} of psoralen. An exception was the 'Monte Gonareddu' population that showed a high content of psoralen (ca. 340 mg kg^{-1}). This last population could be exploited in phytotherapy as source of psoralen, for example in the treatment of psoriasis according to the BALNEO-PUVA methodology (BATH-PUVA) (Scheiba *et al.*, 2011). The 'Monte Gonareddu' population could easily provide the concentration of psoralens necessary for the treatment.

Table 1. Main components of essential oils (EO) and methanolic extracts (ME) of leaves of populations of *B. morisiana*.[1]

Extracts	Components	Populations				
		Monte Gonareddu	Punta Giglio	Siliqua	Burcei	Bitti
EO	Low molecular weight hydrocarbons	0.0^{b}	0.0^{b}	2.6^{a}	0.0^{b}	0.0^{b}
	β-caryophyllene	4.5^{c}	0.6^{e}	9.1^{b}	2.9^{d}	9.9^{a}
	Z)-β-farnesene	37.0^{b}	41.8^{a}	35.1^{c}	34.4^{d}	26.9^{e}
	D-germacrene	5.0^{a}	2.8^{e}	3.8^{b}	3.4^{c}	3.3^{d}
	9,12-octadecadien-1-ol-(Z,Z)	15.4^{b}	17.7^{a}	13.5^{d}	14.7^{c}	9.7^{e}
	9,12,15-octadecatrien-1-ol-(Z,Z,Z)	3.0^{e}	4.6^{b}	3.3^{d}	3.6^{c}	7.5^{a}
	Oleyl alcohol	0.0^{b}	0.0^{b}	0.0^{b}	0.0^{b}	4.5^{a}
ME	Maltol	17.3^{b}	6.9^{e}	19.6^{a}	16.6^{c}	16.2^{d}
	Apocynin trimethyl citrate	4.2^{d}	7.5^{b}	3.7^{e}	8.6^{a}	7.1^{c}
	Apocynin	0.0^{b}	0.0^{b}	1.5^{a}	0.0^{b}	0.0^{b}
	Methyl vanillate	0.0^{b}	0.0^{b}	2.9^{a}	0.0^{b}	0.0^{b}
	Methyl tetradecyl adipate	0.0^{d}	8.4^{b}	2.9^{c}	0.0^{d}	16.9^{a}
	Methyl coumarate	3.9^{c}	3.8^{c}	8.6^{a}	3.8^{c}	8.0^{b}
	Angelicin	17.1^{e}	38.1^{a}	33.0^{b}	30.0^{c}	25.2^{d}
	Psoralen	49.8^{a}	25.0^{b}	21.0^{b}	19.3^{b}	15.0^{b}
	Santonin	7.7^{d}	8.4^{c}	6.7^{e}	17.0^{a}	11.6^{b}
	Methyl linoleate	0.0^{c}	2.1^{b}	0.0^{c}	4.7^{a}	0.0^{c}

[1] Values are expressed in % of the total components. Different letters in each row indicate statistical differences among populations means (Tukey test, $P \leq 0.05$).

Conclusions

The variability in secondary compounds allowed us to differentiate populations of *B. morisiana* for their content in furocoumarins and essential oils components. The population Monte Gonareddu is certainly the most interesting for use in pharmaceutical PUVA applications, due to its high content of psoralen. Accessions for forage production require a low content of secondary compounds. Nonetheless, future experiments are needed to assess the acceptability to animals of leaves of *B. morisiana* containing different levels of secondary compounds.

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Characterizing difference in pollen carriage by bumblebee species in unimproved pastures

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Abstract

Resolving the role of food availability in the decline of bumblebees (*Bombus* sp.) is especially challenging for bumblebee species. We combined Cytochrome Oxidase I (COI) barcoding to aid bee identification and rbcL metabarcoding to characterize pollen carriage to study 269 bumblebees captured in three UK species-rich pastures. Representation of individual bee species and variance in their pollen carriage was both site- and date-dependent. Pollen profiles of bees from the cryptic *Bombus lucorum* complex (*B. terrestris*, *B. cryptarum* and *B. magnus*) were initially indistinguishable but later diverged across all sites. Throughout the season, individuals of most species carried pollen from mature residential gardens >800 m from the capture site. Our findings support landscape-level foraging for all species and suggest differences in the foraging behaviour of cryptic species are patchy and restricted to limited scenarios.

Keywords: bumblebee, foraging behaviour, pollen profile, pastures

Introduction

Pollination is a vital ecosystem service that supports food production and helps maintain biodiversity (Tscheulin *et al.*, 2011). It has been estimated that some 35% of global food production is directly reliant on the action of pollinators (Klein *et al.*, 2007), with the bees considered as the most important contributors (Kearns *et al.*, 1998; Tscheulin *et al.*, 2011). There is growing desire to develop a greater understanding of plant-pollinator interactions at the community level (Ebeling *et al.*, 2008). A range of methodologies have been used to characterise some aspect of pollinator service at the landscape level including pollen morphological analysis (Jha *et al.*, 2013) and pollinator visitation surveys (Garbuzov *et al.*, 2014; Woodcock *et al.*, 2013). These studies all advance our understanding of pollinator service afforded by bees but each provides only a partial picture of pollinator movement and pollen carriage across the landscape. In this study we therefore combine COI DNA barcoding of the bumblebee guild (*Bombus* sp.) with 454 pyrosequencing *rbcL* barcoding of the pollen they carry to characterize landscape-scale changes in pollen carriage over a period of phenological change in three species-rich grassland communities in the UK.

Materials and methods

We surveyed the species content and diversity of three semi-natural wet grassland communities in Mid Wales (UK Grid Refs: SN 667628; SN538650; SN496514). Flowering status and floral abundance of all component species was recorded from mid-June to the end of August. Leaf samples of each species were collected from all species and DNA extracted using the Qiagen DNasy Plant Mini Kit. DNA was stored at -20 °C until needed for reference DNA barcoding. We followed the method of Memmott (1999) to estimate the relative abundance of different bumble bee species at each site on each visitation. During these collection surveys, bees were captured individually in a plastic container (to avoid pollen cross-contamination) and snap cooled on dry ice. The bees were killed and stored at -20 °C.

Forelimbs of each insect were removed and DNA extracted using the Wizard SV 96 Genomic DNA purification System. Each captured bee was placed into 1 ml of nuclease-free water and pollen recovered by vigorous shaking on a Vortex followed by centrifugation at 5,000 rpm for 10 min. Supernatant and insect body parts were removed from each sample, leaving the pollen precipitate originating from all parts of the body of the bee. Pollen DNA was extracted as above using the Qiagen DNasy Plant Mini Kit. DNA barcoding of the bumblebees was performed using the universal primers targeting the Cytochrome Oxidase I (COI) gene:

LEP(F1), 5'-ATTCAACCAATCATAAAGATATTGG-3':

LEP(R1), 5'-TAAACTTCTGGATGTCCAAAAAATCA-.3'.

The amplification protocol comprised: 5 cycles of 94 °C/120 s, 94 °C/40 s, 45 °C/40 s, 72 °C/60 s, 35 cycles of 94 °C/40 s, 51 °C/ 40 s, 72 °C/60 s, and a final 5 min step at 72 °C. Strong amplicons of the appropriate size were subjected to Sanger sequencing. The resultant clean bi-directional sequences were trimmed to 497 bp (the maximum conserved length all samples) using Clustal for alignment comparisons. Reference plant barcodes (from leaf tissues) were generated using the following rbcL primers:

F2 5` ATGTCACCACAAACAGAGACTAAAGC 3`

R2 5` AGTCCACCGCGTAGACATTCAT 3`.

The following thermocycling regime was used for PCR amplification: 94 °C for 2 min, followed by 40 cycles of 94 °C for 30 s, 52 °C for 50 s and 72 °C for 40 s, with a final elongation cycle of 72 °C for 10 min. Strong amplicons of the appropriate size were subjected to DNA sequencing and trimmed as above. DNA barcodes from pollen mixtures recovered from each individual bee were generated by 454 pyrosequencing. For this, tagged primers were used to differentiate 454 sequences originating from different source bees. Barcodes recovered from the 454 pyrosequencing runs were then compared to reference sequences generated above and by reference to data on BOLD systems V3 database.

Results and discussion

In all, 278 of the 288 bumble bees collected from the 3 sites across both years (120 from Rhos Glyn yr Helyg, 105 from Rhos Glandenys and 58 from Rosh Fullbrook) yielded high quality bi-directional DNA CO1 barcode sequence. The remaining 10 samples generated sequence traces that failed to satisfy the 20 threshold PHRED quality score and were discarded. When these CO1 sequences were combined with reference barcodes, the resultant phylogenetic neighbour-joining tree revealed clear structuring of sequences into nine tight, well-supported groups that each corresponded to a different bumble bee species. K2P distances indicated that intraspecific variation was minor in comparison to that seen between species, with overall K2P distance across all samples being just 0.08. There was no instance of incongruity between morphological diagnosis and that revealed by DNA barcoding on either tree, although there were a few instances where morphological diagnosis was ambiguous.

High quality reference *rbcL* barcode sequences were recovered from all but two of the 68 reference flowering plants found in the wet pastures (viz: *Myosotis arvensis* and *Cardamine pratensis*). These gaps were filled by reference to the BOLD Systems database. Collectively these barcodes allowed diagnosis of all species except five sister species pairs. We then used this reference resource to characterize bee pollen carriage. Overall, we generated ~1000 forward and reverse 454 pollen barcode sequence from each of the 267 bumble bees. In total, the 7 bumblebee species carried pollen from 51 species of plant growing within 10 km^2 of the sampling sites. This included pollen from 32 of the 68 plants species found within the study sites themselves but also several species found only in rural gardens at least 800 m from the collection sites. Bee species diverged in their pollen carriage with location and time of year. For instance, for *Bombus terrestris*, pollen carriage diversity changed with site and season, and included garden species such as *Rhododendron simsii* not found in any collection site (Figure 1). Pollen profiles of bees from the cryptic *Bombus lucorum* complex (*B. terrestris*, *B. cryptarum* and *B. magnus*) were initially indistinguishable but later diverged. Our findings support landscape-level foraging for all species and suggest differences in the foraging behaviour of bumblebee species are patchy and restricted to limited scenarios.

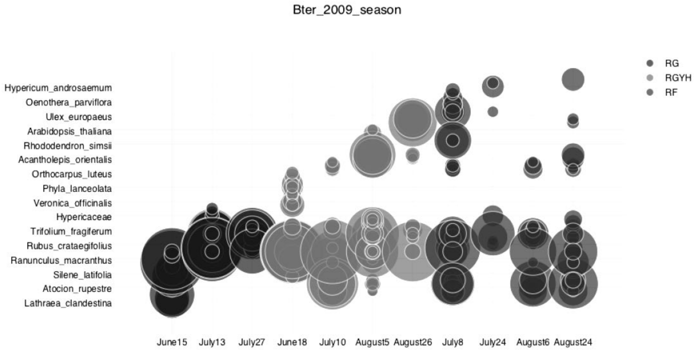

Figure 1. Graphical representation of pollen carriage by *Bombus terrestris* during 2009. Size of circles represents relative pollen abundance corresponding to plant species listed in the left hand column. Circles represent site, with dark grey = Rhos Glandenys; light grey = Rhos Glyn yr Helyg and mid-grey = Rhos Fullbrook. Collection dates are shown on the x-axis.

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