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CICLO: XXX

CHARACTERIZATION, DIVERSITY, AND MANAGEMENT CHALLENGES OF MOUNTAIN PASTURELANDS

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Le bonheur est dans le pré. Cours-y vite, cours-y vite.

Le bonheur est dans le pré. Cours-y vite. Il va filer.

- Paul Fort -

Acknowledgments

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1. Preface

Pasturelands represent a wealth for mountain territory that Europe is committed to preserve, protect, and promote due to their economic, social, cultural, landscape, and environmental roles.[1,2]

These goals can be achieved only through an active and sustainable management of such resources, based on an accurate and careful analysis of mountain farming systems and especially of the vegetation that characterises grassland environments, in its wide variety and complexity.[3,4]

European mountain pasturelands

Pasturelands exploitation and management are among the main factors affecting vegetation dynamics and composition, acting at the same time as a disturbance (i.e. by biomass and nutrient removal, by interfering with the reproductive cycle, by animal trampling) and as a source of fertility and seeds (i.e. by nutrient supplying and seed dispersal, in the case of grazing).[5,6] The result of the complex dynamics produced by the application of agro-pastoral systems in different pedo-climatic conditions is often a heterogeneous mosaic of different grassland vegetation communities, interspersed with shrublands and forestlands and characterised by an overall high plant diversity.[7]

Livestock benefits from the forage produced by these species-rich vegetation communities as the main (and in most of cases unique) feed of their diet, either by direct grazing/browsing or by consumption of harvested/dried fodder. In certain cases, the diet can be composed not only by fresh grass and hay, but also by tree and shrub foliage in different percentages, depending on animal feeding preferences and on foliage availability and quality.[8] Farming system presence and profitability in European mountain environments are based upon the production and the exploitation of high-quality forages.[9]

Forage production and quality

Generally, when evaluating a forage resource, one of the main features to be taken into account is its biomass production. Forage production strongly influences grassland management, by regulating timing, frequency, and regime of its exploitation, either for hay making or for animal grazing/browsing. Biomass production differs in relation to the considered plant species or vegetation community, but can also vary depending on site factors (i.e. nutrient and water availability, soil, light exposure, biotic and abiotic disturbances) and on climatic conditions (i.e. precipitation and temperature).[10,11]

However, a complete evaluation of a forage should not be limited to the assessment of its biomass production, but should also consider its nutrient quality as animal feedstuff. Particularly, animal feed quality is influenced by its chemical composition.[12] The proximate composition consists in the set of elements for which any animal feed is usually analysed: dry matter, ash, crude protein, ether extract, and fibre fractions. These elements are involved in ruminant metabolism and contribute to satisfy animal nutrient requirements for maintenance, growth, gestation, and production. Some chemical compounds, such as fatty acids and plant secondary metabolites (e.g., phenolic compounds), are also linked to lipid biosynthesis and transformation. The molecules resulting from these processes can considerably affect the quality of derived dairy and meat products by conferring them specific sensory, chemical, and nutraceutical attributes.[13–16] The analysis of proximate composition, fatty acid profile, and plant secondary metabolite content is thus of outmost importance when evaluating the quality of a forage.[17,18]

A large number of studies has been conducted in the last decades to analyse forage proximate composition as well as fatty acid and phenolic profiles, but they have often focused only on single plant species or on mono- and bi-specific leys.[19–23] However, in European mountain environments livestock usually feed on species-rich forages. Therefore, it could be meaningful to compare the forage produced by different vegetation communities in terms of proximate, fatty acids, and phenolic composition, trying to identify the most suitable grassland type(s) and fodder species to be used as base feed for obtaining high-quality animal-derived food products.

The first objective of the present thesis was to analyse the relationships between vegetation and chemical composition of some species-rich grassland types, used as forage resource by livestock in an alpine context. This study resulted in the research paper here reported in Chapter 2.

In addition to species composition, another important factor influencing forage chemical composition is plant phenological stage.[23–25] It is a biological event occurring during plant development (e.g. first leaf emission or full flowering) and it depends on the adaptation of a species to environmental features. Irradiance, temperature, and water and nutrient availability are some factors which strongly affect its occurrence and timing.[26] Generally, earlier stages (i.e. before flowering) result in a higher quality of herbage (more crude protein, less fibre and phenolic compounds, better fatty acid profile),[27] while fodder tree and shrub foliage maintain a higher nutrient quality along the vegetative season.[28] Nevertheless, the interest for assessing the nutrient quality and chemical components of fodder tree and shrub foliage raised only recently, although it represents a valuable feeding resource for animal (especially goat) nutrition in European mountains since Neolithic.[29]

In order to supply the first complete data concerning biomass production, chemical composition, and digestibility of some European tree foliages along the vegetative season, a comparative trial on four fodder tree species was carried out. This study was the second objective of the present thesis, which resulted in a research paper and is reported in Chapter 3.

Pastoral regimes: effects on ecosystem

European mountain pastures are among the semi-natural ecosystems with the highest plant diversity.[30–34] The preservation of these environments is ensured by the presence of mountain farming systems, which have actively managed the territory over millennia shaping and modifying the landscape by: tree cutting or plantation, raising buildings and communication routes, cropping, and grassland resource exploitation, through hay making and/or animal grazing and browsing.[35–38]

Farming systems have evolved in mountain heterogeneous environments shaped by human and livestock by developing various management systems, implemented with different livestock species and depending on local traditions, economical sustainability, and environmental suitability. As a result, habitat diversity and landscape heterogeneity have been considerably enhanced.[39] Also ecosystem biodiversity has been positively affected by grassland management, revealing a high variety and variability of animals, plants and micro-organisms, at genetic, species and ecosystem level, which are necessary to sustain the ecosystem itself, its resilience, key functions, structure, and processes.[40–44] Indeed, grassland biodiversity support a number of essential ecosystem services such as pollination and maintenance of soil fertility and biota, which are essential to human survival.[45–48] In addition, a high biodiversity provides habitat and species with the ability to adapt to changing environment and evolve, by increasing their tolerance to frost, high temperature, drought, water-logging, and more generally to climate change, as well as their resistance to particular diseases, pests, and parasites.[49–51]

In the last decades, scientific research largely focused on this subject, but rarely a direct comparison among different management systems was carried out, aiming at understanding which of the investigated systems might be the most valuable for biodiversity maintenance and enhancement.

The third objective of the present thesis was to explore this issue, by assessing the effects produced by different grazer species managed under different grazing systems on grassland multi-taxa species diversity. This study resulted in a research paper and is reported in Chapter 4.

Abstracts of the three research chapters

The abstracts of the next three research chapters are here reported, in order to facilitate the understanding of this thesis outline.

Chapter 2. Relationships between vegetation and chemical composition of forages

Plant composition of species-rich mountain grasslands can affect the sensorial and chemical attributes of dairy and meat products, with implications for human health. A multivariate approach was used to analyse the complex relationships between vegetation characteristics (botanical composition and plant community variables) and chemical composition (proximate constituent and fatty acid profile) in mesophilic and dry vegetation ecological groups, comprising six different semi-natural grassland types in the Western Italian Alps. Mesophilic and dry grasslands were comparable in terms of phenology, biodiversity indices and proportion of botanical families. The content of total fatty acids and that of the most abundant fatty acids (alpha-linolenic, linoleic and palmitic acids) were mainly associated to nutrient-rich plant species, belonging to the mesophilic grassland ecological group. Mesophilic grasslands showed also higher values of crude protein, lower values of fibre content and they were related to higher pastoral values of vegetation compared to dry grasslands. The proximate composition and fatty acid

profile appeared mainly single species dependent rather than botanical family dependent. These findings highlight that forage from mesophilic grasslands can provide higher nutritive value for ruminants and may be associated to ruminant-derived food products with a healthier fatty acid profile.

Chapter 3. Fodder tree species: foliage characterisation

Many tree and shrub species are underestimated fodder resources due to insufficient knowledge about their potential feeding value, especially for goats. This work aimed at assessing productive and nutritional attributes of the foliage of four temperate tree species widespread in Europe: *Acer pseudoplatanus*, *Fraxinus excelsior, Salix caprea,* and *Sorbus aucuparia*. Leaf length and biomass, proximate composition, fatty acid profile, phenolic composition, and *in vitro* true digestibility were determined during the vegetative season. The differences found among the species were remarkable, even if weakly related to seasonal changes, especially when considering fatty acid and phenolic compositions. *Fraxinus* was the most productive species and its foliage showed the lowest phenolic contents, resulting in the highest digestibility. *Sorbus* digestibility was similar, but its lower polyunsaturated fatty acid concentration can reduce the interest for this species as a feeding resource for goat dairy products with healthy properties. The lower digestibility found for *Salix* and *Acer* may be related to their high phenolic concentrations. The four species can represent a complete and good quality feedstuff for goat nutrition, above all in the late summer when herbage quality decreases, particularly in terms of crude protein and fatty acid profile.

Chapter 4. Grazer species and grazing system effects on grassland species diversity

Grazing management is an important tool to preserve insect biodiversity. Although literature has discussed the importance of grazing pressure adjustment to support grassland insect communities for the ecosystem services they provide, little has been published on the economic sustainability of such management adjustments to date. This study compared continuous grazing (CG) to an innovative rotational grazing system (the biodiversity-friendly rotation - BR), where a subplot was excluded from grazing for two months during the main flowering period.

The effects of grazing two different species (cattle and sheep) within both systems were also evaluated. The aims were to assess the effects on butterfly, bumblebee, and ground beetle assemblages, along with the impact on herbage mass and animal performance. The BR enhanced both the abundance and species richness of flower-visiting insect assemblages and it was observed that cattle provided better results than sheep grazing. A multivariate redundancy analysis highlighted that most of the flower-visiting species (including almost all the endangered and locally rare species) were favoured by BR-cattle treatment, mainly due to the high percentage of flower cover and sward heterogeneity involved in this treatment. However, grazing system and grazer species did not affect ground beetle species richness or abundance. Moreover, herbage mass and animal performance (live weight and body condition score) were comparable between CG and BR throughout the grazing season. The BR could be a useful management system to enhance grassland flower-visiting insect assemblages whilst meeting farm production objectives, especially in protected environments where insect conservation is a major target.

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2. Relationships between botanical and chemical composition of forages

Extended paper title: *Relationships between botanical and chemical composition of forages: a multivariate approach to grasslands in the Western Italian Alps*

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Abstract

The abstract of this paper is reported in Chapter 1.4, page 4.

Keywords: ecological group, fatty acids, forage quality, grazing ruminants, pasture, phenology

Introduction

The interest for high-quality and healthy animal products has constantly increased over the last years.[1] Several works highlighted that ruminants fed on high grass based diets provide milk and meat with a remarkable concentration of nutraceutical compounds.[2–5] The high content of polyunsaturated fatty acids (FA), particularly alpha-linolenic acid (C18:3 n-3), and the occurrence of plant secondary metabolites from fresh forages can significantly affect the lipid metabolism in the rumen and in the mammary gland, usually resulting in lower concentrations of hypercholesterolemic saturated FA and higher concentrations of vaccenic acid, rumenic acid and omega-3 FA in the derived products.[6–9] Due to variations in FA and plant secondary metabolites contents, grasslands with different botanical composition can confer specific intrinsic sensory and chemical attributes to dairy and meat products.[10–13]

Several research assessed the proximate composition and FA profile of forages and the factors influencing their modifications, such as genetics, phenological stage, methods of forage conservation, and nutrient supply, focusing on single-plant species or mono- and bi-specific leys.[2,14–19] Conversely, extensive farming systems are dominated by complex and species-rich semi-natural grasslands, which are an important fodder source in most European countries.[20] The high variability of ecological and management conditions in extensive mountain ecosystems (e.g., high degree of variation in climates, slopes, soils, aspects, grazing regimes, etc.) has determined a high number of different grassland communities, characterised by high biodiversity. On the summer pastures of the Western Italian Alps, Cavallero et al.[21] described more than 90 different grassland types, mainly belonging to mesophilic and dry grassland ecological groups. In these environments, only a few recent studies have been conducted to investigate the influence of the botanical composition of pastures on the proximate composition and FA profile of the derived forages.[22,23] However, Revello-Chion et al.[22] focused on the chemical composition of forages within a single grassland type. Peiretti et al.[23] realized a limited number of vegetation surveys and sampling, which did not allow evaluating the complex relationships among chemical and botanical variables with a multivariate approach. Multivariate analyses allow taking into account the complex relationships among several variables and they have been successfully used to evaluate the relationships between the botanical and polyphenolic compositions of permanent pastures in France.[24] Moreover, the effect of different grassland communities on herbage chemical composition is largely unknown.

This work aimed at assessing with a multivariate approach the relationships between vegetation characteristics (botanical composition and plant community variables) and chemical composition (proximate constituents and fatty acid profile) in different species-rich grassland types belonging to contrasting and widespread ecological groups in the Western Italian Alps.

Materials and Methods

Study area

The study was conducted within the Piedmont Region (Western Italian Alps), in two different bioclimatic districts, in order to explore different ecological groups and grassland types, according to Cavallero et al.[21] The first district was located in the Western valleys of Piedmont (Chisone and Susa Valleys), being characterised by an endalpic continental climate (*sensu* Ozenda[25]), with an annual precipitation ranging from 479 to 842 mm (mean value for the years 1996-2014 of the pluviometric stations of Pinerolo and Sestriere[26]) and dominant soils were originated from calcareous parent rock. The second district was located within the Sesia Valley, in northern Piedmont, being characterised by an endalpic suboceanic climate, with higher annual average precipitation (from 1220 to 2077 mm; mean values for the years 1989-2014 of the pluviometric stations of Borgomanero and Alagna) and dominant soils were originated from siliceous parent rock.

Different grasslands, belonging to common alpine and European grassland communities,[21,27,28] were chosen within a similar altitudinal gradient within the two districts (from 500 to 2000 m a.s.l. and from 250 to 1700 m a.s.l., respectively). Grasslands were dominated by *Bromus erectus* Hudson, *Festuca nigrescens* Lam., *Dactylis glomerata* L., *Achillea millefolium* L., *Festuca curvula* Gaudin, and *Poa pratensis* L. and were traditionally grazed under rotational grazing systems and/or mowed once or twice a year. Fresh grass and hay from these grasslands are the prevalent forage resources for dairy cows producing high-quality and typical local products, such as the "Piedmontese Noble Milk".[29]

Vegetation surveys and plant community variables

Thirty-nine vegetation surveys were carried out from September 2013 to September 2014 (Annex 2.A), a few days before each grassland was grazed or mowed, in order to characterise plant species proportion and the phenological stage linked to the traditional grassland management. Botanical composition was determined using the vertical point-quadrat method[30,31] along 25-m transects placed within vegetation patches which were representative of the overall botanical composition of the surveyed areas. One transect was placed for each grassland patch and each grassland was surveyed once. In each transect, at every 50-cm interval, plant species touching a steel needle were identified and recorded (i.e. 50 points of vegetation measurements per transect). The elevation and the Day of Year (DOY) in which each survey was conducted were annotated and the phenological stage of all species occurring along the transects was recorded using the phenological scale of Lambertin (Annex 2.B).[32]

For each recorded plant species, the frequency of occurrence (f_i = number of occurrences/50 points of vegetation measurement), which is an estimate of species canopy cover,[33] was calculated for each transect. Species relative abundance (SRA), a proxy for total above-ground phytomass, was determined at each transect and used to detect the proportion of different species according to the equation of Daget and Poissonet:[30]

$$\mathrm{SRA_i} = \frac{f_i}{\sum_{i=1}^{n} f_i} \times 100(\%)$$

where $\mathrm{SRA_i}$ and f_i are the species relative abundance and the frequency of occurrence of the species *i*, respectively. In addition, the SRA of the botanical families was calculated for each transect and the most abundant families (i.e. those with an average SRA higher than 10% in more than one vegetation survey) were retained for further analyses.

Moreover, each plant species was classified according to the indicator values of Landolt et al.,[34] which are based on a simple ordinal classification of plants according to the position of their realized ecological niche along an environmental gradient ranging from 1 (low requirement for a particular indicator) to 5 (high requirement). More specifically, the species were classified according to the following indicators: soil moisture (i.e. a proxy for the average soil moisture during the growth period), nutrient supply (i.e. a proxy for nutrient content in the soil, referring mostly to nitrogen) and soil reaction (i.e. a proxy for soil pH). The mean values of each transect for each ecological indicator were computed by averaging species values weighted on their SRA.

Plant biodiversity of each transect was expressed according to two indices: species richness (i.e. the total number of species recorded along the transect) and Shannon diversity index.[35]

Each species was also classified according to the Index of Specific Quality (ISQ).[21,30] The ISQ is based on palatability, morphology, structure, and productivity of the plant species found in the Western Italian Alps, and it ranges from 0 (low) to 5 (high). In each transect, forage pastoral value, a synthetic value which summarizes forage yield and nutritive value ranging from 0 to 100, was calculated on the basis of the SRA and the ISQ according to the equation of Daget and Poissonet[30]:

$$\text{Pastoral Value} = \sum_{i=1}^{n} (\text{SRA}_i \times \text{ISQ}_i) \times 0.2$$

where SRA_i and ISQ_i are the species relative abundance and the index of specific quality of the species *i*, respectively.

An average value of the phenological stage, weighted on the SRA, was also calculated for each transect, according to Lambertin.[32]

Sampling and chemical analyses of grass

During each vegetation survey, representative samples of the botanical composition (about 400 g each) were harvested with a MAKITA trimmer UM104D (Makita Corporation, Anjō, Japan) at about 5 cm from the ground, simulating the removal of vegetation by grazing and cutting. The samples were placed in sealed polyethylene bags, immediately stored at 4°C in a portable refrigerator and transported to the laboratory, where each sample was divided into two homogeneous aliquots of about 200 g each. The samples were then frozen at -80°C until analysed for their chemical composition.

The first aliquot of each grass sample was dried at 40°C for 24 h. The samples were then ground with a cutting mill to pass a 1-mm screen sieve (Pulverisette 15 – Fritsch GmbH, Idar-Oberstein, Germany). AOAC[36] procedures were used to determine dry matter (DM, method no. 930.15), crude protein (CP, method no. 984.13) and acid detergent fibre (ADF, method no. 973.18) in the grass samples. Neutral detergent fibre (NDF) was analysed according to Van Soest et al.[37]

The second aliquot of each grass sample was freeze-dried (Edwards MF 1000, Milano, Italy) and ground. These aliquots were used for the assessment of the FA composition using a combined direct transesterification and solid-phase extraction method as described by Alves et al.[38] Separation, identification, and quantification of fatty acid methyl esters were performed as described by Renna et al.[39] The total fatty acids (TFA) concentration was also calculated. The proximate composition and FA profiles were expressed as g kg^{-1} DM.

Statistical analyses

A two-level classification system was used to assign each vegetation survey to a specific grassland type (homogeneous in terms of botanical composition) and ecological group.[21,31] Botanical data were classified by hierarchical cluster analysis performed using the Clustan Graphics 5.27 software. The similarity matrix was calculated using Pearson’s correlation coefficient, while the between-group linkage was selected as agglomeration method.

The relationships between the total and the major individual FA contents in grass samples were analysed with linear regressions. The assumption of normality was tested using the Kolmogorov-Smirnov test. Linear regressions and normality test were performed using SPSS 22.

Two main matrices were arranged: (1) a botanical matrix, with the SRA of the most abundant species (i.e. species occurring in more than one transect with a SRA > 5%) and (2) a chemical matrix, including DM, CP, NDF, ADF, TFA, and the most abundant FA detected in the grass samples (all expressed as g kg^{-1} DM, with the exception of DM which was expressed as %). A Mantel test was used to calculate the correlation between the botanical and chemical matrices (PC-ORD 6 software). A canonical correspondence analysis (CCA) was performed to assess the relationships among chemical (main matrix) and botanical (secondary matrix) data. A third matrix including plant community variables (i.e. pastoral value, biodiversity indices, botanical families, elevation, DOY, and Landolt's ecological indicators) was used as a supplementary matrix to evaluate the gradients associated with the two main axes of the ordination plots. The effect related to exploitation (i.e. first, second or third seasonal growth) was included in the CCA as a covariate. The CCA was performed with the statistical program CANOCO 4.5. Quantitative relationships between vegetation and chemical variables were also assessed by Pearson's correlation analysis using SPSS 22. Independent sample t-tests were performed in order to test for differences on the botanical, chemical and plant community variables between the two ecological groups obtained from the cluster analysis using SPSS 22.

Results and Discussion

Botanical composition of grassland communities

A total of 225 plant species, belonging to 38 botanical families, was detected. However, only a few species and families were the most abundant (38 species and eight families) and considerably contributed to the total above-ground phytomass (72.6 and 86.2%, respectively). The hierarchical cluster analysis identified six grassland types (belonging to five different phytosociological alliances) and two main ecological groups: a) mesophilic grasslands (i.e. grasslands with average soil moisture content), including *P. pratensis*, *Lolium perenne* L. and *F. nigrescens* types and b) dry grasslands (i.e. grasslands with lower soil moisture content), including *B. erectus*, *Brachypodium rupestre* (Host) Roem. & Schult. and *Helianthemum nummularium* L. types (Fig. 2.1). These communities are among the most common grassland communities in the Alps and in other parts of Europe.[21,40–42]

As expected, the grassland types derived from different altitudinal, climatic and management gradients. Within the mesophilic grassland ecological groups, *P. pratensis* and *L. perenne* types were representative of lowlands and valley-bottoms, with a higher management intensity in the second type, while *F. nigrescens* type was located at the highest

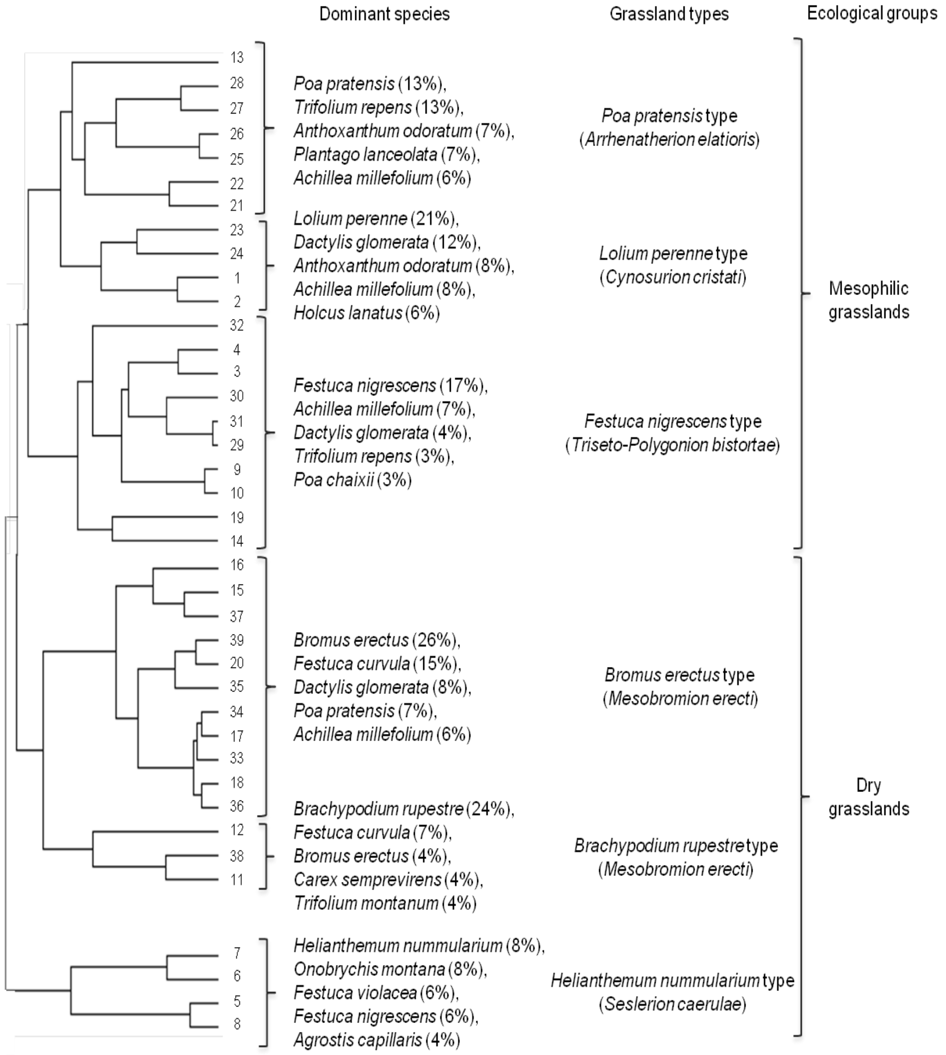

Figure 2.1. Dendrogram with the classification of the vegetation surveys obtained by cluster analysis, with the identification of ecological groups, grassland types (with the corresponding phytosociological alliances in brackets) and their dominant species. Numbers indicate sample codes (see Annex 2.A).

elevations.[43] Similarly, within the dry grassland ecological group, *B. erectus* and *B. rupestre* types were representative of lower elevations, with the second type more related to extremely extensive management and abandonment stages,[44] while *H. nummularium* type was located at the highest elevations. However, the presence of common species within grassland types (e.g., *D. glomerata*, *P. pratensis*, *F. nigrescens* and *A.*

millefolium) revealed the presence of transitional stages, a common condition in grazed grasslands.[45]

Proximate composition and fatty acid profile of grass samples

Due to differences in the botanical composition (Fig. 2.1) and plant phenology (Annex 2.A), the TFA content in the analysed samples was highly variable, ranging from 9.04 to 30.06 g kg^{-1} DM, with a range typically reported for herbage.[46] Seventeen FA were detected in all samples: C12:0, C14:0, C15:0, C16:0, C16:1 *trans*3, C16:1 *cis*9, C18:0, C18:1 *cis*9 (n-9), C18:1 *cis*11, C18:2 *cis*9*cis*12 (n-6), C18:3 *cis*6*cis*9*cis*12 (n-6), C18:3 *cis*9*cis*12*cis*15 (n-3), C20:0, C20:1 *cis*11, C22:0, C20:4 *cis*5*cis*8*cis*11*cis*14 (n-6), C24:0. Among them, five FA [palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 n-9), linoleic acid (C18:2 n-6), and alpha–linolenic acid (C18:3 n-3)] comprised 90 to 95% of TFA and were then considered for further statistical analyses; such percentages were consistent with those observed in other trials.[5,14,22]

The concentrations of C16:0, C18:2 n-6 and C18:3 n-3 varied linearly with changes in the TFA content (Fig. 2.2); the same was not observed for C18:0 and C18:1 n-9. The change in C18:3 n-3 concentration per unit change in TFA content was higher if compared to those observed for C16:0 and C18:2 n-6, as previously observed in grass silages from the Netherlands by Khan et al.[47]

Relationships among botanical, chemical and plant community variables

A significant correlation was detected between the botanical and chemical matrices by Mantel test ($r = 0.28$, $P < 0.01$), highlighting that grasslands with similar botanical composition had similar contents of chemical compounds.

The CCA ordination allowed the visualisation of the relationships among the botanical, chemical and plant community variables considered in this study (Fig. 2.3). Significant correlations among plant species and chemical variables were observed, explaining 79.9% of the distribution fitting with the first axis and 10.7% with the second axis. The grassland types largely overlapped in terms of botanical and chemical composition (Fig. 2.3a), confirming the presence of transitional stages underlined by the hierarchical cluster analysis. Overlapping was also observed between

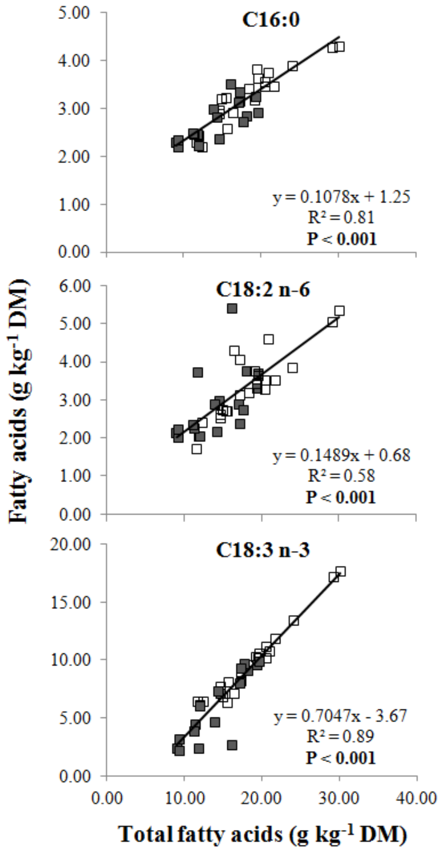

Figure 2.2. Changes in the concentrations of palmitic (C16:0), linoleic (C18:2 n-6) and alpha-linolenic (C18:3 n-3) acids in relation to changes in the total fatty acid content of grass samples. Grey squares represent dry grassland samples, while white squares indicate mesophilic grassland samples.

the two grassland ecological groups; however, differently from what observed for grassland types, mesophilic and dry grasslands separated quite well along a line connecting their geometric centres (Fig. 2.3a). According to Landolt's indicator values, the ecological conditions were significantly different between mesophilic and dry grasslands (Fig. 2.3a; Table 2.1), ranging from mesophilic, weakly acid, and moderately nutrient-rich to moderately dry, weakly neutral, and medium infertile conditions, respectively. Compared to dry grasslands, mesophilic grasslands were located at lower elevations ($P < 0.001$), earlier exploited during the year ($P < 0.05$) and characterised by higher pastoral values (+43%, $P < 0.01$), due to a higher proportion of productive and highly palatable species. All the other plant community variables did not differ between the two ecological groups (Table 2.1). The average phenological stage appeared slightly higher in dry grasslands due to the precocity of their characteristic species,[48] but no significant differences between the two main ecological groups were detected. In particular, mean values

Table 2.1. Botanical families (% of relative abundance) and plant community variables for the two main ecological groups (mesophilic and dry grasslands)

	Mesophilic grasslands Mean ± SE[a]	Dry grasslands Mean ± SE	Independent sample *t*-test *t*	*P*
Botanical families				
Poaceae	50.0 ± 2.26	53.1 ± 3.89	*-0.717*	*NS*[b]
Asteraceae	10.8 ± 1.43	11.6 ± 1.32	*-0.402*	*NS*
Fabaceae	10.8 ± 1.11	10.5 ± 1.44	*0.122*	*NS*
Cyperaceae	2.9 ± 1.21	3.9 ± 1.42	*-0.537*	*NS*
Apiaceae	3.3 ± 0.97	1.9 ± 0.97	*1.186*	*NS*
Plantaginaceae	3.4 ± 1.08	1.7 ± 0.62	*1.334*	*NS*
Caryophyllaceae	3.3 ± 0.96	1.0 ± 0.28	*2.159*	*0.037*
Ranunculaceae	3.0 ± 0.86	0.7 ± 0.23	*2.357*	*0.024*
Other forbs	28.4 ± 2.59	24.7 ± 3.50	*0.860*	*NS*
Plant community variables				
Landolt's soil moisture	2.6 ± 0.08	2.2 ± 0.05	*4.211*	*<0.001*
Landolt's nutrient supply	3.3 ± 0.08	2.6 ± 0.07	*6.471*	*<0.001*
Landolt's soil reaction	3.0 ± 0.02	3.5 ± 0.05	*-8.251*	*<0.001*
Pastoral value	40.0 ± 2.67	27.9 ± 2.26	*3.397*	*0.002*
Elevation	1040.2 ± 136.42	1708.7 ± 57.17	*- 4.519*	*<0.001*
Day of year (DOY)	198 ± 16.0	244 ± 10.6	*- 2.394*	*0.022*
Phenology	259 ± 32.8	373 ± 61.8	*-1.637*	*NS*
Species richness	26 ± 2.1	26 ± 2.4	*-0.159*	*NS*
Shannon diversity index	3.8 ± 0.11	3.7 ± 0.17	*0.543*	*NS*

[a]Standard Error; [b]not significant ($P > 0.05$).

ranged from 30-40% of inflorescences visible (within the mesophilic grasslands) to pre-flowering stage (dry grasslands), which can be considered a negligible difference in terms of forage chemical composition.[22] Species richness, Shannon diversity index and the relative abundance of the most abundant botanical families did not differ between mesophilic and dry grasslands, because both ecological groups were highly biodiverse, a common situation in alpine managed grasslands.[49] The only two families with significant differences ($P < 0.05$) were *Ranunculaceae* and *Caryophyllaceae*, but with negligible average relative abundances.Some dry grassland species, e.g. *B. rupestre*, *F.*

curvula, *Cruciata glabra* (L.) Ehrend., *Onobrychis viciifolia* Scop., and above all *B. erectus*, were set on the right side of the line connecting the geometric centres of both ecological groups and were associated with a high content of DM, NDF and ADF. By the opposite, mesophilic grassland species, e.g. *Silene vulgaris* (Moench) Garcke, *Plantago lanceolata* L., *Trifolium repens* L., *Anthoxantum odoratum* L., *Festuca pratensis* Huds., *Lolium perenne*, *Trifolium pratense* L., *Holcus lanatus* L., *Agrostis capillaris* L. were set on the left side of the line connecting the geometric centres of ecological groups and were mainly associated with higher C18:3 n-3 contents.

Total fatty acids and CP share a common location within the photosynthetic organs of the plants.[47,50] Particularly, FA in forages are mainly located in leaf chloroplasts and, for this reason, the TFA concentration of forages is also usually negatively correlated with the concentrations of plant fibre contents[14,22], as also highlighted both in the CCA (Fig. 2.3b) and by Pearson's correlation analysis (TFA and NDF: $r = -0.82$, $P < 0.001$; TFA and ADF: $r = -0.70$, $P < 0.001$).

The univariate analysis provided quantitative information about the differences between the two ecological groups in terms of their chemical composition (Table 2.2). Mesophilic grassland species determined an average higher CP content (+33%, $P < 0.001$) and lower DM, NDF and ADF (-41%, -13% and -19%, respectively; $P \leq 0.001$) than dry grasslands species, which is in accordance with previous literature.[16,51–53] These proximate compositions confirmed also the observed significantly higher pastoral value of mesophilic than dry grasslands (Table 2.1). Mesophilic grasslands also showed significantly higher concentrations of C16:0 (+22%, $P = 0.001$), C18:2 n-6 (+21%, $P < 0.05$), C18:3 n-3 (+64%, $P < 0.001$) and TFA (+33%, $P < 0.01$) and significantly lower concentration of C18:1 n-9 (-38%, $P < 0.01$) if compared to dry grasslands. The concentration of C18:0 did not significantly differ between ecological groups. As expected C18:3 n-3 was by far the most abundant detected FA in both alpine ecological groups.[14] The TFA content was located nearby the geometric centre of the mesophilic ecological group; the positive relationship between TFA, C18:3 n-3 and mesophilous species confirmed the results obtained in previous trials.[50] It is noteworthy that a significant Pearson's correlation was found between the pastoral value and the plant concentration of C18:3 n-3 ($r = 0.36$, $P < 0.05$), the latter being considered as one of the most important FA strongly influencing the quality of grazing animal products.[54,55] Since the pastoral value is based on a not-analytic factor (the Index of Specific Quality of plant species),[21] this finding may give an additional confirmation of the reliability of this vegetation index for the evaluation of the quality of grassland forages, and merits further investigation.

The proximate composition and FA profile appeared mainly single species dependent rather than botanical family dependent. In contrast, Reynaud et al.[24] and Peiretti et al.[23] found that botanical families were statistically linked to total phenolic content and FA profile, respectively. However, the latter often focused on botanical families with very low relative abundances (e.g., *Cyperaceae*, *Ranunculaceae*, *Geraniaceae*, *Roseceae*, and *Valerianaceae*). In our work, the same botanical families comprised different exclusive plant species between the two ecological groups, e.g. *T. repens* versus *O. viciifolia* for the *Fabaceae* family, in the mesophilic and dry grasslands, respectively. Therefore, the species assemblage appeared to be more related to forage proximate composition and FA profile than to botanical families.

Table 2.2. Proximate composition (g kg^{-1} DM, unless otherwise stated) and fatty acid profile (g kg^{-1} DM) of the two main ecological groups (mesophilic and dry grasslands).

	Mesophilic grasslands	Dry grasslands	Independent sample *t*-test	
	Mean ± SE[a]	Mean ± SE	*t*	*P*
Proximate composition				
DM[b] (g kg^{-1})	223 ± 14.2	381 ± 17.5	*-7.088*	*<0.001*
CP[c]	136 ± 5.7	102 ± 4.5	*4.460*	*<0.001*
NDF[d]	489 ± 12.9	563 ± 16.9	*-3.553*	*0.001*
ADF[e]	295 ± 8.2	366 ± 10.2	*-5.547*	*<0.001*
Fatty acid profile				
C16:0	3.32 ± 0.122	2.72 ± 0.097	*3.747*	*0.001*
C18:0	0.38 ± 0.028	0.40 ± 0.030	*-0.431*	*NS*[f]
C18:1 n-9	0.71 ± 0.065	1.15 ± 0.134	*-2.963*	*0.007*
C18:2 n-6	3.44 ± 0.198	2.84 ± 0.209	*2.074*	*0.045*
C18:3 n-3	9.82 ± 0.700	6.00 ± 0.673	*3.903*	*<0.001*
TFA[g]	18.77 ± 1.040	14.14 ± 0.842	*3.396*	*0.002*

[a]Standard Error; [b]Dry Matter; [c]Crude Protein; [d]Neutral Detergent Fibre; [e]Acid Detergent Fibre; [f]not significant ($P > 0.05$); [g]Total Fatty Acids.

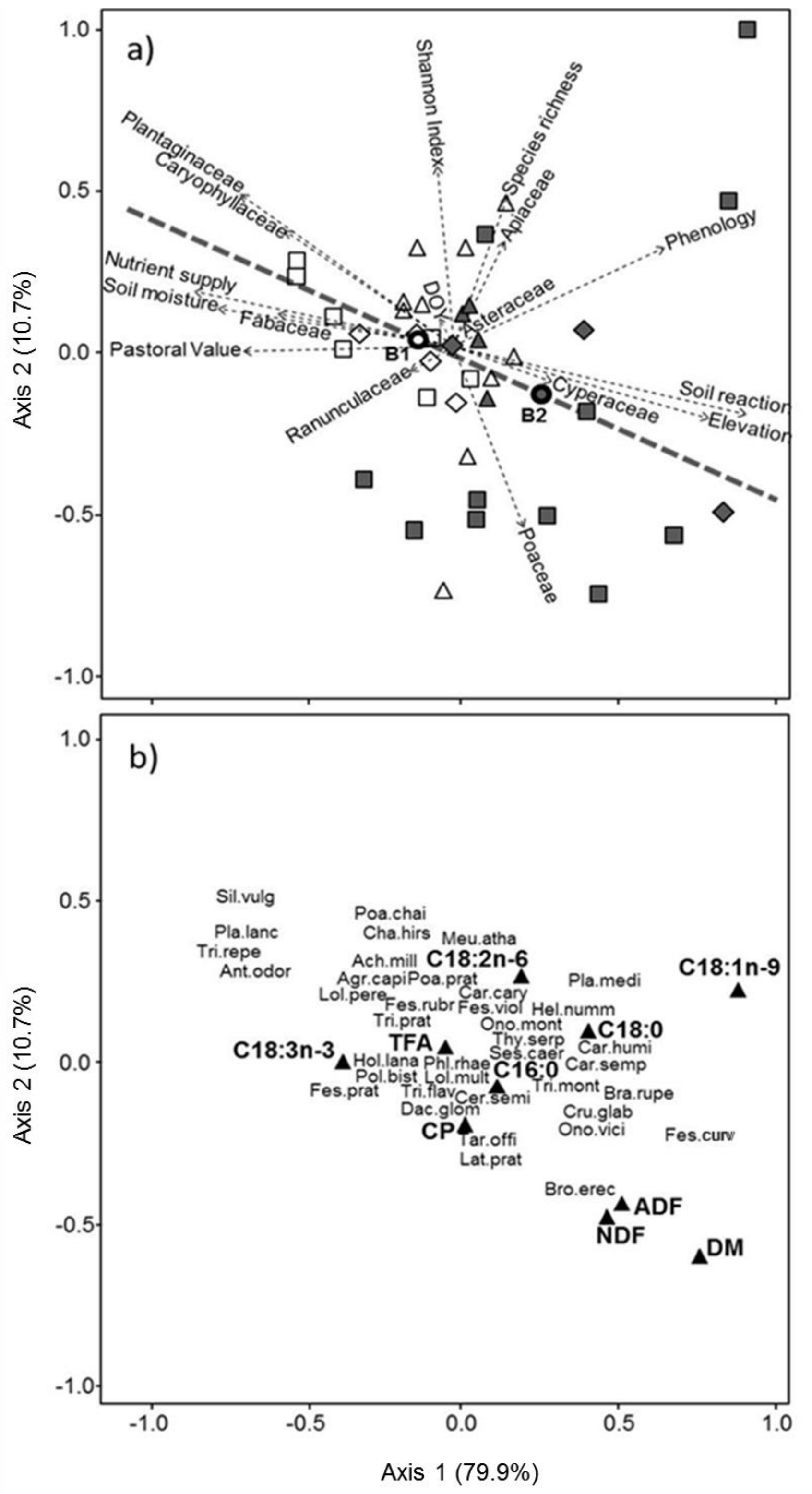
a)
b)
Axis 2 (10.7%)
Axis 1 (79.9%)
Shannon Index
Species richness
Plantaginaceae
Caryophyllaceae
Apiaceae
Phenology
Nutrient supply
Soil moisture
Fabaceae
DOY
Asteraceae
Pastoral Value
B1
B2
Ranunculaceae
Cyperaceae
Soil reaction
Elevation
Poaceae
Sil.vulg
Pla.lanc
Tri.repe
Ant.odor
Poa.chai
Cha.hirs
Meu.atha
Ach.mill
C18:2n-6
C18:1n-9
Agr.capi
Poa.prat
Pla.medi
Lol.pere
Car.cary
Fes.rubr
Fes.viol
Hel.numm
Tri.prat
Ono.mont
C18:0
TFA
Thy.serp
C18:3n-3
Hol.lana
Car.humi
Phl.rhae
Ses.caer
Car.semp
Pol.bist
Lol.mult
C16:0
Fes.prat
Tri.flav
Tri.mont
Bra.rupe
Cer.semi
Dac.glom
Cru.glab
CP
Ono.vici
Fes.curv
Tar.offi
Lat.prat
Bro.erec
ADF
NDF
DM

Figure 2.3. a) CCA ordination bi-plot showing the distribution of the 39 vegetation surveys and the corresponding grass samples, and their relationships with plant community variables (dotted arrows). The length of the arrows is proportional to their importance and the directions of the arrows show their correlation with the axes. The dashed line connects the geometric centres of both ecological groups, identified by circles (i.e. B1, white circle representing mesophilic grasslands and B2, grey circle representing dry grasslands). Mesophilic grassland types: □ *Poa pratensis*; ◇*Lolium perenne*; △ *Festuca nigrescens*; dry grassland types: ■ *Bromus erectus*; ◆ *Brachypodium rupestre*; ▲ *Helianthemum nummularium*. b) CCA ordination bi-plot showing the relationships between chemical data (identified by triangles) and the most abundant grassland species.

Chemical matrix variables: DM = Dry Matter; CP = Crude Protein; NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre; C16:0 = palmitic acid; C18:0 = stearic acid; C18:1 n-9 = oleic acid; C18:2 n-6 = linoleic acid; C18:3 n-3 = alpha-linolenic acid; TFA = total fatty acids.

Botanical matrix species: Ach.mill = *Achillea millefolium*; Agr.capi = *Agrostis capillaris*; Ant.odor = *Anthoxanthum odoratum*; Bra.rupe = *Brachypodium rupestre*; Bro.erec = *Bromus erectus*; Car.cary = *Carex caryophyllea*; Car.humi = *Carex humilis*; Car.semp = *Carex sempervirens*; Cer.semi = *Cerastium semidecandrum*; Cha.hirs = *Chaerophyllum hirsutum*; Cru.glab = *Cruciata glabra*; Dac.glom = *Dactylis glomerata*; Fes.curv = *Festuca curvula*; Fes.prat = *Festuca pratensis*; Fes.nigr = *Festuca nigrescens*; Fes.viol = *Festuca violacea*; Hel.numm = *Helianthemum nummularium*; Hol.lana = *Holcus lanatus*; Lat.prat = *Lathyrus pratensis*; Lol.mult = *Lolium multiflorum*; Lol.pere = *Lolium perenne*; Meu.atha = *Meum athamanticum*; Ono.mont = *Onobrychis montana*; Ono.vici = *Onobrychis viciifolia*; Phl.rhae = *Phleum rhaeticum*; Pla.lanc = *Plantago lanceolata*; Pla.medi = *Plantago media*; Poa.chai = *Poa chaixii*; Poa.prat = *Poa pratensis*; Pol.bist = *Polygonum bistorta*; Ses.caer = *Sesleria caerulea*; Sil.vulg = *Silene vulgaris*; Tar.offi = *Taraxacum officinale*; Thy.serp = *Thymus serpyllum*; Tri.flav = *Trisetum flavescens*; Tri.mont = *Trifolium montanum*; Tri.prat = *Trifolium pratense*; Tri.repe = *Trifolium repens*.

Conclusions

The proximate composition and fatty acid profile of grasslands in the Western Italian Alps were significantly influenced by the botanical composition of the vegetation. Analysing a wide and representative variety of grassland types, our data showed that the abundance of single plant species affected the chemical composition of forages more than the abundance of botanical families. Significant differences in the chemical composition were observed between two ecological groups comprising six different grassland types: the mesophilic grasslands, characterised by a higher soil moisture content and more intensive pastoral management and the dry grasslands, characterised by a lower soil moisture content and more extensive management systems. The main lipid precursors (C18:2 n-6 and above all C18:3 n-3) for the synthesis of fatty acids considered beneficial to human health (e.g., vaccenic, rumenic and omega-3 fatty acids) were significantly higher in the grasslands belonging to the mesophilic ecological group, which was also characterised by a higher relative abundance of productive and palatable plant species compared to the grasslands belonging to the dry ecological group. Mesophilic grasslands showed higher values of crude protein, lower values of fibre and they were related to higher pastoral values than dry grasslands. These results suggest that high quality forage resources can provide higher nutritive value and higher concentration of precursors for the production of dairy and meat products rich in nutraceutical compounds.

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Annex 2.A

Details about the 39 vegetation surveys conducted: sample code, ecological group, grassland type, elevation, sampling date, Day of Year (DOY), average Lambertin's phenology (weighted on species relative abundances), exploitation (first, second or third seasonal growth), latitude and longitude (coordinates UTM WGS84).

Sample code	Ecological Group	Grassland type	Elevation (m a.s.l.)	Sampling date	DOY	Lambertin's Phenology	Exploitation	Latitude	Longitude
1	Mesophilic grasslands	Lolium perenne	555	24 October 2013	297	214,58	3rd	4.976.373,47	361.586,08
2	Mesophilic grasslands	Lolium perenne	570	10 April 2014	100	214,29	1st	4.976.438,96	361.645,76
3	Mesophilic grasslands	Festuca nigrescens	563	10 April 2014	100	418,40	1st	4.976.383,77	361.668,83
4	Mesophilic grasslands	Festuca nigrescens	1.955	15 July 2014	196	115,87	1st	4.989.738,63	352.250,74
5	Dry grasslands	Helianthemum nummularium	2.091	5 September 2013	248	678,00	1st	4.990.388,98	352.266,61
6	Dry grasslands	Helianthemum nummularium	2.137	14 August 2014	226	523,44	1st	4.990.618,79	352.044,70
7	Dry grasslands	Helianthemum nummularium	2.165	14 August 2014	226	515,13	1st	4.990.638,15	352.117,11
8	Dry grasslands	Helianthemum nummularium	2.075	5 September 2013	248	597,56	1st	4.990.269,47	352.292,76
9	Mesophilic grasslands	Festuca nigrescens	552	24 October 2013	297	114,58	3rd	4.976.335,07	361.579,56
10	Mesophilic grasslands	Festuca nigrescens	1.969	15 July 2014	196	421,50	1st	4.989.773,51	352.141,53
11	Dry grasslands	Brachypodium rupestre	1.828	12 September 2013	255	746,48	2nd	4.980.650,40	328.325,69
12	Dry grasslands	Brachypodium rupestre	1.748	14 August 2014	226	433,18	1st	4.981.177,56	328.162,01
13	Mesophilic grasslands	Poa pratensis	1.794	14 August 2014	226	462,75	1st	4.981.229,83	328.279,69
14	Mesophilic grasslands	Festuca nigrescens	1.818	12 September 2013	255	696,50	2nd	4.980.674,70	328.307,10
15	Dry grasslands	Bromus erectus	1.568	18 October 2013	291	110,80	2nd	4.980.818,97	327.117,22
16	Dry grasslands	Bromus erectus	1.568	18 October 2013	291	115,17	2nd	4.980.860,98	327.126,16
17	Dry grasslands	Bromus erectus	1.512	21 May 2014	141	219,96	1st	4.980.642,75	326.574,47
18	Dry grasslands	Bromus erectus	1.509	21 May 2014	141	203,83	1st	4.980.723,96	326.586,87
19	Mesophilic grasslands	Festuca nigrescens	1.670	14 July 2014	195	424,59	1st	4.980.128,37	327.800,77
20	Dry grasslands	Bromus erectus	1.565	14 July 2014	195	446,60	1st	4.980.864,83	326.875,29
21	Mesophilic grasslands	Poa pratensis	836	14 October 2013	287	109,75	3rd	5.072.060,10	424.550,95
22	Mesophilic grasslands	Poa pratensis	841	14 October 2013	287	112,87	3rd	5.072.146,47	424.549,76
23	Mesophilic grasslands	Lolium perenne	841	28 May 2014	148	237,06	1st	5.072.662,33	424.578,03
24	Mesophilic grasslands	Lolium perenne	853	28 May 2014	148	221,70	1st	5.072.070,25	424.970,32
25	Mesophilic grasslands	Poa pratensis	240	21 October 2013	294	109,09	3rd	5.049.089,24	453.240,43
26	Mesophilic grasslands	Poa pratensis	241	21 October 2013	294	110,66	3rd	5.049.099,03	453.238,79
27	Mesophilic grasslands	Poa pratensis	240	11 April 2014	101	212,40	1st	5.048.974,78	453.263,77
28	Mesophilic grasslands	Poa pratensis	240	11 April 2014	101	243,93	1st	5.049.003,11	453.259,80
29	Mesophilic grasslands	Festuca nigrescens	1.514	28 May 2014	148	221,15	1st	5.072.749,93	415.379,40
30	Mesophilic grasslands	Festuca nigrescens	1.489	28 May 2014	148	250,57	1st	5.072.806,55	415.462,81
31	Mesophilic grasslands	Festuca nigrescens	1.514	19 June 2014	170	260,26	1st	5.072.759,93	415.375,40
32	Mesophilic grasslands	Festuca nigrescens	1.549	19 June 2014	170	262,88	1st	5.072.934,22	415.348,92
33	Dry grasslands	Bromus erectus	1.480	29 September 2014	272	116,79	3rd	4.980.684,60	326.465,96
34	Dry grasslands	Bromus erectus	1.510	29 September 2014	272	117,12	3rd	4.980.867,85	326.560,73
35	Dry grasslands	Bromus erectus	1.525	29 September 2014	272	114,29	3rd	4.980.364,94	326.736,50
36	Dry grasslands	Bromus erectus	1.563	29 September 2014	272	777,71	3rd	4.980.375,17	327.085,96
37	Dry grasslands	Bromus erectus	1.560	29 September 2014	272	114,36	3rd	4.980.318,56	326.994,70
38	Dry grasslands	Brachypodium rupestre	1.640	29 September 2014	272	122,38	2nd	4.981.218,20	327.514,41
39	Dry grasslands	Bromus erectus	1.712	29 September 2014	272	768,33	2nd	4.981.274,74	327.886,68

Annex 2.B

Lambertin’s phenological scale used to record the phenological stages of vegetation during the botanical surveys (Lambertin, 1990, traduced).

Poaceae and *Cyperaceae*		Other species	
Stage	**Value**	**Stage**	**Value**
	-	Snow melting	001
	-	Bud swelling	010
	-	First leaves growth	025
	-	Some plants completely developed	050
Snow melting	075	50% of plants completely developed	075
Bud swelling	100	All plants completely developed	100
Beginning of sprouting	125	Drafts of flower buds	125
Flag leaf sheaths swollen	150	Some flower buds visible	150
Inflorescences not yet emerged (flag leaf sheaths opening)	175	50% of flower buds visible, no one opened	175
70% of inflorescences in flag leaves	200	70% of flower buds visible	200
Some inflorescences emerged	225	Sepals stretch	225
30-40% of inflorescences emerged	250	30-40% of flower buds opened	250
50% of inflorescences emerged	275	Androecium and gynaecium non yet visible	275
70% of inflorescences emerged. Spikelets still close to inflorescence axis	300	70% of flower buds opened. Androecium and gynaecium barely visible	300
Spikelets start to distance from inflorescence axis	325	Petals not yet stretched but androecium and gynaecium well visible	325
Spikelets show a clear angle with inflorescence axis	350	Some flower buds remain. Petals stretch	350
Styles appearance, not yet unfolded by filament tips	375	50% of plant flowering. Corollas reach their maximum lengthening	375
Full flowering	400	Full flowering	400
Styles have lost their colours	425	Styles start to change colour	425
Styles falling (filaments remain)	450	Some flowers withered	450
Some seeds appear	475	50% of flowers withered	475
Milky ripe	500	All flowers withered	500
No more plants in flower	525	No opened flowers are yet visible	525
30-40% of seeds in dough stage	550	Some fruits barely developed	550
50% of seeds in dough stage	575	50% of fruits completely developed	575
All seeds in dough stage	600	Start of fruiting: all fruits completely developed	600
Inflorescences lose their colours	625	Fruit swelling and colouration	625
30-40% hard seeds	650	Fruit colouration	650
50% hard seeds	675	50% of fruits fully-ripe	675
All hard seeds	700	All fruits are fully-ripe	700
All seeds have the same colour	725	All fruits have the same colour	725
Beginning of dissemination	750	Fruit opening	750
No seeds in spikelets yet	775	50% of fruits are empty	775
End of vegetation	800	End of vegetation	800
NB: it's frequent to observe spikelets starting dissemination prematurely (525-775)		NB: some stages could be very brief	

3. Fodder tree species: foliage characterisation

Extended paper title: *Seasonal variation in leaf traits, chemical composition, and in vitro true digestibility of four temperate fodder tree species*

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Abstract

The abstract of this paper is reported in Chapter 1.4, page 5.

Keywords: Allometric equations; Browses; Fatty acids; Goat; Phenolic compounds; Tannins

Introduction

Tree and shrub foliage is an important component of small ruminant diet in many parts of the world and plays an essential role for browsing animals, especially where livestock systems are based on rangeland and grazable forestland exploitation for a remarkable part of the year.[1] The importance of fodder tree species is particularly relevant during dry periods, when herbage quality decreases as a consequence of reduced water availability and/or the advancement of plant phenological stage, while in the meantime tree foliage maintains a higher nutrient quality.[2] For this reason, an improved evaluation of fodder tree species is of outmost importance for a sustainable ruminant production in marginal areas, and also considering future climate changes, with increasing drought periods over large European areas.[3]

Generally, when evaluating a forage resource, the main features to take into account are its biomass production and chemical composition. A simple method to estimate foliage production of a tree or shrub species could be represented by allometric equations, which relate leaf biomass to an easy recordable in-field leaf trait, such as leaf length.[4]

Concerning its chemical composition, tree and shrub foliage can be considered a total feed in ruminant nutrition, as they may represent: i) a source of protein that escapes rumen degradation to be digested in the intestines, enhancing the protein status of the animal, ii) a source of vitamins and minerals, able to cover herbage deficiencies, iii) an improvement of microbial growth and digestion of cellulosic biomass in the rumen, iv) a source of plant secondary metabolites, which can alter the balance of microorganisms in the rumen, and v) a source of fatty acids (FA), and therefore energy, for the animal.[1] Due to variations in FA and plant secondary metabolite contents, fresh foliage from different fodder tree species can also confer specific intrinsic sensory and chemical attributes to ruminant-derived dairy and meat products.[1] However, a great number of fodder tree species contain also particular plant secondary metabolites characterized by a potential anti-nutritional effect, such as phenolic compounds.[5] Among them, tannins can cause either adverse or beneficial effects on nutrient utilization, health, and animal production, in relation to their molecular characteristics and concentration.[6] As a consequence of their low protein levels and high contents of cell wall constituents and plant secondary metabolites (especially tannins), fodder tree leaves are generally characterized by low ruminal digestibility.[7]

The knowledge of the importance of tree foliage for ruminant nutrition due to the above mentioned features has led to several studies aimed at evaluating leaf forage productivity, chemical composition, and digestibility, since they can be considered as useful indicators for the evaluation of feeding resources.[8] These parameters have been largely assessed for various fodder tree species in several environments such as savannah,[9] tropics,[10] sub-tropics,[11] American temperate region,[12] Mediterranean region,[13] and non-European alpine areas,[14] where foliage is a major feeding resource for the breeding of local grazing ruminants.

In each region, the investigated fodder tree species have been chosen in relation to local vegetation abundance and browsing animal species. Seasonal variations have also been analyzed, since tree foliage productivity, chemical composition, and digestibility can significantly change during the vegetative season, due to phenological advancement and climatic variations.[2,13,15]

In the last decades extensive goat rearing has spread in European alpine areas, and particularly in marginal areas, for rangeland and grazable forestland management,[16] playing a key role in cost-effective production of high-quality animal-derived food products.[17] Here, due to their abundance, availability, browsing and drought resistance, and nutritive quality, extensive shrublands and small trees can provide leaf fodder, which represents the basic component of goat diet. Goat diet composition changes seasonally, according to plant species availability, phenological stage, and nutritive value, with a general increase of fodder tree leaf consumption when the availability and nutritive value of herbage reduce.[18] Moreover, goats are well adapted to exploit low-quality forages due to their ruminal bacterial population, better suited for degradation of highly lignified material and resistance to tannin toxicity, resulting in an increased tree foliage digestibility.[19]

To date, different research has been conducted on European temperate tree species,[20–26], which have been a widespread feeding resource since

Neolithic,[27–30] especially for goat nutrition.[1,18] However, the leaf biomass production of fodder tree species in these environments is poorly documented and only a limited number of authors experienced the assessment of foliage production using allometric equations based on leaf traits.[31] In addition, there is a lack of knowledge on the FA profile of such tree foliage, even if this feature is of increasing interest for a complete screening of forage quality.[32]

The present study aimed to fill the gap of knowledge about the above mentioned topic, by selecting four fodder tree species which are widely common for goat nutrition in different European mountain areas, either directly by browsing or fed after cutting. The objective was to characterize tree leaves along the vegetative season in terms of leaf traits, proximate composition, fatty acid profile, phenolic compound content, and digestibility.

Materials and Methods

Study area

The study was conducted at Oasi Zegna, located in the Piedmont Region, north-western Italy (latitude 45°40' N, longitude 8°09' E), within the boundaries of Valle Sessera Site of Community Interest (SCI IT11300002). The study area is characterized by a sub-oceanic climate (Köppen's classification: Cfb), with annual mean temperature of 7.3°C and precipitation of 1,700 mm (mean value for the years 2002–2015 of the pluviometric station of Bielmonte). Dominant soils were originated from siliceous parent rock. Tree stands were mixed broadleaved populations, dominated by *Fagus sylvatica* L. (European beech) and *Betula pendula* Roth (silver birch), currently seldom managed by selective cutting.

Data collection for leaf traits

Four tree species were selected among the browses most preferred by goats [18,27,28,30,33–35] and largely widespread on European uplands:[36] *Acer pseudoplatanus* L. (sycamore maple), *Fraxinus excelsior* L. (ash), *Sorbus aucuparia* L. (rowan), and *Salix caprea* L. (goat willow) (hereafter the species are referred to by their genus name).

A group of four trees was selected for each species in December 2014, during the vegetative dormancy, in a 500 × 150 m^2 area with homogeneous exposure (N-NW) and elevation (1,270 to 1,320 m a.s.l.). Within each species group, the trees were located at a maximum distance of 125 m each other and had similar height, stem diameter, age, and no disease evidence. Moreover, similarity of phenological stage within each species group was verified at each leaf sampling date. Four HOBO data loggers (Onset Corp., Pocasset, MA), placed near the centroid of each species group, recorded air temperature every 30 minutes for the whole trial. Four sprouts at bud stage per each tree were selected at a maximum height of 1.80 m (i.e. as far as goats can browse buds and leaves, assuming a bipedal stance).[35] Each sprout was located in a different cardinal direction to avoid differences due to light exposure. The elongation of emerging leaves was monitored on each sprout, from the budburst of the earliest sprout until all trees reached the maximum vegetative phenological stage, according to the extended BBCH scale.[37] At each survey date, following the survey schedule provided in Annex 3.A, the phenological stage (vegetative and, when present, reproductive) of each individual was recorded and one sprout, comparable to the monitored one in terms of leaf number and development, was harvested and transported to the laboratory. The emitted leaves were then separated from the sprout and weighed. Leaf traits (i.e. leaf length and biomass) were computed by cumulating the values of all unfolded leaves per each sprout in each survey date.

Leaf sampling and laboratory analyses

As soon as all the selected sprouts ended leaf emission (i.e. on June, the 4th), approximately corresponding to the start of goat browsing season in these grazable forestlands, one leaf sample per tree was collected for four dates from June to August, as detailed in Annex 3.A. About 400 g of fresh leaves (including petioles and rachises)[24,26] were harvested all around the canopy bottom-up to 1.80 height, to simulate goat browsing.[35] All leaves damaged by pathogens (insects, fungi) were avoided. The samples were placed in sealed polyethylene bags, immediately stored at 4°C in a portable refrigerator and transported to the laboratory, where each sample was divided into two homogeneous aliquots of about 200 g each. The samples were then frozen at -80°C until analyzed for their proximate, FA, and phenolic compositions, and *in vitro* true digestibility (IVTD). The samples were freeze dried (Freeze Drying Equipments, Criofarma, Torino, Italy) and then ground with a cutting mill to pass a 1-

mm screen sieve (Pulverisette 15 – Fritsch GmbH, Idar-Oberstein, Germany).

AOAC procedures were used to determine dry matter (DM, method no. 930.15), ash (method no. 942.05), crude protein (CP, method no. 984.13), acid detergent fiber, and acid detergent lignin (ADF and ADL, respectively; method no. 973.18).[38] Ether extract (EE) was determined following method no. 920.39 of AOAC.[39] Neutral detergent fiber (NDF) was analyzed according to Van Soest et al.;[40] α-amylase (Sigma Aldrich, Saint Louis, MO, USA), but no sodium sulphite, was added, and results were corrected for residual ash content. The proximate composition was expressed as g $100g^{-1}$ DM.

The FA composition was assessed using a combined direct transesterification and solid-phase extraction method as described by Alves et al.[32] Fatty acid methyl esters were separated, identified, and quantified as detailed by Renna et al.[41] The FA composition was expressed as mg $100g^{-1}$ DM.

Total extractable phenols (TEP) and phenol fractions (non-tannin phenols, NTP; condensed tannins, CT) were determined using standard protocols, as detailed in Iussig et al.[34] The absorbance was recorded at 725 nm (TEP and NTP, expressed as gallic acid equivalents) and 550 nm (CT, expressed as leucocyanidin equivalents) using a UV–vis spectrophotometer (Shimadzu UVmini-1240, Shimadzu Corporation, Kyoto, Japan). Total tannins (TT) were computed as the difference between TEP and NTP. Hydrolysable tannins (HT) were estimated as the difference between TT and CT. The phenolic composition was expressed as g kg^{-1} DM.

The IVTD was determined according to the ANKOM DAISY procedure.[42] Leaf samples (0.25 g) were weighed into filter bags (ANKOM® Corp. #F57; pore size 25 µm) and heat-sealed. For each incubation, fresh rumen fluid was collected from slaughtered adult male Alpine goats fed in alpine environments rich in fodder tree species. Rumen fluid was diluted into the buffer medium in proportion 1:4 (v/v), then 2 L of buffered rumen fluid were transferred in 5-L jars at 39 °C under anaerobic conditions. Each jar, containing leaf samples and one blank, was placed in a revolving incubator (ANKOM Daisy^{II} digestion system, ANKOM Technology Corp., Fairport, NY, USA) at 39 °C for 48 h under continuous rotation. After incubation, the samples were rinsed with cold water and subjected to an extraction with NDF solution at 100

°C for 1 h, in order to remove microbial debris and any remaining endogenous products.

Calculations and statistical analysis

Heat units from January 1st to the end of the trial, expressed as cumulative growing degree days (GDD), were calculated for each species group from daily air temperature, by cumulating all mean daily temperatures above 5 °C, according to previous studies performed on temperate species.[43] For each sprout, the GDD corresponding to the 25, 50, 75, and 100% of the final leaf length and biomass were assessed by data interpolation.

A one-way ANOVA was performed on the final leaf length and biomass values. The relationships between leaf length and biomass were explored with linear regressions, using a separate dataset for each species. To evaluate species precocity in leaf development, one-way ANOVA was used to analyze the differences among the GDD corresponding to the budburst and to the 25, 50, 75, and 100% of the final leaf trait values. For all these analyses, sprout was considered as statistical unit and tree species as fixed factor.

General linear models accounting for repeated measures were performed on proximate composition, FA profile [namely, total FA (TFA), groups of FA, and main represented individual FA], phenolic composition, and IVTD of each tree species. Tree was considered as statistical unit, species as fixed factor, and sampling date as repeated measure.

For each of the four sampling dates, a one-way ANOVA was performed on the same variables, using tree as statistical unit and tree species as fixed factor. Additionally, the temporal variations in terms of proximate, FA, phenolic compositions, and digestibility within the same species were tested performing a one-way ANOVA, with tree as statistical unit and sampling date as fixed factor.

Assumptions of normality and homogeneity of variance were checked with Shapiro-Wilk and Levene's tests, respectively. Variables which were not normally distributed were log-transformed prior to further statistical analysis. However, results are presented as non-transformed data. When normal distribution or homogeneity of variances were not met, even after log-transformation, the non-parametric Kruskal-Wallis test or Welch one-way ANOVA were used, respectively. When significant differences were found, Tukey's, Steel's, and Tamhane's *post* *hoc* tests were performed for ANOVA, Kruskal-Wallis, and Welch one-way ANOVA, respectively

All statistical analyses were performed using SPSS 24.[44] Significance was set at $P<0.05$.

Results and Discussion

Leaf traits

A total of 479 leaves was monitored during the trial. Mean number of emitted leaves per sprout was five for *Sorbus*, eight for *Acer* and *Salix*, and nine for *Fraxinus*.

The vegetative season started at 135 GDD (105th day of the year), with *Salix* unfolding of the first leaves (Annex 3.A). Budburst occurred first (at a lower GDD) for *Salix* and *Sorbus*, followed by *Fraxinus* and then by *Acer* (Table 3.1). Budburst values for *Fraxinus* and *Sorbus* were consistent with results obtained in Swiss environments,[45] while the same authors report a later budburst for *Acer*. *Salix* and *Sorbus* developed earlier in terms of leaf length and biomass for most of the considered stages, followed by *Fraxinus* and, then, by *Acer*.

For each of the four species, leaf length was significantly correlated with leaf biomass, with a remarkable amount of variance explained by the regressions leaf length *vs* biomass (regression parameters and equations are reported in Figure 3.1). The estimate of leaf biomass via allometric equations based on leaf length could thus represent a useful tool to evaluate tree foliage production, as it offers a non-destructive and time-affordable method.[4]

Differences among tree species in terms of final leaf traits were similar between leaf length and biomass, with *Fraxinus* showing the greatest values, followed by *Acer*, *Sorbus*, and *Salix* (Figure 3.2). Therefore, precocity of leaf development was inversely proportional to final values of both leaf traits, i.e. leaves developed earlier in the species with shorter and lighter leaves at the end of the season (*Salix* and *Sorbus*).

These considerations concerning leaf length and biomass variations along the vegetative season can be used as proficient tools for fodder tree management, since one of the basic criteria for selecting a particular tree species as feeding resource in a certain environment is productivity.[46] Indeed, in European alpine regions *Fraxinus* trees growing close to farm households were often used to forage ruminants.[29] Trees were managed with pollard practice and yearling sprouts were given to animals either as fresh (in summer) or conserved (in winter) forage.[30]

Table 3.1. Mean growing degree days (± standard error) for each tree species provided for budburst and cumulative leaf length and biomass per sprout at 25, 50, 75, and 100% of the final recorded value. *ns*, P≥0.05; different letters within a row indicate significant differences among tree species.

		Acer pseudoplatanus		***Fraxinus excelsior***		***Sorbus aucuparia***		***Salix caprea***	
Budburst	P<0.001	466.8 ± 4.55	**c**	266.5 ± 2.96	**b**	167.7 ± 2.81	**ab**	166.0 ± 2.14	**a**
Leaf length									
25%	P<0.001	481.0 ± 4.74	**c**	333.9 ± 2.50	**b**	203.2 ± 2.64	**a**	218.6 ± 2.09	**a**
50%	P<0.001	514.3 ± 4.96	**c**	411.5 ± 2.34	**b**	281.2 ± 4.17	**a**	301.2 ± 2.78	**a**
75%	P<0.001	589.5 ± 5.42	**c**	493.8 ± 3.13	**b**	401.6 ± 2.62	**a**	424.6 ± 4.09	**a**
100%	ns	1136.9 ± 18.95		1257.9 ± 17.70		1107.7 ± 14.90		1109.5 ± 14.50	
Leaf biomass									
25%	P<0.001	571.7 ± 6.81	**c**	444.1 ± 2.77	**b**	334.2 ± 3.94	**a**	385.2 ± 4.48	**ab**
50%	P<0.001	700.2 ± 7.31	**c**	648.8 ± 16.86	**bc**	492.9 ± 5.27	**a**	569.1 ± 7.11	**ab**
75%	ns	1003.6 ± 16.46		968.1 ± 23.08		912.0 ± 24.56		820.1 ± 18.68	
100%	P<0.001	1901.4 ± 13.49	**b**	1720.1 ± 23.52	**a**	1759.9 ± 17.23	**ab**	1725.6 ± 16.63	**a**

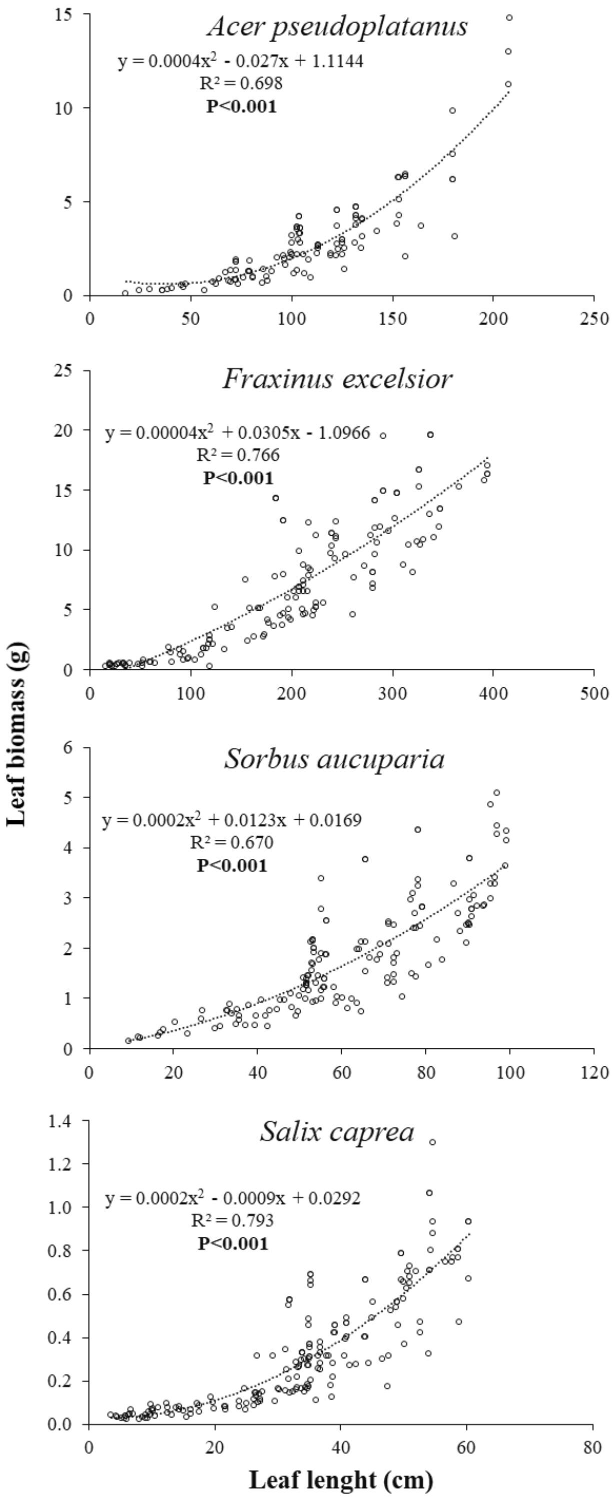

Figure 3.1. Changes in cumulative leaf biomass per sprout in relation to changes in cumulative leaf length per sprout.

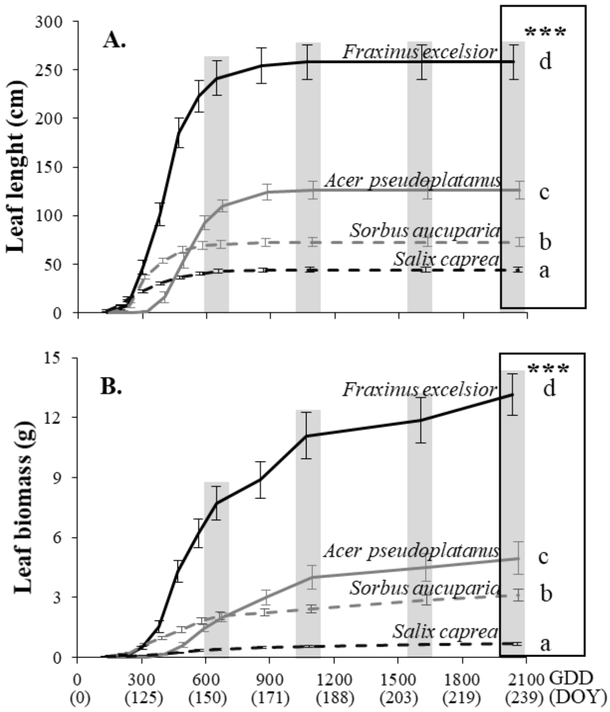

Figure 3.2. Increase of cumulative leaf length (A) and leaf biomass (B) per sprout in relation to growing degree days (GDD) and day of the year (DOY, expressed as a mean for the four tree species). Grey highlights indicate leaf sampling dates for laboratory analyses. ***, P<0.001; error bars represent the standard error of the means, while different letters indicate significant differences among tree species.

Proximate composition

The proximate composition was significantly different among the considered fodder tree species (Figure 3.3). The average DM content of *Sorbus* and *Salix* leaves was higher if compared to that of *Acer* and *Fraxinus* leaves. The ash content in *Sorbus* leaves was always lower than in the other species. In *Fraxinus* samples ash increased along time, displaying the highest values at the end of the season (Annex 3.B). The highest EE values were observed in *Acer*, followed by *Salix*, *Fraxinus*, and *Sorbus*. The results obtained for the above mentioned chemical parameters are consistent with those already reported by other authors.[12,13,20]

The CP contents of the four species were higher than the minimum level of 7-8 g $100g^{-1}$ DM required for optimum rumen function and feed intake in goats,[47,48], with *Acer* showing the highest values. The range of CP content in tree foliage was comparable to the one of herbage growing in similar alpine environments along the vegetative season.[25,49,50] Conversely, in dry to arid environments, other authors reported higher CP contents in foliage of deciduous fodder tree species than in local grass or hay.[51]

Salix was found as the most fibrous species, with highest contents of NDF, ADF, and ADL. *Fraxinus* showed the lowest contents of ADF and ADL. In all considered species NDF values were always lower than 60 g 100 g^{-1} DM, a threshold reported for ruminants to limit feed intake due to rumen fill.[52] Conversely, the average ADF content of the four species was always over 18 g 100 g^{-1} DM, so that, as reported by Santini et al.,[53] goats feeding only on the considered foliage may reduce their feed intake. More recent research showed that ADF concentrations of 18-20 g 100 g^{-1} DM or NDF concentrations of 41 g 100 g^{-1} DM are nutritionally adequate for high-producing lactating dairy goats.[54]

Other studies provided results sometimes different for proximate composition, even if leaf samples were collected from the same tree species, in similar environments, and at comparable dates. In particular, regarding *Fraxinus*, Emile et al. found lower DM and NDF and, together with Luske and van Eekeren, comparable CP contents, while Masson et al. reported lower DM and higher ash and CP contents.[20,25,26] Hejcman et al., instead, reported similar ADF but lower NDF, ADL, and ash contents for *Sorbus* leaves in Iceland.[23]

The statistical analyses performed on the temporal variation of chemical compounds highlighted a general variability of proximate composition related to season advancement with, in particular, an overall decrease in CP and an increase in fiber contents (Annex 3.B). It is common to observe similar trends for these parameters in most of fodder tree species, also in other than European environments,[2,12,13,51] and in fresh grass from semi-natural pastures.[55]

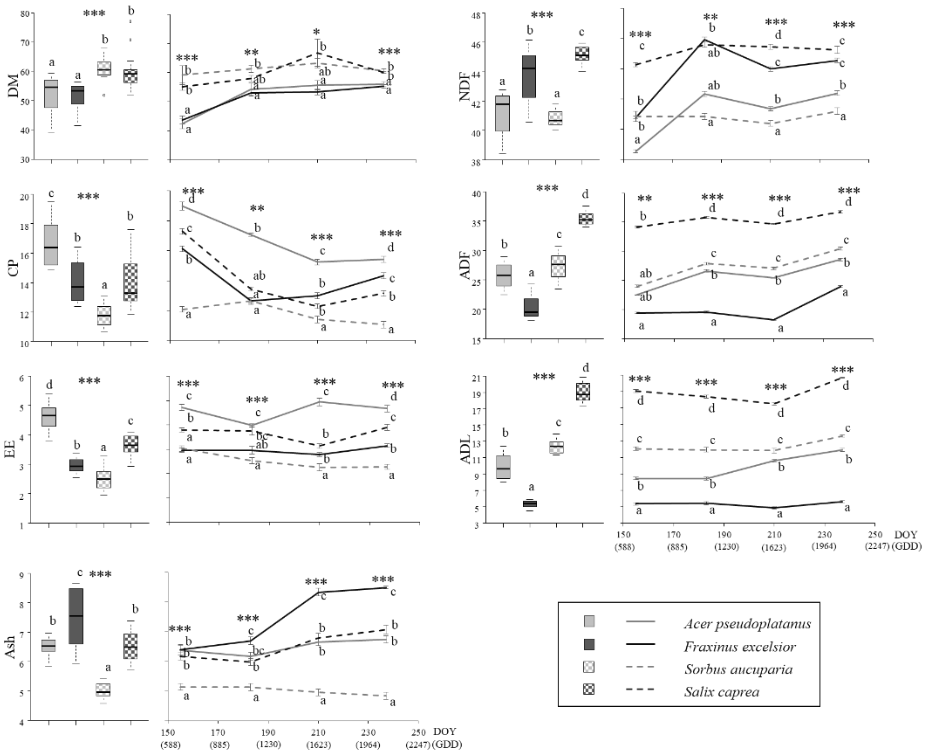

Figure 3.3. Proximate composition of the four tree species: overall ranges (boxplots) and seasonal variations (line charts) at the four survey dates. Provided data are expressed as g 100 g^{-1} dry matter (DM), except for DM which is expressed in g 100 g^{-1} fresh matter. CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin. DOY, day of the year; GDD, growing degree days, expressed as a mean for the four tree species. ***, P<0.001; **, P<0.01; *, P<0.05; error bars represent the standard error of the means while different letters, within each sampling date, indicate significant differences among tree species.

Fatty acid profile

Fifteen FA were detected in all samples: C12:0, C14:0, C15:0, C16:0, C16:1 *c*9, C18:0, C18:1 *c*9 (n9), C18:1 *c*11, C18:2 *c*9*c*12 (n6), C18:3 *c*6*c*9*c*12 (n6), C18:3 *c*9*c*12*c*15 (n3), C20:0, C20:1 *c*11, C22:0, C20:4 *c*5*c*8*c*11*c*14 (n6). Among them, six FA [palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 n9), linoleic acid (C18:2 n6), α–linolenic acid (C18:3 n3), and γ-linolenic acid (C18:3 n6)] comprised 92 to 97% of

TFA and were then considered for further statistical analyses, while the remaining FA were cumulated in the ‘Other FA’ group. In all tree species, C18:3 n3, C18:2 n6, and C16:0 were the main detected FA (Figure 3.4); this is consistent with findings for other fodder tree species and with grassland fodder values, also from environments other than the alpine ones.[15,49,50,56]

In *Sorbus* samples the lowest total monounsaturated fatty acids (MUFA - together with *Salix*), polyunsaturated fatty acids (PUFA), and TFA contents were detected (Figure 3.4). Concerning individual fatty acids, *Sorbus* showed the highest C18:0 and the lowest C18:3 n6 and C18:3 n3 concentrations. The C18:3 n3 concentration in *Sorbus* leaves was also lower than that observed in herbage collected in the same environment.[34] *Acer* leaves had the highest C16:0 content, while *Fraxinus* leaves showed the highest MUFA and C18:1 n9 concentrations. No differences were found for the ‘Other FA’ group among the species. The majority of individual FA and FA groups showed differences among the considered species during the whole season. However, for total SFA, C16:0, C18:3 n6, C18:3 n3, and ‘Other FA’, the differences among species tended to wane as season advanced.

Rosenqvist and Laakso reported comparable FA profile and main FA percentages to those of the present study for *Salix caprea* in northern Finland.[57] Concerning *Acer*, *Fraxinus* and *Sorbus*, no data on FA profile of leaves are currently available in literature. Other studies conducted in dryer environments found similar amounts of some individual FA and FA groups when compared to the fodder tree species studied, while higher SFA and lower PUFA contents were reported.[15,56]

Variations among sampling dates in FA profiles (see Annex 3.B) showed few significant variations. The concentration of linoleic acid was significantly higher ($P<0.001$) at the first sampling date for all the species. Concerning *Fraxinus* and *Salix*, the sampling date influenced the concentration of total PUFA, which was significantly higher (both $P<0.05$) at the first sampling date if compared to the third (*Fraxinus*) or to the second and third sampling dates (*Salix*).

According to the obtained results on FA profile, *Acer*, *Fraxinus*, and *Salix* leaves can represent a partial or a complete and good quality feedstuff for goat nutrition, also in late summer, when quality (and particularly C18:3 n3 concentration) of grassland species decreases.[15,49,50,56,57] Indeed, the lipid metabolism in the rumen and in the mammary gland can be affected by such decrease in quality of grassland species, usually resulting in higher concentrations of hypercholesterolaemic saturated FA and lower concentrations of beneficial FA (i.e. vaccenic acid, rumenic acid, and ω3 FA) in the derived dairy and meat products.[58]

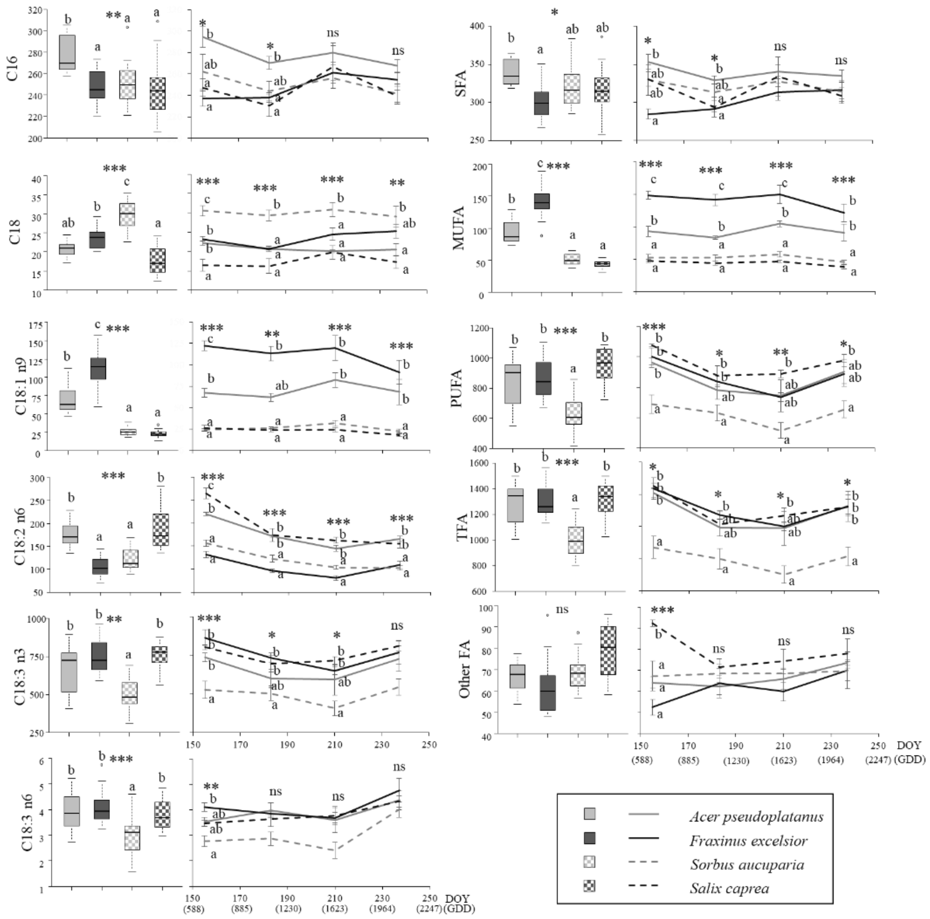

Figure 3.4. Fatty acid profile of the four tree species: overall ranges (boxplots) and seasonal variations (line charts) at the four survey dates. Provided data are expressed in mg 100 g^{-1} dry matter. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, total fatty acids; Other FA, C12:0 + C14:0 + C15:0 + C16:1 n9 + C18:1 n11 + C20:0 + C20:1 n11 + C22:0 + C20:4 n6. DOY, day of the year; GDD, growing degree days, expressed as a mean for the four tree species. ***, P<0.001; **, P<0.01; *, P<0.05; ns, P≥0.05; error bars represent the standard error of the means, while different letters within each sampling date indicate significant differences among tree species.

Phenolic composition

Based on their mean phenolic compound values, the fodder tree leaves were significantly different (box plots in Figure 3.5). *Acer* and *Salix* showed higher concentrations of TEP, TT, and HT if compared to *Fraxinus* and *Sorbus*. The concentration of CT was lower in *Acer* than in *Sorbus* and *Salix*; CT were not detected in *Fraxinus*. No significant differences in NTP contents were detected among the species throughout the whole season.

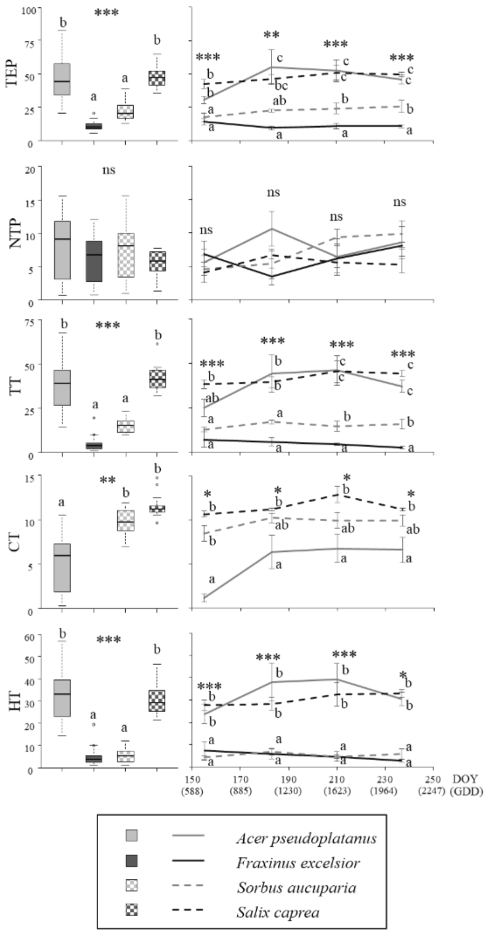

Figure 3.5. Phenolic compound content of the four tree species: overall ranges (boxplots) and seasonal variations (line charts) at the four survey dates. Provided data are expressed in g kg^{-1} dry matter, as gallic acid equivalents, except for CT which is expressed as leucocyanidin equivalents. TEP, total extractable phenols; NTP, non-tannin phenols; TT, total tannins; CT, condensed tannins; HT, hydrolysable tannins. *Fraxinus excelsior* values for CT were null and not represented. DOY, day of the year; GDD, growing degree days, expressed as a mean for the four tree species. ***, $P<0.001$; **, $P<0.01$; *, $P<0.05$; ns, $P\geq0.05$; error bars represent the standard error of the means, while different letters within each sampling date indicate significant differences among tree species.

Phenolic compound data for the leaves of these four species were unavailable in literature before the present study. However, the TEP, NTP, TT, CT, and HT concentrations reported for other fodder tree species from drier regions were similar or even higher than those of the species here studied.[13,59–61] Variations in phenolic composition among sampling dates were not significant or negligible (Annex 3.B).

Overall CT contents were lower than the threshold of 20 g kg^{-1}, identified by Min et al. as the limit from low to medium CT concentrations for ruminant nutrition.[6] According to these authors, such CT values should have a limited negative effect on ruminal digestion, especially for goats, which prefer tannin-rich foliage and produce tannin-binding proteins in saliva overcoming the negative impacts on digestibility in the rumen.[62] It has been suggested that tannins, depending on their type, chemical characteristics, and amount ingested can influence the composition and quality of the derived milk and meat products, as well as ruminant nutrition and health, by (i) increasing intestinal escape protein availability, (ii) reducing methane production, (iii) defending against bloat, and (iv) waning gastrointestinal parasites.[5,6,63,64] Francisco et al. reported also that low levels (up to 16.3 g kg^{-1} DM) of CT inclusion in lamb diet could wax antioxidant properties in meat, improving its stability after storage.[65] Similarly, milk from goats feeding on a low HT feed (comparable to values obtained for *Fraxinus* and *Sorbus* leaves) showed an increasing trend of conjugated linoleic acid isomers and oleic acid, as a result of an enhancement of Δ9-desaturase activity.[66]

Digestibility

In vitro true digestibility of leaves significantly differed among the fodder tree species, ranging from 27.5 in *Acer* to 76.5 g 100 g^{-1} DM in *Fraxinus* (Figure 3.6). Based on leaf mean digestibility values, *Sorbus* and *Fraxinus* were significantly more digestible than *Acer* and *Salix*. The obtained results were similar to those of some Mediterranean tree and shrub species, either deciduous or evergreen, investigated by other *in vitro* studies.[59,60]

Differences in digestibility among the considered fodder tree species may be attributed to a complexity of interactions among several factors, such as proximate composition (especially NDF, ADF, ADL), fatty acid profile (particularly PUFA), and phenolic compounds (above all CT and HT).[5,6,8] Concerning proximate composition, structural carbohydrates (mainly represented by hemicellulose and cellulose) are the dominant feed fraction for grazing ruminants, supplying energy for maintenance and productions.[54] However, they have a slower ruminal passage rate than other dietary components due to their chemical conformation difficult to cleave or even indigestible, resulting in a filling effect over time.[54] The association between low ADF and ADL concentrations and high digestibility pointed out by previous authors was observed also in the present study for *Fraxinus*,[59,60,67] whereas in Van Soest it appears to be less consistent.[48] The different PUFA concentrations could have also played an important role in digestibility, since they can inhibit and/or alter the microbial activity and biohydrogenation pathways within the rumen.[58] For this reason, the high PUFA contents (especially C18:3 n6, C18:3 n3, and C18:2 n6) in *Salix* and *Acer* leaves could have contributed to reduce the degradation capacity of goat microbiome. Instead, the higher *Fraxinus* IVTD was probably less affected by comparable PUFA concentrations, thanks to the lower ADF and ADL amounts. Concerning phenolic components, HT can generate toxic compounds with chronic or systemic effects, while CT, due to their structure heterogeneity (i.e. chemical composition, molecular weight, and flavanol monomers) can lead to different binding activity intensities interfering with nutrient utilization.[5,64] Moreover, CT can also create insoluble complexes with protein and fiber reducing degradation and fermentation in the rumen by microorganisms, and consequently digestibility.[64] For this reason, the highest HT concentration found in *Acer* and *Salix* leaves may have negatively influenced their digestibility, resulting in low IVTD levels. In addition, the lower IVTD associated to lower CT concentrations observed in *Acer* leaves may be due to a higher binding activity of CT molecules, while the higher IVTD associated to higher CT concentration in *Sorbus* leaves is probably due to a lower CT activity. *Fraxinus* leaves showed a phenolic profile more suitable for ruminal digestion, since they were totally free of CT and with low HT concentrations.

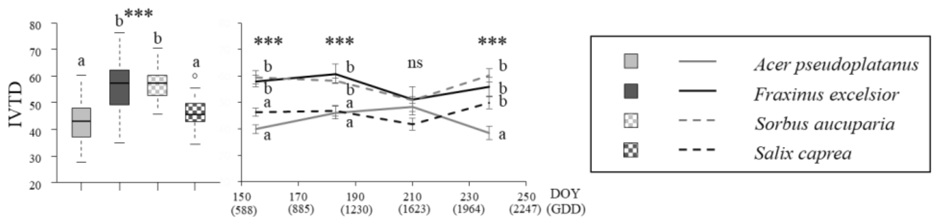

Figure 3.6. *In vitro* true digestibility (IVTD) of the four tree species: overall ranges (boxplots) and seasonal variations (line charts) at the four survey dates. Provided data are expressed in g 100 g^{-1} of dry matter. DOY, day of the year; GDD, growing degree days, expressed as a mean for the four tree species. ***, $P<0.001$; ns, $P\geq0.05$; error bars represent the standard error of the means, while different letters within each sampling date indicate significant differences among tree species.

Sampling date affected *Acer* (highest value: third sampling date; lowest value: fourth sampling date) and *Sorbus* (highest values: first and final sampling dates; lowest value: third sampling date) digestibility, whereas *Fraxinus* and *Salix* leaves were characterized by a more stable digestibility throughout the season (Annex 3.B). Another study on a Mediterranean tree species provided contrasting results, with a progressive decline of leaf digestibility with dry season advancement, probably because it dealt with leaves of an evergreen species which generally shows a lower quality at late phenological stages.[17].

Final considerations

In situ, chemical, and *in vitro* measurements can be considered as useful tools in initial screening studies to rank forages according to their nutritive quality.[8] According to the present results, the investigated species, due to their foliage production, proximate composition, fatty acid profile, phenolic composition, and digestibility can represent a complete and good quality feedstuff for goat nutrition, above all in the late summer when herbage quality decreases, particularly in terms of CP and FA profile.[50]

The studied species can supply goat feedstuff either by direct browsing or with fresh or dried fodder, also depending on each foliage production. More specifically, earlier and less productive species (i.e. *Sorbus* and *Salix*) could be exploited in advance and when present in higher densities, such as in browsing hedges, while more productive species (i.e. *Acer* and *Fraxinus*) could be better managed by pollarding single trees for fresh or dried fodder.

However, the chemical and digestibility values assessed by the present study appeared occasionally in contrast to those from the same or other fodder tree species in different environments (e.g., the semi-arid African region).[25,51] These discrepancies could be attributed to the sampling method and to the differences in ecotypes, genotypes, seasons, ecological zone, soil type, and age of the trees.[64]

The differences among the four selected species were remarkable, even if weakly related to season advancement, especially when considering FA and phenolic composition. The high digestibility showed by *Fraxinus* leaves along the season could be due to the positive influence of low ADL and phenolic compounds irrespective of low CP contents. This species could be regarded as a potential highly nutritive feedstuff, also improving the quality of the derived dairy products for human nutrition, as confirmed by its traditional use in European mountain areas.[29,30] Also *Sorbus* leaves showed similar digestibility values, although, the low CP and PUFA concentrations (especially C18:3 n3) can partially reduce the interest for this species as a feeding resource for goat dairy and meat products with healthy properties. Conversely, a lower digestibility was found for *Salix* samples, which was also the less productive species, especially at the beginning of the season, as a consequence of its high phenolic and ADL contents. Nevertheless, the FA profile of this species highlighted high C18:2 n6, C18:3 n6, and C18:3 n3 levels, generally recognized as main lipid precursors in ruminant metabolism for the synthesis of FA considered beneficial to human health (e.g. vaccenic acid, rumenic acid, and ω3 FA). A modest digestibility was recorded for *Acer* leaves, despite their high CP and medium-low ADL and CT contents, which were probably insufficient to contrast the high phenolic concentrations (TEP, TT, and HT), even if they displayed a good level of the same ‘healthy precursors’ reported for *Salix* foliage.

Additional research on production, chemical, and digestibility features of other tree species selected by browsing goats from European environments [e.g., *Alnus viridis* (Chaix) DC., *Betula pendula* Roth, *Corylus avellana* L., *Fagus sylvatica* L., *Quercus* sp., *Tilia cordata* Mill., *Ulmus* sp.] would be advisable.[18,27,28,33] Moreover, the preferences by goats for the investigated tree species should be taken into consideration. For this reason, further *in vivo* studies appear advisable to assess their influence on voluntary intake, total tract digestion (e.g. protein sparing effect by CT), and goat milk quantitative and qualitative production.

Acknowledgments

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Annex 3.A

Survey schedule, with the indication of the phenological stage (vegetative and, when present, reproductive; average values per species) at each survey date for the four tree species following the extended BBCH scale. All surveys have been conducted in 2015. DOY, day of the year; GDD, growing degree days, expressed as the mean values of the four tree species (± standard error).

Date	DOY	GDD	Survey	Phenological stages: *Acer pseudoplatanus*	*Fraxinus excelsior*	*Sorbus aucuparia*	*Salix caprea*
April, 15	105	137.7 ± 2.76	Leaf traits (1st)	Buds show green tips	Beginning of bud swelling; Flower buds visible	Buds show green tips	First leaves separated; Beginning of flowering
April, 23	113	205.0 ± 3.90	Leaf traits (2nd)	Buds show green tips	First leaves separated; 30% of flowers open	First leaves separated	First leaves separated; Full flowering
April, 28	118	238.6 ± 3.94	Leaf traits (3rd)	Buds show green tips	First leaves separated; 40% of flowers open	First leaves separated; Beginning of heading	Four true leaves unfolded; Flowering finishing
May, 6	126	309.0 ± 4.23	Leaf traits (4th)	Buds show green tips	Four true leaves unfolded; 60% of flowers open	Four true leaves unfolded; Beginning of heading	Seven true leaves unfolded; Flowering finishing
May, 12	132	391.4 ± 4.86	Leaf traits (5th)	First leaves separated; Half of inflorescences emerged	Five true leaves unfolded; End of flowering: fruit sets visible	Four true leaves unfolded; Half of inflorescence emerged	Eight true leaves unfolded End of flowering, fruit sets visible
May, 19	139	482.0 ± 5.27	Leaf traits (6th)	Three true leaf pairs unfolded; First flowers open	Five true leaves unfolded; Fruits have reached 10% of final size	Five true leaves unfolded; End of heading	Eight true leaves unfolded Fruits have reached 10% of final size
May, 29	149	576.6 ± 5.82	Leaf traits (7th)	Four true leaf pairs unfolded; 40% of flowers open	Five true leaves unfolded; Fruits have reached 30% of final size	Five true leaves unfolded; Full flowering	Nine true leaves unfolded; Beginning of fruit coloration
June, 4	155	661.3 ± 5.98	Leaf traits (8th) and chemical analyses (1st)	Four true leaf pairs unfolded; End of flowering: fruit sets visible	Five true leaves unfolded; Fruits have reached 40% of final size	Five true leaves unfolded; Fruits have reached 10% of final size	Nine true leaves unfolded; Fruits begin to soften
June, 18	169	869.5 ± 6.16	Leaf traits (9th)	Five true leaf pairs unfolded; Fruits have reached 10% of final size	Shoot development completed; Fruits have reached 60% of final size	Shoot development completed; Fruits have reached 50% of final size	Shoot development completed; Fully ripe
July, 2	183	1086.0 ± 5.99	Leaf traits (10th) and chemical analyses (2nd)	Shoot development completed; Fruits have reached 30% of final size	Shoot development completed; Fruits have reached 70% of final size	Shoot development completed; Nearly all fruits have reached the final size	Shoot development completed; Fully ripe
July, 29	210	1623.5 ± 6.16	Leaf traits (11th) and chemical analyses (3rd)	Shoot development completed; Fruits have reached 60% of final size	Shoot development completed; Fruits have reached 70% of final size	Beginning of leaf fall; Beginning of fruit coloration	Shoot development completed
August, 25	237	2054.3 ± 6.61	Leaf traits (12th) and chemical analyses (4th)	Shoot development completed; Nearly all fruits have reached the final size	Shoot development completed; Nearly all fruits have reached the final size	Beginning of leaf fall; Fully ripe	Shoot development completed

Annex 3.B

Proximate composition [g 100 g^{-1} dry matter (DM), except for DM which is expressed in g 100 g^{-1} fresh matter], fatty acid composition (mg 100g^{-1} DM), phenolic composition (g kg^{-1} DM), and digestibility (g 100 g^{-1} DM) at each sampling date (DOY, day of the year) expressed as the mean values of the four tree species (± standard error). DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, total fatty acids; Other FA, C12:0 + C14:0 + C15:0 + C16:1 n9 + C18:1 n11 + C20:0 + C20:1 n11 + C22:0 + C20:4 n6; TEP, total extractable phenols; NTP, non-tannin phenols; CT, condensed tannins; HT, hydrolysable tannins; TT, total tannins; IVTD, *in vitro* true digestibility; *nd*, not detected. For each tree species, different letters within a column indicate significant differences among the survey dates according to Tukey's test.

		Proximate composition						
	DOY	DM	CP	EE	Ash	NDF	ADF	ADL
Acer pseudoplatanus	155	42.4 ± 1.64 **a**	19.0 ± 0.27 **c**	4.7 ± 0.13 **b**	6.4 ± 0.16	38.6 ± 0.10 **a**	22.6 ± 0.04 **a**	8.5 ± 0.17 **a**
	183	54.3 ± 2.09 **b**	17.1 ± 0.11 **b**	4.0 ± 0.07 **a**	6.2 ± 0.14	42.3 ± 0.15 **c**	26.6 ± 0.22 **ab**	8.4 ± 0.20 **a**
	210	55.6 ± 1.57 **b**	15.2 ± 0.19 **a**	5.0 ± 0.16 **b**	6.7 ± 0.13	41.4 ± 0.14 **b**	25.4 ± 0.05 **ab**	10.6 ± 0.17 **b**
	237	56.0 ± 0.87 **b**	15.4 ± 0.20 **a**	4.7 ± 0.14 **b**	6.7 ± 0.12	42.4 ± 0.15 **c**	28.6 ± 0.20 **b**	11.9 ± 0.19 **c**
		P<0.001	P<0.05	P<0.001		P<0.001	P<0.01	P<0.001
Fraxinus excelsior	155	43.6 ± 1.34 **a**	16.1 ± 0.17 **b**	3.0 ± 0.09	6.4 ± 0.17 **a**	40.8 ± 0.08 **a**	19.4 ± 0.15 **b**	5.4 ± 0.17
	183	53.0 ± 1.11 **b**	12.7 ± 0.15 **a**	3.0 ± 0.16	6.7 ± 0.13 **a**	45.9 ± 0.17 **b**	19.6 ± 0.14 **b**	5.4 ± 0.18
	210	53.3 ± 1.07 **b**	13.0 ± 0.22 **a**	2.8 ± 0.08	8.3 ± 0.13 **b**	44.0 ± 0.16 **ab**	18.3 ± 0.10 **a**	4.9 ± 0.19
	237	55.2 ± 0.57 **b**	14.3 ± 0.20 **ab**	3.2 ± 0.08	8.5 ± 0.05 **b**	44.6 ± 0.15 **ab**	24.0 ± 0.16 **c**	5.6 ± 0.13
		P<0.001	P<0.01		P<0.001	P<0.01	P<0.001	
Sorbus aucuparia	155	59.1 ± 3.29	12.1 ± 0.19 **bc**	3.0 ± 0.12 **b**	5.1 ± 0.11	40.9 ± 0.33	24.0 ± 0.23 **a**	12.1 ± 0.20 **a**
	183	61.2 ± 1.26	12.6 ± 0.21 **c**	2.5 ± 0.12 **ab**	5.1 ± 0.12	40.9 ± 0.21	27.9 ± 0.20 **ab**	11.9 ± 0.25 **a**
	210	63.2 ± 1.58	11.4 ± 0.22 **ab**	2.3 ± 0.14 **a**	5.0 ± 0.12	40.4 ± 0.18	27.1 ± 0.26 **ab**	11.8 ± 0.27 **a**
	237	60.5 ± 0.73	11.1 ± 0.22 **a**	2.3 ± 0.11 **a**	4.8 ± 0.13	41.2 ± 0.22	30.5 ± 0.24 **b**	13.6 ± 0.11 **b**
			P<0.001	P<0.01			P<0.01	P<0.001
Salix caprea	155	55.1 ± 1.23 **a**	17.3 ± 0.19 **c**	3.8 ± 0.09 **b**	6.2 ± 0.12 **a**	44.3 ± 0.13 **a**	34.2 ± 0.17 **a**	19.1 ± 0.19 **b**
	183	57.9 ± 1.05 **ab**	13.4 ± 0.12 **b**	3.8 ± 0.16 **b**	6.0 ± 0.12 **a**	45.6 ± 0.18 **b**	35.8 ± 0.18 **b**	18.4 ± 0.19 **b**
	210	66.8 ± 4.61 **b**	12.3 ± 0.16 **a**	3.1 ± 0.09 **a**	6.8 ± 0.15 **b**	45.5 ± 0.16 **b**	34.7 ± 0.10 **a**	17.5 ± 0.17 **a**
	237	60.0 ± 0.35 **ab**	13.2 ± 0.19 **b**	3.9 ± 0.12 **b**	7.1 ± 0.14 **b**	45.3 ± 0.23 **b**	36.8 ± 0.25 **c**	20.7 ± 0.05 **c**
		P<0.05	P<0.001	P<0.01	P<0.001	P<0.01	P<0.001	P<0.001

	DOY	Fatty acid composition C16	C18	C18:1c9	C18:2n6	C18:3n3	C18:3n6	SFA	MUFA	PUFA	TFA	Other FA
Acer pseudoplatanus	155	294.4 ± 9.59	22.2 ± 0.63	67.2 ± 5.11	219.5 ± 3.54 **c**	739.1 ± 28.89	3.5 ± 0.17	353.1 ± 9.94	93.1 ± 7.92	964.1 ± 31.97	1410.3 ± 30.45	64.3 ± 2.62
	183	270.2 ± 6.19	20.7 ± 0.70	61.8 ± 4.99	171.9 ± 2.26 **b**	601.9 ± 59.08	4.0 ± 0.30	329.2 ± 5.33	82.9 ± 2.37	780.7 ± 59.15	1192.9 ± 53.97	62.4 ± 3.78
	210	280.0 ± 9.15	20.1 ± 0.44	82.0 ± 8.35	144.9 ± 6.43 **a**	592.7 ± 100.59	3.6 ± 0.49	340.2 ± 9.26	104.5 ± 4.37	744.6 ± 105.14	1189.2 ± 105.28	65.9 ± 4.66
	237	267.6 ± 5.77	20.5 ± 1.61	68.2 ± 14.9	165.8 ± 5.61 **b**	729.4 ± 82.57	4.4 ± 0.37	335.1 ± 7.79	90.9 ± 12.94	903.6 ± 79.27	1329.6 ± 64.51	73.8 ± 1.32
					$P<0.001$							
Fraxinus excelsior	155	237.0 ± 7.27	23.2 ± 0.65	121.8 ± 5.75	132.0 ± 6.82 **c**	865.4 ± 50.86	4.1 ± 0.17	284.3 ± 6.72	148.4 ± 6.33	1003.4 ± 56.67 **b**	1436.1 ± 65.90	52.5 ± 3.66
	183	237.8 ± 4.51	20.7 ± 0.27	112.9 ± 8.63	96.3 ± 3.47 **ab**	734.5 ± 31.40	3.9 ± 0.11	291.1 ± 4.26	141.1 ± 8.85	837.8 ± 34.21 **ab**	1270.0 ± 26.37	64.0 ± 6.95
	210	261.2 ± 6.94	24.5 ± 1.60	119.4 ± 14.36	80.8 ± 5.29 **a**	649.7 ± 24.52	3.7 ± 0.20	313.5 ± 11.04	149.7 ± 13.97	736.0 ± 29.30 **a**	1199.2 ± 26.70	60.0 ± 4.60
	237	254.8 ± 6.37	25.2 ± 1.69	91.2 ± 14.08	109.2 ± 8.28 **bc**	769.8 ± 77.07	4.8 ± 0.46	316.4 ± 11.79	121.2 ± 14.02	887.5 ± 79.45 **ab**	1325.1 ± 94.63	70.2 ± 8.72
					$P<0.001$					$P<0.05$		
Sorbus aucuparia	155	262.2 ± 16.04	30.5 ± 1.33	24.1 ± 1.64	156.2 ± 6.91 **b**	527.6 ± 56.54	2.8 ± 0.21 **a**	329.7 ± 20.18	52.3 ± 4.83	688.6 ± 60.26	1070.6 ± 70.14	67.3 ± 7.10
	183	244.1 ± 8.82	29.3 ± 1.39	26.1 ± 1.53	122.8 ± 7.41 **a**	504.8 ± 45.99	2.9 ± 0.27 **ab**	313.5 ± 10.60	52.2 ± 4.46	633.0 ± 53.06	998.6 ± 62.88	68.6 ± 4.43
	210	256.3 ± 4.76	30.9 ± 1.81	31.6 ± 3.58	103.7 ± 4.21 **a**	407.3 ± 47.37	2.4 ± 0.33 **a**	327.5 ± 6.65	57.1 ± 4.28	516.1 ± 50.85	900.8 ± 54.17	68.6 ± 2.63
	237	241.3 ± 9.46	29.0 ± 2.74	22.3 ± 2.84	102.6 ± 5.35 **a**	545.5 ± 52.70	4.0 ± 0.31 **b**	313.8 ± 12.60	45.3 ± 2.93	655.4 ± 54.44	1014.5 ± 58.30	69.8 ± 4.46
					$P<0.001$		$P<0.05$					
Salix caprea	155	247.1 ± 8.36	16.5 ± 1.57	26.2 ± 3.10	264.1 ± 11.81 **b**	803.7 ± 14.67	3.5 ± 0.13	330.7 ± 9.69	47.4 ± 2.50	1075.5 ± 6.29 **b**	1453.7 ± 17.37 **b**	92.6 ± 1.54
	183	230.4 ± 10.1	16.3 ± 1.83	23.6 ± 2.16	174.0 ± 14.09 **a**	695.7 ± 51.36	3.7 ± 0.30	293.8 ± 13.09	44.3 ± 3.36	877.2 ± 62.75 **a**	1215.3 ± 76.33 **a**	71.7 ± 3.90
	210	266.5 ± 20.0	19.8 ± 1.76	24.3 ± 2.63	162.4 ± 6.39 **a**	716.6 ± 26.46	3.8 ± 0.35	334.1 ± 25.31	46.7 ± 1.71	887.1 ± 25.61 **a**	1267.9 ± 47.47 **ab**	74.4 ± 5.76
	237	239.3 ± 5.75	17.3 ± 1.67	18.2 ± 2.15	155.5 ± 9.83 **a**	810.3 ± 31.46	4.3 ± 0.22	308.8 ± 10.07	38.2 ± 3.12	976.0 ± 39.93 **ab**	1323.0 ± 44.85 **ab**	78.1 ± 7.01
					$P<0.001$					$P<0.05$	$P<0.05$	

		Phenolic composition				
	DOY	**TEP**	**NTP**	**TT**	**CT**	**HT**
Acer pseudoplatanus	155	30.5 ± 2.81	5.6 ± 1.97	24.9 ± 4.75	1.1 ± 0.44	23.8 ± 4.32
	183	55.0 ± 12.92	10.6 ± 2.59	44.4 ± 10.37	6.4 ± 1.92	38.0 ± 8.48
	210	52.5 ± 8.18	6.4 ± 2.74	46.1 ± 8.27	6.8 ± 1.57	39.3 ± 6.97
	237	45.7 ± 3.27	8.6 ± 2.38	37.1 ± 3.29	6.6 ± 1.42	30.5 ± 2.94
Fraxinus excelsior	155	14.0 ± 2.40	6.8 ± 1.95	7.2 ± 4.13	*nd*	7.2 ± 4.13
	183	9.4 ± 1.46	3.5 ± 1.25	5.9 ± 2.36	*nd*	5.9 ± 2.36
	210	10.7 ± 2.14	6.2 ± 2.22	4.6 ± 0.67	*nd*	4.6 ± 0.67
	237	10.8 ± 1.01	8.1 ± 0.56	2.7 ± 0.54	*nd*	2.7 ± 0.54
Sorbus aucuparia	155	17.4 ± 3.16	4.6 ± 2.02	12.8 ± 1.21	8.5 ± 0.89	4.3 ± 0.68
	183	22.4 ± 1.19	5.4 ± 2.22	17.0 ± 1.10	10.3 ± 0.55	6.8 ± 1.38
	210	24.0 ± 3.99	9.3 ± 1.26	14.6 ± 2.82	10.0 ± 0.92	4.7 ± 2.08
	237	25.7 ± 4.72	9.8 ± 1.97	15.8 ± 2.83	10.0 ± 0.66	5.9 ± 2.26
Salix caprea	155	42.3 ± 3.26	4.1 ± 1.50	38.3 ± 2.46	10.7 ± 0.35 **a**	27.6 ± 2.26
	183	46.2 ± 3.38	6.6 ± 0.58	39.5 ± 2.96	11.2 ± 0.15 **ab**	28.3 ± 2.89
	210	51.0 ± 5.77	5.5 ± 0.76	45.4 ± 6.09	12.9 ± 0.93 **b**	32.5 ± 5.23
	237	49.5 ± 1.8	5.2 ± 1.29	44.3 ± 1.64	11.2 ± 0.16 **ab**	33.1 ± 1.74
					P<0.05	

		Digestibility
	DOY	**IVTD**
Acer pseudoplatanus	155	39.97 ± 1.6 **ab**
	183	46.18 ± 2.4 **ab**
	210	48.36 ± 2.9 **b**
	237	38.53 ± 2.6 **a**
		P<0.05
Fraxinus excelsior	155	57.98 ± 2.2
	183	60.80 ± 3.6
	210	51.25 ± 4.8
	237	55.83 ± 3.5
Sorbus aucuparia	155	59.51 ± 2.5 **b**
	183	58.32 ± 0.9 **ab**
	210	50.89 ± 1.3 **a**
	237	60.29 ± 2.5 **b**
		P<0.01
Salix caprea	155	46.37 ± 1.4
	183	46.92 ± 2.1
	210	41.79 ± 2.2
	237	49.85 ± 2.2

4. Grazer species and grazing system effects on grassland species diversity

Extended paper title: *A biodiversity-friendly rotational grazing system enhancing flower-visiting insect assemblages while maintaining animal and grassland productivity*

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Abstract

The abstract of this paper is reported in Chapter 1.4, page 5.

Keywords: Butterflies, Cattle, Grazing Management, Flowering Intensity, Ground Beetles, Sheep

Introduction

The sustainability of animal production systems has become a major issue over the last few years,[1–4] emphasizing the need to optimize land-use, mitigate and adapt to climate change and to reduce biodiversity loss.[5,6] Agro-pastoral systems play a pivotal role in this context as they must maximize the benefits provided to human society and the biosphere, such as food production and ecosystem functioning.[7,8]

After several millennia of land management, agro-pastoral systems have contributed to create a wide variety of semi-natural habitats, often characterised by high biodiversity levels.[9] Mountain grasslands, which have been mainly created and maintained by extensive cattle and sheep grazing and/or mowing, are among the most biodiverse habitats in Europe[10] and the sustainability of the traditional management of these ecosystems is currently under constant threat due to socio-economic and market changes.[11,12] Indeed, the increase in production costs and reduction in product sale incomes have often led to an intensification of grassland management within the most productive sites, along with grassland abandonment when management has become unprofitable.[13,14] In both cases, changes in management led to changes in grassland productivity and in an overall decrease in plant and animal diversity.[9,15–17] Moreover, the highest biodiversity in these semi-natural ecosystems is generally associated to intermediate levels of management intensity, in agreement with the intermediate disturbance hypothesis.[18–20] Within permanent mountain pastures, optimal livestock pressure for biodiversity conservation can be achieved by using specific pastoral practices[21,22] and/or by adjusting the number of grazing animals, the area available for grazing, the grazing schedule and system (e.g. rotational or continuous grazing).[23,24] Nowadays, a major challenge is that of applying innovative management systems able, not only to preserve plant and animal diversity but also to maintain levels of animal and grassland productivity.

Several studies focused on grassland insect communities so as to monitor the effects of different grazing regimes produced on grassland biodiversity as they can be considered key groups due to the fact that their assemblages are immediately and severely affected by habitat changes.[25] Moreover, grassland insect communities include a wide variety of species threatened by habitat loss and modification,[26] including several protected by local, national or EU legislation, such as the Habitat Directive (92/43/EEC). Livestock pressures on grassland habitats may have varying effects on insect communities in different ways, as reported by van Klink et al.[27], including: i) the modification of the abiotic conditions (modification of vegetation patches, a decrease in vegetation height, an alteration in structural complexity, and changes in soil conditions), ii) varying the feeding resource availability (flower and herbage mass reduction, the rate of dung depositions, and live tissue accessibility), and iii) ingestion or trampling by the grazing animals. Each of these actions depends on livestock species and management, due to grazer/browser feeding preferences, live weight and social behaviour.[27,28] Amongst the most common grazer species, the higher selectivity of sheep for legumes and forbs and flowering plant parts can lead to grass-dominated plant communities with a lower diversity of nectar-dependent insect taxa than cattle-grazed grasslands.[29,30]

Furthermore, Sjödin et al.[16] highlighted that it is essential to consider different insect taxa simultaneously in a systemic research as the effects of livestock pressure on insect diversity and abundance may differ when more than a single insect group is taken into consideration. Nevertheless, while multi-taxon approaches have been largely applied to compare variations in diversity and abundance for various insect groups at variable grazing pressures,[16,31,32] the simultaneous effects of different grazing systems and grazer species on a given plant community have, to date, been only scantily evaluated. Scohier et al.[33] focused only on sheep grazing and observed that a particular rotational grazing system, with sheep exclusion from pasture during the main flowering period as proposed by Farruggia et al.[34], was more beneficial for bumblebees than it was for butterflies. Zhu et al.[35] focused on rationed grazing system with cattle, sheep and goats and recorded different responses of six insect groups (grasshoppers, homopterans, beetles, dipterans, hemipterans and butterflies) according to the grazer species, without considering grassland or animal performance during the grazing season. Contrasting results were reported in other studies that focused only on grassland and animal performance under continuous and rotational grazing systems, with contrasting results, without considering their effect on insect diversity.[36]

The present study aimed at assessing the effects produced by two grazer species (cattle and sheep) managed at the same stocking density under two grazing systems, i.e. continuous grazing (CG) and an innovative rotational grazing system to enhance biodiversity (the biodiversity-friendly rotational grazing system - BR), on three insect taxa (butterflies, bumblebees and ground beetles), as well as on herbage mass and animal performance. Butterflies and bumblebees were chosen for their role in pollination as flower-visiting insect taxa, whilst ground beetles were chosen as they represent a large insect taxon related to grassland structure, with different feeding behaviours (often carnivorous)[27] and as indicators of invertebrate abundance and Coleoptera richness.[37] The following hypotheses were tested: i) insect abundance and diversity would be enhanced by the BR, ii) sheep grazing would be detrimental for flowering intensity and, consequently, for insect assemblages, iii) benefits would differ among insect taxa, and iv) BR would not differ from CG in terms of herbage mass or animal performance.

Materials and Methods

Study area

The grazing experiment was established in semi-natural mountain pastures managed by INRA (Institut National de Recherche Agronomique) in the upland area of central France, within the Volcans d'Auvergne Natural Park (Massif Central, 45°15'N, 2°51'E). The study area was located at 1,100 m a.s.l. and it was characterised by volcanic soils and sub-Atlantic climate (Köppen's classification: Cfb)[38] with average annual temperature of 7.0 °C and precipitation of 1,169 mm (average values for the period 1965-2010 according to the Marcenat weather station). Pastures without mineral fertilization had been extensively grazed by cattle since 1992.[39] The dominant plant community belonged to the *Cynosurion cristati* alliance, *sensu* Braun-Blanquet et al.[40]

Experimental design

In the years 2011, 2012 and 2013, continuous grazing (CG) was compared to an innovative rotational grazing system (hereafter referred to as 'biodiversity-friendly rotation', BR), i.e. a system in which enclosures (plots) were divided into four subplots (A, B, C, and D), each one grazed for 35 days per year, with subplot D excluded from grazing for 63 days during the main flowering period, i.e. from early-June to early-August (see Annex 4.A). Two grazer species in the experimental design were compared i.e. cattle and sheep and each grazing system × grazer species treatment was replicated three times in a complete randomized design, so that 12 plots were set up (see Annex 4.B). A total of six 3.6 ha plots were grazed by seven Charolais heifers (corresponding to 6.30 livestock units) each and six 0.6 ha plots were grazed by seven Limousine ewes (corresponding to 1.05 livestock units) each, providing a comparable stocking density (1.75 livestock units ha^{-1}), which is in line with the local stocking density commonly applied in the region.

The plots were chosen with similar elevation, exposure, roughness and slope and in each one had a randomly positioned water source to meet animal requirements. Moreover, grassland botanical composition was evaluated before setting the experiment up according to the characterisation made by a botanist (see Acknowledgements), to ensure that both plots and subplots were set-up on a similar plant community.

Data collection

Flowering intensity and sward structure

The detailed botanical composition of the plots and subplots was recorded only once in July 2011, as no significant changes in plant community composition due to the grazing treatments were expected in the time span under investigation (2011 to 2013), as the vegetation dynamics in these permanent mountain grasslands are slow.[29] The botanist carried out botanical surveys during the main flowering period, i.e. at the maximum trophic availability for flower-visiting insects. In each plot, ten 1-m^2 quadrats were set and the relative abundance (%) of each plant species was assessed along eight quadrat points within each quadrat, so that a total of 80 quadrat points per plot were performed. A minimum value of 0.3% was assigned to occasional species,[41] i.e. to the species not recorded along the quadrat points but occurring within a range of 5 m from the quadrat itself. Grassland plant diversity was assessed according to the Shannon diversity index[42] for each plot and subplot and the relative abundance (%) of species pollinated by butterflies and bumblebees[43,44] was calculated.

During the flowering peak (July), the flowers cover percentage was visually estimated by the same observer in eight 30 × 30 m squares within each plot (two per BR subplot), twice yearly[34] (see Annex 4.A). The percentage covers of yellow, white and purple-pink flowers in each square were noted during each observation and then used to calculate an overall flowering intensity.

Sward surface heights were measured monthly during the exclusion period (see Annex 4.A) with a graduated stick[45] along regular transects at 500 points per plot and 125 points per subplot and the average values were calculated.[34] Sward height data were then used to assess the sward height heterogeneity by calculating: i) the coefficient of variation (CV) and ii) Pielou's equitability index[46] on three height classes (< 7 cm, between 7 and 25 cm, and > 25 cm, according to Dumont et al.,[47] adapted), calculated as follows:

$$J = \frac{H'}{\log_2(S)}$$

where H' is Shannon diversity index among the three height classes and S is the number of classes.

Insect sampling

Butterflies (true butterflies: *Rhopalocera* and burnet moths: *Zygaenidae*) and bumblebees (*Apidae*: *Bombus*) were recorded by a specialist (see Acknowledgements) using the ‘Pollard walk’[48] along 50-m by 5-m fixed transects, four per each CG and BR plot (one per BR subplot). The surveys were made between 11 a.m. and 3 p.m., under good weather conditions (temperature > +15 °C, gentle wind, cloudless sky) and were repeated twice a year during the exclusion of subplot D from grazing, at 2- or 3-week intervals between early July and early August (see Annex 4.A), corresponding with the peak of flight activity for most species.

Ground beetles (*Coleoptera*: *Carabidae*) were sampled once a year with 12 fixed pitfall traps per each CG and BR plot (three per BR subplot). The traps were filled with a solution of 2/3 ethanol and 1/3 water at the beginning of the trapping period in mid-July; the liquid was topped up every 3-4 days and the traps emptied after 15 days (see Annex 4.A).

Butterflies, bumblebees and ground beetles were counted and identified at species level, so that abundance and species richness were analysed at both a plot and subplot scale.

Herbage mass and animal performance

The average weather conditions over the three year experiment period were compared to a 40-year climatic database. All records were registered by the Marcenat weather station.

Seasonal herbage mass changes were evaluated by cutting 0.5 m^2 strips (0.1 × 5 m) at ground level five times a year (see Annex 4.A), with eight samplings per CG- and BR-cattle plots (two per BR-cattle subplot) and four per CG- and BR-sheep plots (one per BR-sheep subplot). Herbage mass (g_{DM}) was weighted by drying samples at 60 °C for 48 h and then aggregating them to express herbage mass in t_{DM} ha^{-1}.

Animal performance was assessed recording live weight and body condition score (BCS)[49,50] for each animal in five periods (see Annex 4.A).

Data analysis

Plant communities were classified by two hierarchical cluster analyses, for plots and BR subplots separately, using the PAST version 3.11.[51] The similarity matrix was calculated using the Euclidean distance, whilst the complete linkage was selected as agglomeration method. Moreover, the homogeneity of Shannon diversity index and of the relative abundance of plant species pollinated by butterflies and bumblebees between grazing systems, grazer species and among subplots was verified at the set-up of the experiment performing a mixed model (SAS Inst. Inc., Cary, NC, USA).

Two mixed models were used to analyse any differences in flower cover, insect counts, as well as sward height and heterogeneity (i.e. CV and Pielou's equitability index). The first one considered the plot as the statistical unit, the year as a random factor, and grazing system and grazer species and all possible interactions were considered fixed factors. The second one considered the subplot as the statistical unit, the year as a random factor, and grazer species and all possible interactions as fixed factors. When significant interactions were observed, mixed models were also performed to detect statistical differences amongst the factor combinations. Tukey's *post-hoc* tests were performed when significant differences amongst subplots were found.

The responses of insect species to treatments were analysed using redundancy analysis (RDA) in CANOCO version 4.5.[52] Insect data were arranged in species matrices, whilst the four treatments (two grazing systems × two grazer species) were considered to be the environmental categorical variables and coded as dummy variables. Mantel tests with 9,999 permutations were used to calculate the correlations between insect taxa (butterfly, bumblebee, and ground beetle) and treatment matrices (PAST version 3.11). A third matrix including flower cover, sward height and sward heterogeneity (CV) was used as a supplementary matrix to evaluate the gradients associated with the two main axes of the ordination plots.[52]

Herbage mass at plot scale was analysed at each sampling date, using a mixed model with year as a random factor and grazing system, grazer species and all possible interactions as fixed factors. In the BR system, herbage mass was also analysed at subplot scale at each sampling date by performing a mixed model with year as a random factor and subplot, grazing animal and all possible interactions as fixed factors. The same analyses were performed on cattle and sheep animal live weight and BCS at plot scale, but grazing animal was not considered to be a fixed factor.

Results

Botanical composition, flower cover and sward structure

The dominant plant species detected during vegetation surveys were *Agrostis capillaris* L. (18.7 %), *Festuca nigrescens* Lam. (13.0 %) and *Trifolium repens* L. (6.1 %). The hierarchical cluster analyses showed ordinations without clear plot or subplot agglomerations based on grazing system or grazer species (see Annex 4.C). At the experiment set-up, the Shannon diversity index (average value: 4.5) and the relative abundance of species pollinated by butterflies (15.0 %) and bumblebees (35.7 %) did not significantly differ between CG and BR, cattle and sheep plots, or among BR subplots.

Over the three year study period, the percentage of the flower cover was significantly higher in BR than in CG, in cattle than in sheep plots, and in D than A, B, and C subplots (Figure 4.1). Moreover, positive interactions between the grazing system and the grazer species ($P < 0.05$) and between year and grazer species ($P < 0.001$) were observed, highlighting a significantly higher flower cover in BR- than in CG-sheep plots (Figure 4.1a') as well as in cattle over sheep plots in 2012 and 2013 (Figure 4.1a''). The average sward height was 24.1 cm and no differences between CG and BR plots, cattle and sheep plots, or among BR subplots were detected (Figures 4.2a and 4.2b). Sward heterogeneity (CV and Pielou's equitability index) was comparable between the grazing system and grazer species and among BR subplots, except for the higher CV in cattle than in sheep plots (Figures 4.2c and 4.2d).

Insect abundance, diversity and response to treatments

A total of 1,913 butterflies from 37 different species were sampled during the experiment period. Only one protected species from European, national and regional lists was collected, i.e. *Maculinea arion* (Linnaeus 1758), which was only found in three cattle plots (two managed under BR and one under CG), whilst nine species were classified as 'locally rare' according to Bachelard and Fournier's abundance scale[53]. There were three most abundant species over the three year period, namely *Zygaena purpuralis* (Brünnich 1763, 504 individuals, 26% of the total), *Thymelicus lineola* (Ochsenheimer 1808, 404, 21%) and *Coenonympha pamphilus* (Linnaeus 1758, 275, 14%). Butterfly abundance and species richness were significantly higher in BR than in CG, in cattle than in sheep plots and in D than A, B and C subplots (Figures 4.3a to 4.3d).

A total of 4,672 ground beetles, belonging to 22 species, were collected. Neither rare nor protected species from national or regional list species were found. The most abundant species were *Carabus monilis* Fabricius 1762 (1,101 individuals, 24%), *C. violaceus* Linne 1758 (1,087, 23%), *Pterostichus melanarius* (Illiger 1798, 937, 20%) and *Amara lunicollis* Schiodte 1837 (585, 13%). Ground beetle abundance and species richness did not differ between grazing systems, grazer species, or among subplots, as reported in Figures 4.3i to 4.3l.

Mantel's tests, performed before the RDA, showed significant correlations between treatment matrix with butterfly (r: 0.18; $P < 0.05$) and bumblebee matrices (r: 0.39; $P < 0.001$) but not with ground beetle matrix. Thus, only butterfly and bumblebee matrices were retained and assembled in a unique flower-visiting insect matrix to explore the response of these two insect groups simultaneously. The latter matrix was still correlated with treatment matrix (r: 0.19; $P < 0.05$) and was used to perform the RDA analysis. The RDA ordination biplot, shown in Figure 4.4, allows the visualisation of the first two axes, explaining 54.9% and 3.5% of the distribution, respectively. The ordination biplot showed a clear distinction among the four treatments, highlighting that the interaction between grazing system and grazer species affected butterfly and bumblebee species. The BR-cattle treatment plots separated well on the first axis, in contrast to CG- and BR-sheep treatments. The highest number of insect species was related to BR-cattle treatment, with 30 species (66.7% of total butterfly and bumblebee species) displaying positive scores of the perpendicular projection onto this treatment vector[52]. Noteworthy is the fact that among them there were the only endangered butterfly species (*M. arion*) and 89% of the 'locally rare' butterfly species, i.e. *Adscita geryon* (Hübner 1813), *Mellicta parthenoides* Keferstein 1851, *M. arion*, *Pyrgus alveus* (Hübner 1803), *P. carthami* (Hübner 1813), *P. malvae* (Linnaeus 1758), *Spialia sertorius* (Hoffmannsegg 1804), and *Zygaena purpuralis* (Brünnich 1763). This treatment and the related insect species fitted with high flowering intensities, low sward height and high sward heterogeneity. In contrast, insect species clearly fitting with other treatments accounted for fewer individuals (see Annex 4.D for the complete species-abundance report). Moreover, according to Bachelard and Fournier[53], the three species associated with CG-sheep treatments, i.e. *Aricia agestis* (Denis and Sciffermüler, 1775), *Colias hyale* (Linnaeus 1758), and *Gonepteryx rhamni* (Linnaeus 1758), were very common species, locally frequenting a range of habitats and were found in small numbers (one, one, and three, respectively). The butterfly species *Plebejus idas* (Linnaeus 1761), reported as 'locally rare', though related to BR-sheep treatment, was found only once. The CG-cattle treatment showed the weakest relationship with species, as indicated by its short arrow on the biplot[52]. Three species, namely *Cyaniris semiargus* (Rottemburg 1775), *Ochlodes venatus* (Bremer & Grey, 1853) and *B. hortorum*, were strongly related to this treatment, as they were exclusively collected in CG-cattle plots over the three years, even if with only a few individuals (two, one and three, respectively).

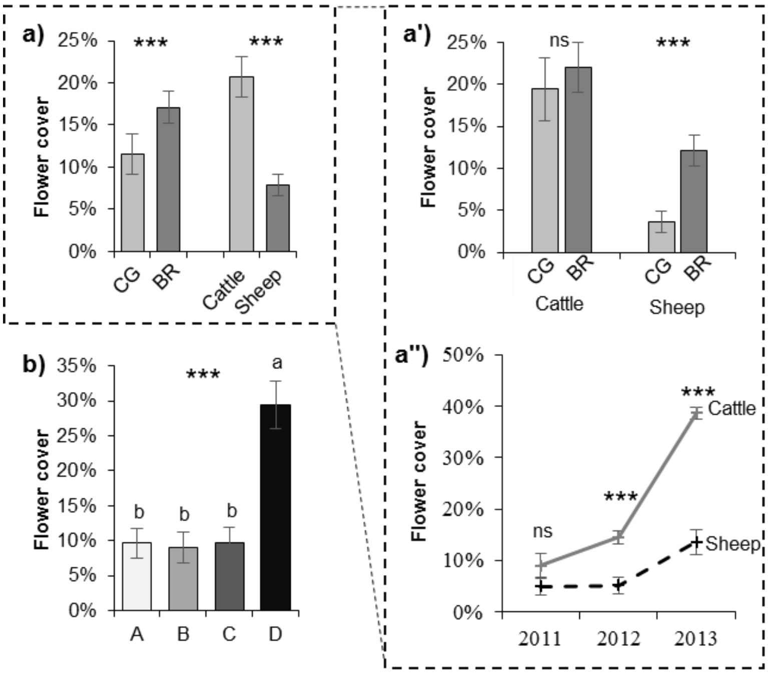

Figure 4.1: flower cover (average flower cover percentage) during the exclusion period according to (a) grazing system and grazer species, (b) BR subplot, and the interactions between grazer species and (a') grazing system and (a'') year. CG, continuous grazing; BR, biodiversity-friendly rotation; A, B, C, biodiversity-friendly rotation subplots without exclusion period; D, biodiversity-friendly rotation subplot with exclusion period. ***, $P < 0.001$; ns, $P \geq 0.05$. Error bars represent the standard error of the averages, while letters above histograms indicate significant differences among BR subplots according to Tukey's test. Number of replicates (per year) = 36.

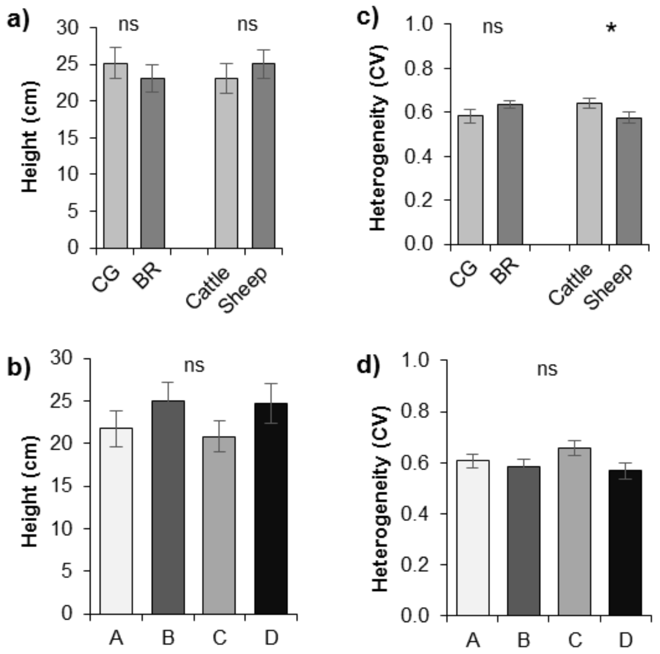

Figure 4.2: average sward height (a and b) and heterogeneity (c and d) during the exclusion period according to the grazing system and the grazer species (a and c) and BR subplot (b and d). CV, coefficient of variation; CG, continuous grazing; BR, biodiversity-friendly rotation; A, B, C, ecological rotation subplots without exclusion period; D, biodiversity-friendly rotation subplot with exclusion period. *, $P < 0.05$; ns, $P > 0.1$. Error bars represent the standard error of the averages. Number of replicates (per year) = 18.

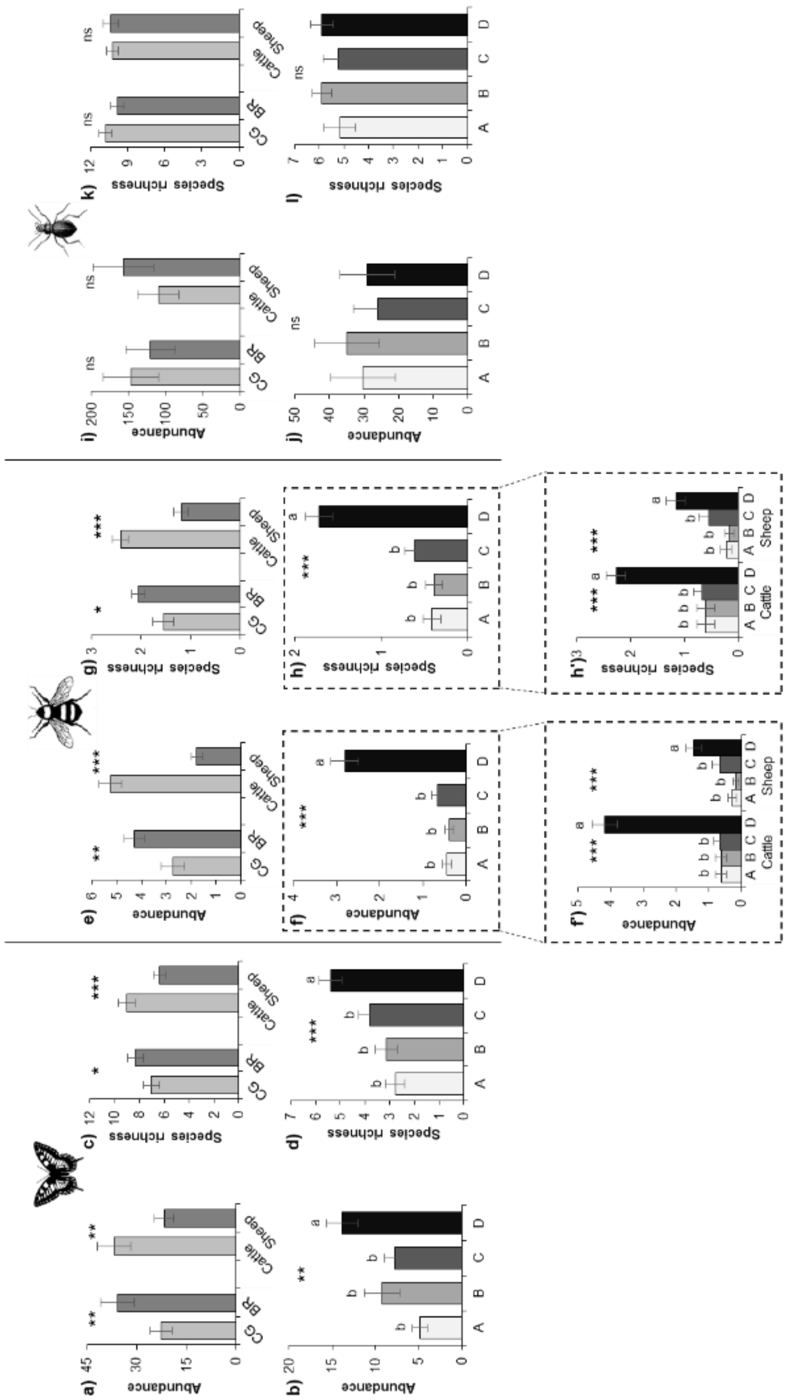

Figure 4.3: insect abundance according to the grazing system and the grazer species (a, butterflies; e, bumblebees; i, ground beetles), BR subplot (b, butterflies; f, bumblebees; j, ground beetles) and the

interaction between grazer species and BR subplot (f ', bumblebees); insect species richness according to the grazing system and the grazer species (c, butterflies; g, bumblebees; k, ground beetles), BR subplot (d, butterflies; h, bumblebees; l, ground beetles) and the interaction among grazer species and BR subplot (h', bumblebees). CG, continuous grazing; BR, biodiversity-friendly rotation. ***, P < 0.001; **, P < 0.01; *, P < 0.05; ns, P ≥ 0.05. Error bars represent the standard error of the averages while letters above histograms indicate significant differences among BR subplots according to Tukey's test. Number of replicates = 36 (butterflies and bumblebees) and 18 (ground beetles).

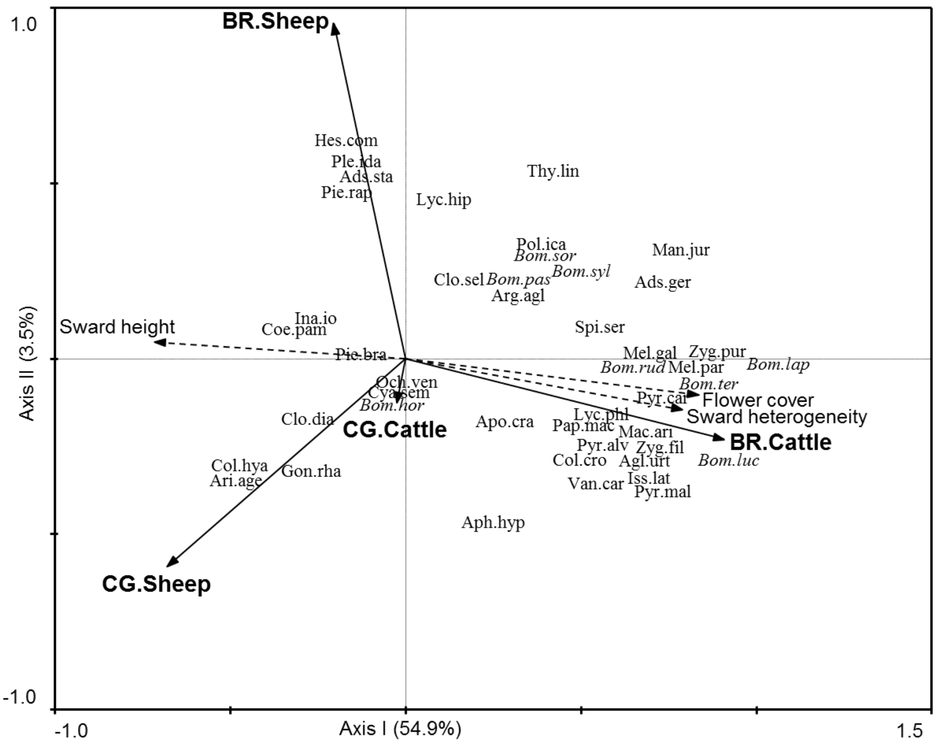

Figure 4.4: RDA ordination biplot showing the effect of the four treatments (solid arrows) on butterfly (regular font) and bumblebee (italics) distribution. Flower cover, sward height and heterogeneity (coefficient of variation) are projected as passive variables (dashed arrows). The variance explained by each axis is given within brackets. CG, continuous grazing; BR, biodiversity-friendly rotation; for insect species abbreviations see Annex 4.D.

Herbage mass and animal performance

The study area was characterised in 2011 and 2012 by lower precipitation (-100 and -86 mm) and higher temperatures (+1.3 and +0.4 °C), whilst in 2013 by higher precipitation (+162 mm) and lower temperatures (-0.3 °C) compared to 1965-2010.

Starting and ending dates of grazing periods were set according to herbage availability, weather conditions and traditional habits of the local farmers. Consequently, cattle and sheep started grazing on May 18th, 2011, May 23rd, 2012 and on June 5th, 2013, whilst they finished on October 4th, 2011, on October 9th, 2012 and on October 22th, 2013, accounting for 140 grazing days per year.

The average annual herbage mass amounted to 2.93 t_{DM} ha^{-1} and did not differ between CG and BR (Figure 4.5a) or among subplots throughout the whole grazing season. Conversely, it was significantly lower in cattle than in sheep plots, except at the beginning of the grazing season (Figure 4.5b). Nevertheless, herbage mass was always comparable when the interaction between the grazing system and grazer species was considered (Figure 4.5c).

No differences in animal live weights were recorded, except for the higher weight of sheep under CG in July, whereas BCS was always comparable along the grazing season for both cattle and sheep (Figures 4.6 and 4.7).

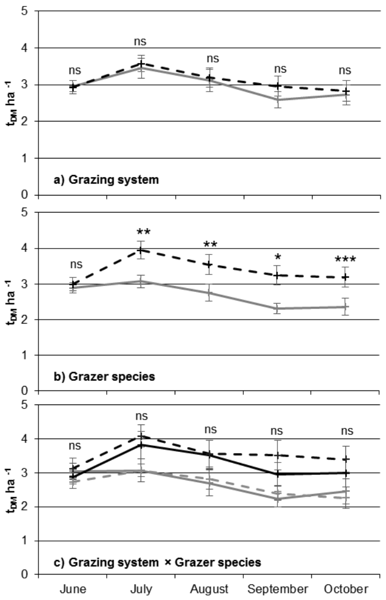

Figure 4.5: Herbage mass during the grazing season according to (a) the grazing system (grey solid line represents continuous grazing - CG - and black dashed line biodiversity-friendly rotation - BR), (b) the grazer species (grey solid line represents cattle and black dashed line sheep) and (c) the grazing system × the grazer species (grey solid line represents CG-cattle, grey dashed line BR-cattle, black solid lines CG-sheep, black dashed line BR-sheep). DM, dry matter based. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; ns, $P \geq 0.05$. Error bars represent the standard error of the averages. Number of replicates = 18 (grazing system and grazer species) and 9 (grazing system × grazer species).

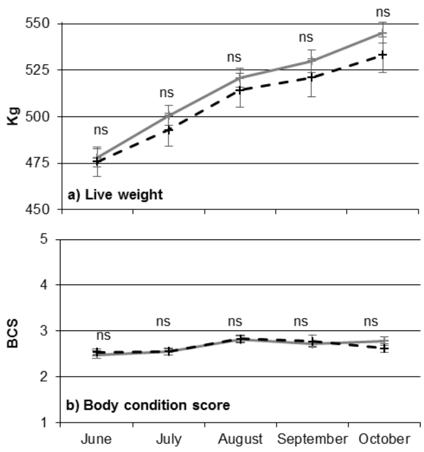

Figure 4.6: Cattle performance during the grazing season in terms of (a) animal live weight and (b) body condition score (BCS); grey solid line represents continuous grazing and black dashed line biodiversity-friendly rotation. ns, $P \geq 0.05$. Error bars represent the standard error of the averages. Number of replicates (per date) = 63.

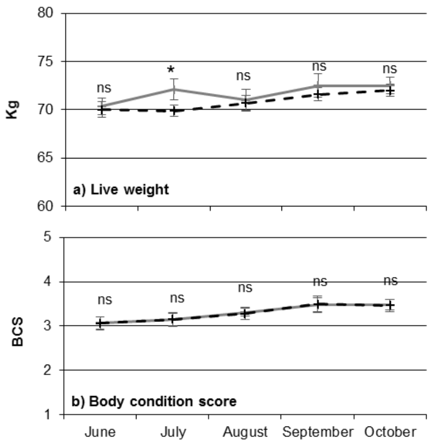

Figure 4.7: Sheep performance during the grazing season in terms of (a) animal live weight and (b) body condition score (BCS); grey solid line represents continuous grazing and black dashed line biodiversity-friendly rotation. *, $P < 0.05$; ns, $P \geq 0.05$. Error bars represent the standard error of the averages. Number of replicates (per date) = 63.

Discussion

The present study evidenced the beneficial effects produced by the implementation of a biodiversity-friendly rotational grazing system, which led to an increase in butterfly and bumblebee abundance and diversity, whilst, at the same time, meeting animal and grassland production objectives. These noteworthy findings likely resulted from the combination of appropriate stocking rate and length of the grazing exclusion period. Both butterfly and bumblebee abundance and diversity showed similar responses to treatments, as both taxa were attracted in D subplots by the temporary increase in resource availability and lack of livestock disturbances, such as grazing and trampling. Moreover, the excluded area may have represented a suitable nesting place for bumblebees (since all species were ground-nesting) as well as for egg-laying and larval development for butterflies during the two-month exclusion period. Indeed, the experiment confirmed that flower cover (mainly forbs and legumes) was strongly affected by grazer species, due to specific intake behaviour, with sheep preferring forbs and legumes and flowering plant parts, whilst cattle are less selective.[29,56] Moreover, the positive interaction found between year and grazer species might indicate that the lower selection for legumes, forbs and flowers by cattle may enhance the overall flower cover, above all in years with favourable weather conditions (e.g. in 2013). Conversely, the BR system in sheep grazed plots allowed for a temporary increase in flower cover, which was, however, insufficient to reach cattle grazed plot levels. The positive effects on insect assemblages were only ascribable to the grazing system applied regardless of grazer species, highlighting that the improvement in insect abundance and diversity can be determined by the implementation of the BR regime or by cattle grazing, independently. However, the multivariate analysis on flower-visiting species evidenced that most of them (including almost all the endangered and locally rare species) were supported by the BR-cattle treatment, due to high flower cover and sward heterogeneity, as suggested by the so-called 'trophic level' hypothesis.[30,57] Nevertheless, since a few different species were advantaged by other treatments (e.g. *B. hortorum* by CG-cattle treatment and *Pieris rapae* (Linnaeus 1758) by BR-sheep treatment), a mosaic of management strategies would be likely to increase flower-visiting insect diversity on a wider scale, as well as other insect taxa diversity (e.g. ants).[58] However, so as to obtain better understanding of butterfly assemblages in further research it would be important to assess also the effects of grazing treatments on the abundance of host plants, which are needed for butterfly spawning and larvae feeding, i.e. to complete their life cycle.[59] Moreover, the effect of the BR system on insect assemblages should also be examined at the end of the growing season, as D subplots may turn into an 'ecological trap'[60] when re-grazed after the exclusion period. Indeed, although the subplots which were not grazed during the main flowering period did attract adult insects, their eggs and larvae or nests might later have suffered from livestock disturbances in August. Thus, it would be important to discriminate if the observed increase in flower-visiting insect abundance and diversity only constituted a temporary concentration of adults (the so-called 'concentration effect') and not a real and sustained population-level effect.[61,62] A longer monitoring period across years would allow to disentangle these effects by evaluating to what extent an increase in butterfly and bumblebee populations occurs in the long-term, whereas the non significant interactions among treatments and years suggested a concentration effect over the timespan considered. However, an annual increase in both abundance and biodiversity of flower-visiting insects, even if limited to the two-month exclusion period, still can enhance the level of ecosystem services provided, such as pollination.

Unexpectedly, the average sward height was not affected by the grazing system, maybe due to the relatively high stocking rate applied and the homogeneity of grassland composition and distribution, which determined a homogeneous exploitation by livestock under both systems. This result was also confirmed by the lack of differences in sward heterogeneity between BR and CG and among BR subplots. Consequently, grassland structural homogeneity may have determined the lack of effects in ground beetle assemblages, since these taxa are markedly affected by grassland heterogeneity[63].

The differences in herbage mass levels observed between cattle and sheep grazing from July onwards was an unforeseen result, as stocking rate was comparable between cattle and sheep at the beginning of each year. However, the cattle stocking rate involved heifers, that increased their live weight during each grazing season (on average + 58 kg, + 12%), whilst the sheep stocking rate involved dry ewes, that had a much more stable live weight (on average + 1.5 kg, + 2%). This is why herbage intake and mass could have been partly affected by different live weight gains. Nevertheless, the interaction between the grazer species and the grazing system was not significant for herbage mass, which was comparable between CG and BR. Studies carried out in other biogeographic areas and environments did not detect differences in herbage mass when CG was compared to rotational grazing systems.[64–68] Even if in the CG animal live weight was higher in the mid-grazing season for sheep, differences in terms of kilograms were negligible, as they were less than 3.5% of the live weight. Moreover, the BCS on the same recording date was not affected by these small variations in animal live weight. Similarly, recent studies carried out in European mountain semi-natural grasslands reported comparable outputs in animal performance between CG and rotational grazing systems.[23,69] Thus, not only did the BR system provide remarkable results as to flower-visiting insects, but it also maintained animal production levels, ensuring unvaried economic returns for farmers, whilst, at the same time, enhancing ecosystem diversity. Moreover, the implementation of a BR system is not only biologically but also economically sustainable, as it requires limited additional costs and work for the farmers, who have to fence the subplots about twice a month.

Grazing exclusion repeated several years over the same area could affect plant species competition, vegetation dynamics, leading to change in species relative abundance, with cascade effects on insect communities, herbage mass and animal performance.[70] Therefore, so as to allow for a homogeneous distribution of the benefits of BR over the whole grazed area, it might well be advisable to implement a rotation of the grazing exclusion area amongst the four BR subplots.

Conclusions

The present study demonstrated the effectiveness of the innovative ‘biodiversity-friendly rotational’ grazing system for the enhancement of flower-visiting insect abundance and diversity in semi-natural grassland environments, when compared to a continuous grazing system. The beneficial effects on butterflies and bumblebees from grazing exclusion of one quarter of the BR enclosures for two months during the flowering peak (June to July) were more remarkable under cattle than sheep grazing. Moreover, most flower-visiting species, including rare species, were positively influenced by the BR-cattle treatment, as they were attracted by its high flower cover and sward heterogeneity. Conversely, the BR grazing system was not effective in enhancing ground beetle assemblages. Neither herbage mass nor animal performance were negatively affected by the BR system, confirming the promising opportunities offered by this innovative grazing system to maintain the economic returns for farmers whilst enhancing ecosystem diversity. However, additional research on the type and extent of the effects of the ‘biodiversity-friendly rotational’ grazing system on insect assemblages, botanical composition, herbage mass, and animal performance in the long-term appears warranted.

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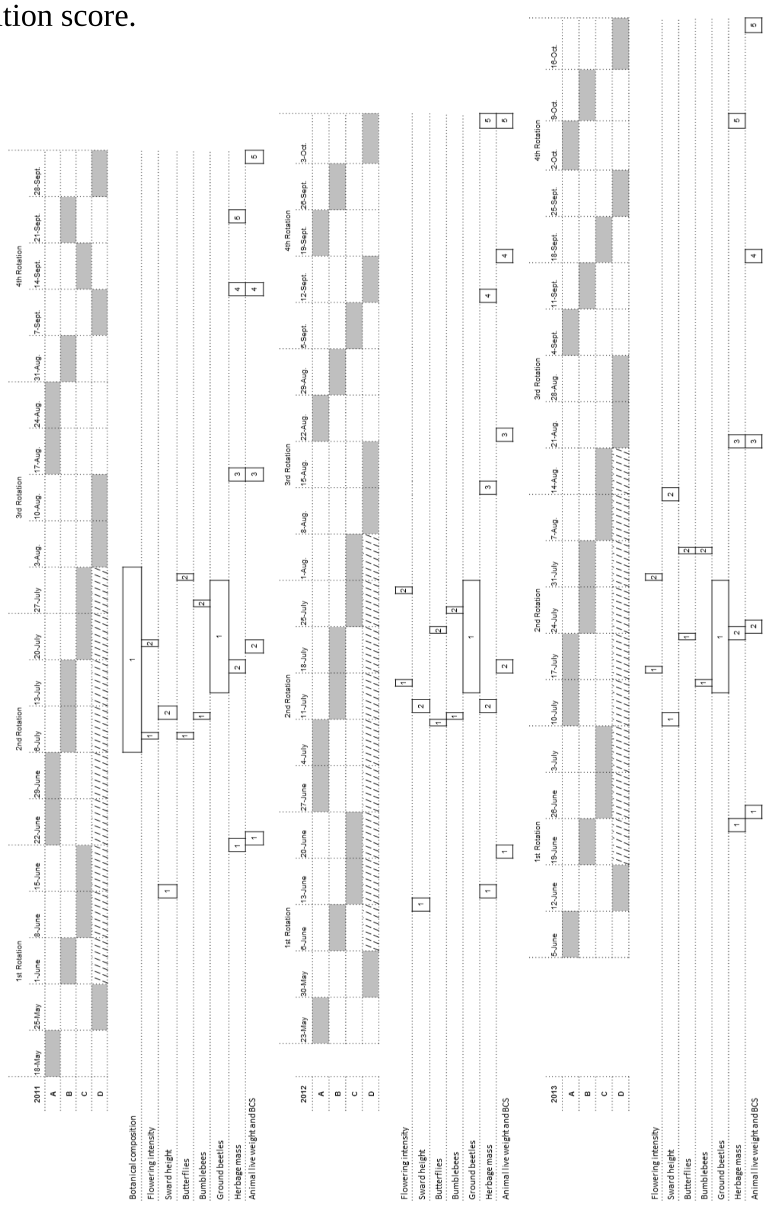
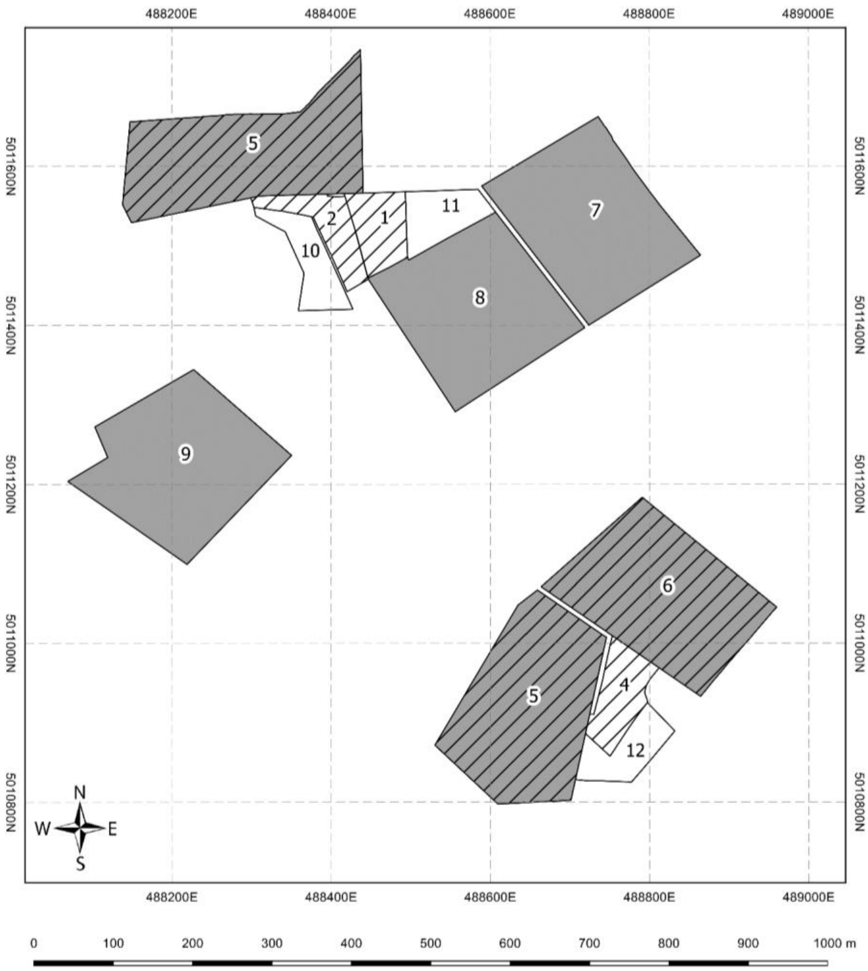
Annex 4.A

The grazing schedule for each year of the experiment, detailed for biodiversity-friendly rotation subplots. Grey rectangles indicate when a subplot is under grazing, while dashed rectangles indicate subplot D exclusion from grazing during the main flowering period. Plots managed under continuous grazing followed the same starting and ending dates. Dates of each surveyed variable are represented by white rectangles at the bottom, where numbers indicate the replicates within the year. BCS, body condition score.



Annex 4.B

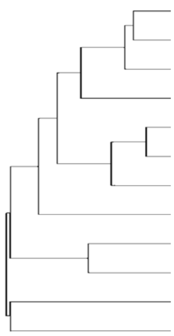
A map of the experimental plots. Coordinates are provided in the WGS 84 / UTM zone 31N. Each number indicates one plot: grey plots, cattle grazing; white plots, sheep grazing; dashed plots, biodiversity-friendly rotation plots.



Annex 4.C

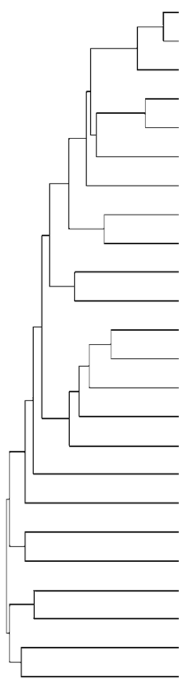
Dendrograms with the ordinations obtained by hierarchical cluster analyses of the plot (a) and biodiversity-friendly rotation subplot (b) plant communities, with the indication of the grazing system, the grazer species and the biodiversity-friendly rotation subplot. CG, continuous grazing; BR, biodiversity-friendly rotation; A, B, C, ecological rotation subplots without exclusion period; D, biodiversity-friendly rotation subplot with exclusion period. Botanical composition (relative abundance percentage of dominant species) is provided for each plot and subplot.

a)



Grazer species	Grazing system	Botanical composition
Sheep	BR	*Agrostis capillaris* 22.3%; *Festuca nigrescens* 13.3%; *Trifolium repens* 9.5%
Sheep	CG	*Agrostis capillaris* 19.3%; *Festuca nigrescens* 15.4%; *Festuca lemanii* 7.7%
Cattle	BR	*Agrostis capillaris* 22.1%; *Festuca nigrescens* 14.9%; *Festuca lemanii* 8.8%
Cattle	BR	*Agrostis capillaris* 19.3%; *Festuca nigrescens* 13.1%; *Helianthemum nummularium* 7.7%
Cattle	CG	*Agrostis capillaris* 16.6%; *Festuca nigrescens* 13.3%; *Festuca lemanii* 10.7%
Cattle	BR	*Agrostis capillaris* 17.9%; *Festuca nigrescens* 11.8%; *Trifolium repens* 10.1%
Cattle	CG	*Agrostis capillaris* 17.5%; *Festuca nigrescens* 15.1%; *Trifolium repens* 13.4%
Cattle	CG	*Agrostis capillaris* 14.4%; *Festuca nigrescens* 8.2%; *Trifolium repens* 6.4%
Sheep	BR	*Agrostis capillaris* 19.7%; *Festuca nigrescens* 16.3%; *Achillea millefolium* 7.1%
Sheep	CG	*Festuca nigrescens* 14.4%; *Agrostis capillaris* 13.1%; *Poa pratensis* 11.0%
Sheep	CG	*Agrostis capillaris* 15.1%; *Festuca nigrescens* 11.7%; *Helianthemum nummularium* 9.7%
Sheep	BR	*Agrostis capillaris* 18.8%; *Festuca nigrescens* 11.8%; *Galium verum* 6.9%

b)



Grazer species	Plot	Subplot	Botanical composition
Sheep	1	A	*Agrostis capillaris* 23.5%; *Festuca nigrescens* 18.6%; *Poa pratensis* 10.8%
Sheep	1	B	*Agrostis capillaris* 20.0%; *Festuca nigrescens* 20.0%; *Achillea millefolium* 12.0%
Sheep	2	A	*Festuca nigrescens* 22.3%; *Agrostis capillaris* 18.4%; *Poa pratensis* 12.6%
Cattle	3	C	*Agrostis capillaris* 32.9%; *Festuca nigrescens* 19.3%; *Thymus pulegioides* 8.7%
Sheep	4	B	*Agrostis capillaris* 33.2%; *Festuca nigrescens* 11.7%; *Festuca lemanii* 7.8%
Sheep	2	D	*Agrostis capillaris* 28.1%; *Galium verum* 12.6%; *Festuca nigrescens* 11.6%
Cattle	5	A	*Agrostis capillaris* 22.4%; *Festuca nigrescens* 16.3%; *Festuca lemanii* 9.2%
Cattle	3	A	*Agrostis capillaris* 17.0%; *Festuca lemanii* 17.0%; *Festuca nigrescens* 11.7%
Cattle	5	B	*Festuca lemanii* 21.0%; *Agrostis capillaris* 18.9%; *Festuca nigrescens* 12.6%
Cattle	6	B	*Agrostis capillaris* 24.8%; *Koeleria pyramidata* 11.6%; *Festuca nigrescens* 9.5%
Sheep	1	D	*Agrostis capillaris* 18.0%; *Polygonum bistorta* 12%; *Koeleria pyramidata* 11.0%
Cattle	6	C	*Trifolium repens* 20.2%; *Festuca nigrescens* 17.0%; *Agrostis capillaris* 16.0%
Sheep	4	C	*Trifolium repens* 19.2%; *Agrostis capillaris* 17.3%; *Festuca nigrescens* 14.4%
Cattle	5	D	*Trifolium repens* 15.3%; *Avenula pratensis* 11.5%; *Achillea millefolium* 8.6%
Sheep	2	B	*Agrostis capillaris* 13.4%; *Trifolium repens* 12.5%; *Cirsium arvense* 10.5%
Cattle	5	C	*Agrostis capillaris* 18.2%; *Trifolium repens* 12.1%; *Festuca nigrescens* 9.9%
Sheep	4	A	*Agrostis capillaris* 18.9%; *Festuca nigrescens* 15.1%; *Helianthemum nummularium* 9.4%
Cattle	3	D	*Agrostis capillaris* 18.8%; *Festuca nigrescens* 13.1%; *Helianthemum nummularium* 11.0%
Cattle	6	A	*Festuca nigrescens* 14.8%; *Helianthemum nummularium* 11.9%; *Agrostis capillaris* 9.9%
Sheep	1	C	*Festuca nigrescens* 17.6%; *Agrostis capillaris* 13.5%; *Helianthemum nummularium* 6.2%
Cattle	6	D	*Agrostis capillaris* 15.4%; *Festuca nigrescens* 10.6%; *Helianthemum nummularium* 9.7%
Sheep	2	C	*Helianthemum nummularium* 13.9%; *Sanguisorba minor* 12.9%; *Agrostis capillaris* 10.9%
Cattle	3	B	*Agrostis capillaris* 15.3%; *Festuca nigrescens* 12.2%; *Helianthemum nummularium* 11.2%
Sheep	4	D	*Agrostis capillaris* 14.6%; *Helianthemum nummularium* 12.6%; *Briza media* 8.7%

Annex 4.D

Insect species and their abundance of the three insect taxa recorded during the three-year experiment.

Insect species	Abbreviation[a]	Frequency
Butterflies		
Adscita geryon	Ads.ger	62
Adscita statices	Ads.sta	1
Aglais urticae	Agl.urt	19
Aphantopus hyperanthus	Aph.hyp	152
Aporia crataegi	Apo.cra	4
Argynnis aglaja	Arg.agl	38
Aricia agestis	Ari.age	1
Clossiana dia	Clo.dia	5
Clossiana selene	Clo.sel	17
Coenonympha pamphilus	Coe.pam	275
Colias crocea	Col.cro	4
Colias hyale	Col.hya	1
Cyaniris semiargus	Cya.sem	2
Gonepteryx rhamni	Gon.rha	3
Hesperia comma	Hes.com	3
Inachis io	Ina.io	9
Issoria lathonia	Iss.lat	44
Lycaena hippothoe	Lyc.hip	32
Lycaena phlaeas	Lyc.phl	1
Maculinea arion	Mac.ari	3
Maniola jurtina	Man.jur	111
Melanargia galathea	Mel.gal	35
Mellicta parthenoides	Mel.par	176
Ochlodes venatus	Och.ven	1
Papilio machaon	Pap.mac	1
Pieris brassicae	Pie.bra	4
Pieris rapae	Pie.rap	5
Plebejus idas	Ple.ida	1
Polyommatus icarus	Pol.ica	5
Pyrgus alveus	Pyr.alv	62
Pyrgus carthami	Pyr.car	53
Pyrgus malvae	Pyr.mal	8
Spialia sertorius	Spi.ser	15
Thymelicus lineola	Thy.lin	404
Vanessa cardui	Van.car	5
Zygaena filipendulae	Zyg.fil	18
Zygaena purpuralis	Zyg.pur	504
Total butterflies		1913

Insect species	Abbreviation[a]	Frequency
Bumblebees		
Bombus hortorum	Bom.hor	3
Bombus lapidarius	Bom.lap	76
Bombus lucorum	Bom.luc	8
Bombus pascuorum	Bom.pas	3
Bombus ruderarius	Bom.rud	7
Bombus soroeensis	Bom.sor	6
Bombus sylvarum	Bom.syl	13
Bombus terrestris	Bom.ter	137
Total bumblebees		253
Ground beetles		
Amara aenea		56
Amara aulica		1
Amara convexior		31
Amara familiaris		3
Amara lunicollis		586
Calathus fuscipes		105
Calathus melanocephalus		27
Carabus auronitens		231
Carabus cancellatus		9
Carabus violaceus		1091
Cicindela campestris		1
Harpalus latus		89
Nebria brevicollis		1
Poecilus cupreus		20
Poecilus kugelanni		3
Poecilus versicolor		165
Pseudoophonus rufipes		3
Pterostichus madidus		185
Pterostichus melanarius		939
Total beetles		4685

[a] abbreviations for insect species used in the RDA ordination biplot showed in Figure 4.4.

5. Overall considerations and perspectives

The outcomes of this thesis highlight the remarkable importance for European mountain territory of pastureland diversity, which contributes i) to provide forage for livestock nutrition, as the base for high-quality animal products, and ii) to maintain and enhance habitat biodiversity. Mountain farming systems are thus closely connected to these environments, from which they are sustained and which they preserve in their wide heterogeneity.

The relevance of this thesis was to contribute to extending the current research concerning forage quality and grassland biodiversity conservation to more complex or unexplored situations. The results achieved by each trial allowed significant considerations and provided helpful suggestions for a proficient and sustainable management of mountain pasturelands.

Relationships between vegetation and chemical composition of forages

Since the appeal of food with healthy properties is increasing in current developed countries, the identification of raw materials for the production of high-quality animal-derived food products is an ongoing challenge.[1] It is well acknowledged that livestock feeding on species-rich forages can supply excellent animal products, particularly milk.[2] As an example, the 'Piedmont Noble Milk' project (2013-2014) highlighted that cows grazing on alpine pastures (the same mentioned in Chapter 2) provided milk with superior nutraceutical characteristics if compared to that one usually available on the market (see also Annex A). In grass-fed milk total conjugated linoleic acid (CLA) content was more than 0.25g 100 g^{-1} fat while the ratios between linoleic and α-linolenic acids and between PUFA of the omega-6 and omega-3 series were close to 1:1 (i.e. the best desirable value from a nutritional/health point of view). These latter values were more than four-fold lower than the corresponding values obtained for commercial milk, bearing out the grass-fed milk as a healthy food for human consumption.

Unfortunately, it was not possible to analyse and study the differences among the milks produced by cows grazing on the different grassland types mentioned in Chapter 2, but previous research recorded remarkable results in similar trials.[3–5] Nevertheless, the comparison among the studied mesophilic and dry grasslands highlighted that forage quality can significantly change among different vegetation communities and sites. Grassland diversity, in terms of plant species composition, corresponded to a diversity in forage potentials, in terms of chemical attributes. All the considered grassland types showed a good proximate composition and fatty acid profile, but the mesophilic ones appeared more suitable as base feed for animal-derived products with healthy properties. Therefore, it would be of great interest to encourage the exploitation of mesophilic grasslands aiming at valuable productions. At the same time, it should be advisable to promote the implementation of agricultural practices for improving the development and maintenance of mesophilic grassland types.[6,7]

For instance, a case study of a pastoral strategy aiming at restoring the cover of mesophilic vegetation is reported in Annex B. In this experiment, sub-alpine and alpine vegetation communities were shrub encroached: an extreme but widespread situation in European mountain pastures linked to the decline (or cessation) of pastoral activity.[8,9] The implementation of a pastoral technique such as the arrangement of temporary night camp areas was a sustainable strategy to reverse shrub encroachment and increase meso-eutrophic species cover and related forage quality. More generally, the management (i.e. distribution, stocking rate, and use frequency) of animal resting areas and water and salt sources together with manure management could be effective (and in many cases essential) tools to the maintenance and restoration of productive and high-quality forage resources.[10–12]

This diversity in high-quality forages has to be preserved over European mountains, since it represents a strength for farming systems, from which they are able to obtain the optimal animal products, in terms of both quality and differentiation.[13,14]

Fodder tree species: foliage characterisation

'Palatable trees on a property can make the difference between survival and disaster for stock during drought'.[15] Although this statement dates from 1969 and tree and shrub foliage is largely recognised as an important forage resource in many part of the world since an even longer time,[16–25] their quality evaluations are lacking, especially in Europe.

The exploitation of tree and shrub foliage as forage, especially by goats, is an increasingly rediscovered tool in Europe, able to contribute to farming system sustainability and enhancement.[26–28] Nevertheless, in

European environments only in Greece this matter has been widely explored for Mediterranean tree and shrub species in the last decades,[29–33] while other authors dealt with temperate plants,[34–37] but carrying out only preliminary or historiographic studies. The few works analysing the nutritional potential of European fodder tree species focused only on a limited number of chemical features or did not consider their interactive effects on ruminant digestion.[38–42]

In Chapter 3 the first production and chemical (especially FA) records for *Fraxinus excelsior*, *Acer pseudoplatanus*, *Sorbus aucuparia*, and *Salix caprea* foliage are reported. Results suggest that these four species can be regarded as good feedstuff for goat nutrition, due to their proximate, fatty acid, and phenolic profile and their digestibility values. Among them, *Fraxinus* showed the best values, also in terms of foliage production. This study can be considered a starting point for the complete evaluation of the interactions between fodder tree species and ruminant nutrition in Europe and further research appears advisable. However, these first outcomes suggest that the use of fodder tree species can be implemented and encouraged also in European environments to support mountain farming systems.

More specifically, fodder tree and shrub species can contribute to achieve some agricultural and environmental objectives, such as:[43]

i) to provide high-quality forage along the vegetative season, especially when herbage quality decreases due to reduced water availability and/or advancement of plant phenological stage;
ii) to allow the diversification of the derived animal products, if compared to traditional forages;
iii) to cope with Global Climate Change, as forage resource with a more stable chemical quality than herbage in drought periods;
iv) to allow multi-layer grazing-browsing systems, in association with grasslands;
v) to reduce the grazing pressure on grassland areas.

Nowadays trees and shrubs are also considered diversification factors in the agricultural landscape, encouraged by European politicy and legislation, whether actively managed and interspersed with open vegetation communities.[44–46] Indeed, woody structures can provide habitats and resources for a wide variety of ordinary, heritage, or threatened species, contributing to landscape and biodiversity conservation.[47]

Grazer species and grazing system effects on grassland species diversity

As above mentioned, grassland ecosystems are among the most species-rich habitats in European environments, but they are more and more threatened by agricultural intensification, abandonment, and land fragmentation.[48–51] The evaluation and implementation of new or dismissed pastoral strategies and management practices for preserving these complex semi-natural environments are becoming essential nowadays.[52–55]

An example of management technique able to contrast abandonment and to enhance species richness is that reported in Annex B. The implemented strategy was able to reverse shrub encroachment and to improve plant diversity through the management of cattle night camp areas, without any other mechanic or chemical action. This experiment confirmed that pastoral management is one of the main factors able to restore plant diversity, which is also an acknowledged base-condition for grassland productivity and ecosystem diversity.[56–60]

Also, the 'biodiversity-friendly' rotational grazing system reported in Chapter 4 could be a valuable strategy for the enhancement of grassland biodiversity. The novelty of this experiment was to compare different grazing regime (as the combination of grazer species and grazing system) effects on different grassland insect taxa. The results achieved by this innovative strategies are promising, especially for the enhancement of flower-visiting insect assemblages. This could be considered a major objective within biodiversity conservation programmes, since grassland insects have a key role in food chain (as plant eaters as well as feedstuff for entomophagous species) and allow entomogamous plant reproduction (as pollinator agents).[61–63]

The biodiversity-friendly rotational grazing system was compared to extensive continuous grazing, since the latter can be considered as one of the most common grazing system, especially applied for cattle, which replaced the traditional shepherded grazing, mainly because of its lower management costs.[64,65] Even if less expensive, continuous grazing is generally unfavourable for both grasslands and farming system, since it does not allow a rational and complete use of pastoral surfaces, shaping the pastures in under- and overgrazed areas, with negative effects on grassland biodiversity and forage value.[66,67] The second noteworthy outcome of the BR grazing strategy was to ensure unvaried economic returns for farmers, by allowing forage and livestock productions comparable to those of the continuous grazing regime. A win-win management strategy like this could be proposed particularly in territories committed to biodiversity conservation, such as natural parks. In these areas, management authorities could address the farmers to choose and apply the best grassland management solution, as the appropriate combination of grazer species and grazing management, with fulfilling outcomes for both the environment and farming system. Indeed, although there are often local or European projects financing initiatives of biodiversity conservation, it would be advisable to assess the economic sustainability of the implemented strategies, in order to allow the post-project prosecution as well as the extension to other territories.[68]

Conclusions

The author hopes that this thesis could contribute to improve the scientific knowledge concerning the valorization of pasturelands and livestock farming systems in European mountains. In these environments, an appropriate management should aim at the optimization of the available resources, to obtain high-quality food products while preserving ecosystem values, such as plant and animal diversity. The accomplishment of this aim could definitively let mountain users to gain and enjoy the wealth and variety of pasturelands and all their positive externalities.

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General appendix

Annex A

Production regulations and characteristics of cow *Piedmontese Noble Milk*

An edited version of this oral contribution has been published in the *Italian Journal of Animal Science* 2015, 14, supplement 1: *ASPA 21st Congress, Milano (Italy), 9-12 June 2015 – Book of Abstracts*, Giovanni Savoini ed., p. 33 and it is here reproduced with permission from the authors:

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The need to combine the quality of animal products with the human health as well as to revitalize the milk chain has recently led to the development of a new model based on milk and dairy products characteristics and specificity. The model, called *Latte Nobile* (Noble Milk), was conceived in South Italy in 2011, and it is now fast spreading in other parts of the country. In Piedmont (NW Italy) a two-year project, developed on different research lines (agronomy, animal nutrition, chemical and microbiological features of the product, heat treatment processes, shelf-life and traceability), aimed to adapt the *Noble Milk* model to the environmental and productive conditions of this region. Thanks to the results of the project, *Piedmontese Noble Milk* (PNM) production regulations have been proposed and the product is expected to enter the milk market in 2015. According to the regulations, the PNM is obtained from animals yielding maximum 6000 kg of milk per lactation and fed fresh grass and/or hay from local mixed grasslands (minimum 70% of daily dry matter intake). Silages and genetically modified feedstuffs are forbidden and only pastures or meadows with at least four dominant species are allowed for animal feeding. Pastures and meadows must also constitute at least the 50% of the farm forage system. Cows must graze for at least 150 days per year under a stocking rate not exceeding 1.5 animal unit ha^{-1} $year^{-1}$. Animal welfare has to be guaranteed in accordance with the Welfare Quality standards. PNM contains >0.25g 100 g^{-1} fat of total conjugated linoleic acid (CLA) and >0.50g 100 g^{-1} fat of total omega-3 fatty acids (n3 FA). According to the results of the project, typical values range from 0.29 (winter) to 1.71 (summer) g 100 g^{-1} fat and from 0.60 (winter) to 1.99 (summer) g 100 g^{-1} fat for CLA and n3 FA, respectively. The linoleic/alpha-linolenic acids ratio must be lower than 4 all year round. The PNM can be sold raw or pasteurized; the findings of the heat treatment trials suggest that, to preserve the best chemical, nutritional and organoleptic characteristics of the product, PNM should be pasteurized at 72°C for at least one minute. A specific software has been implemented to guarantee the traceability of all the PNM production process, from the forage system to the derived *Piedmontese Noble Milk* chemical and microbiological characteristics.

Annex B

Temporary night camp areas: an effective way to restore shrub-encroached grasslands using livestock

An edited version of this oral contribution has been published in *Mountain pastures and livestock farming facing uncertainty: environmental, technical and socio-economic challenges – Proceedings of the 19th Meeting of the Sub-Network on Mediterranean Pastures of the FAO-CIHEAM International Network for the Research and Development of Pastures and Fodder Crops*, Zaragoza (Spain), 14-16 June 2016, Isabel Casasús and Giampiero Lombardi eds., pp. 241-245 and it is here reproduced with permission from the authors:

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Abstract

Over the last decades, the decline of agro-pastoral activities in many European mountain regions has led to an extensive tree and shrub-encroachment of semi-natural grasslands, with a reduction of the ecosystem services provided by these open habitats. In 2011, temporary night camp areas (TNCA) for cattle were arranged in shrub-encroached areas to reverse this process and to restore semi-natural sub-alpine grasslands within the Val Troncea Natural Park in the western Italian Alps. Vegetation surveys were conducted along permanent transects from 2011 to 2015 and the effects on vegetation structure (cover and height), vegetation composition (cover of species belonging to different phytosociological units and species richness), and pastoral value of forage were assessed. Four years after their implementation, TNCA were effective in reducing the cover of shrubs and increasing herbaceous cover and height ($p < 0.01$). Moreover, the cover of species typical of mesophilic and nutrient-rich grasslands and the cover of fringe and tall herb grassland species significantly increased ($p < 0.05$). Conversely, plant biodiversity did not change over time, but pastoral value was significantly enhanced ($p < 0.001$). These findings highlight that the establishment of TNCA can be an effective and sustainable practice to restore shrub-encroached grasslands in steep and rugged mountain locations.

Keywords. Alps – Grazing - Pastoral value – Plant biodiversity – Semi-natural grasslands

Aires de repos nocturne temporaires: un moyen efficace pour restaurer les prairies envahies par les arbustes en utilisant le bétail.

Résumé

Dans les dernières décennies, le déclin des pratiques agro-pastorales au sein de nombreuses régions européennes de montagne a amené à un empiétement extensif des prairies semi-naturelles par les arbres et les arbustes, avec une réduction des services écosystémiques fournis par ces habitats ouverts. En 2011, des aires de repos nocturne temporaires (ARNT) pour les bovins ont été arrangées dans des zones envahies par arbustes afin de renverser ce processus et de restaurer les prairies semi-naturelles subalpines au sein du Parc Naturel Val Troncea (Alpes italiennes occidentales). Entre 2011 et 2015, nous avons effectué des relevés de végétation le long des transepts permanents et nous avons déterminé les effets provoqués sur la structure de la végétation (couverture et hauteur), sur la composition botanique (couverture des espèces reconductibles à différentes unités phytosociologiques et indices de biodiversité) et sur certaines variables de la communauté des plantes (valeur pastorale et valeur de disponibilité des nutriments du sol d'après Landolt). Après quatre années d'arrangement, les ARNT se sont révélées efficaces dans la réduction de la couverture des arbustes et dans l'augmentation de couverture et hauteur de l'herbe ($p < 0.01$). De plus, la couverture des espèces typiques des prairies mésophiles et grasses et la couverture des espèces hautes et d'écotone ont augmenté significativement ($p < 0.05$). Au contraire, la biodiversité générale n'a pas changé au cours de l'expérimentation, mais la valeur pastorale a été améliorée significativement ($p < 0.001$). Ces résultats soulignent que l'arrangement des ARNT peut être une pratique efficace et soutenable pour restaurer les prairies envahies par les arbustes en zones de montagne raides et accidentées.

***Mots-clés**. Alpes – Biodiversité des plantes –Pâturage ciblé – Prairies semi-naturelles –Valeur Pastorale*

Introduction

Since the end of the Second World War, agro-pastoral abandonment has resulted in an extensive tree and shrub-encroachment of former semi-natural grasslands in different European mountain chains.[1] Sub-alpine meso-eutrophic grasslands have been one of the most abandoned habitats, above all in the south-western Italian Alps, where nowadays they amount to about 15% of total grassland area.[2]

The implementation of temporary night camp areas (TNCA) for cattle in shrub-encroached areas can be used to reduce shrub cover and restore meso-eutrophic grassland vegetation, as described by Pittarello et al.[3] In this study the effects on vegetation were examined three years after treatments. However, to better understand the effects of this restoration practices on vegetation, a longer period of monitoring is often needed. Therefore, the aim of this research was to assess the effects produced by TNCA on i) vegetation structure and ii) botanical composition to identify their potential to restore sub-alpine meso-eutrophic grassland vegetation over a longer period (i.e. four years after treatment).

Materials and methods

Study area and experimental design

The study area was located in Val Troncea Natural Park, south-western Italian Alps, with altitudes ranging from 1,960 to 2,360 m a.s.l. Grasslands were mainly dominated by *Festuca curvula* Gaudin, *Nardus stricta* L. and *Festuca* gr. *rubra* and they were encroached by *Juniperus nana* Willd. and *Rhododendron ferrugineum* L. The area (about 75 ha) was grazed for three weeks in July 2011 by 160 beef cows. The paddock was stocked at the same stocking rate in the same period in 2012, 2013, 2014, and 2015. Four TNCA of about 1,100 m^2 each were established within large patches of shrub-encroached grasslands at comparable altitudes, as described in Tocco et al.[4] All cattle were confined for two consecutive nights within each TNCA, which was bordered by electric fences and an area of 7 m^2 per night was available to each cow, resulting in a stocking density of 1200 AU ha^{-1}. Each TNCA was considered as a treatment site and paired with a control site, which was not fenced.

Vegetation surveys

Botanical composition was determined using the vertical point-quadrat method along permanent linear transects[4] and surveys were carried out in late June in 2011 (pre-treatment survey), 2012, 2013, 2014, and 2015. Within 1-m buffer around the transect line, the percentages of shrub and herbaceous covers were visually estimated. Furthermore, 20 measurements of the height of the herbaceous layer were randomly carried out with the sward stick method.

Data analysis

For each plant species recorded in each transect, the percent frequency of occurrence (i.e. an estimate of Species canopy Cover, %SC) and the Species Relative Abundance (SRA) were calculated as described in Pittarello et al.[3] Each plant species was related to its phytosociological optimum at the class level, according to Aeschimann et al.[5] Groups of classes with physiognomic, ecological and floristic similarity (called 'vegetation units') were defined and the sum of the %SC of the species belonging to each unit was computed.[3] Moreover, an Index of Specific Quality (ISQ) was attributed to each species according to Cavallero et al.[2] and forage pastoral value was calculated in each transect on the basis of SRA and ISQ.

Generalized Linear Mixed Models (GLMMs) were used to test for annual differences between treatment and control sites for vegetation variables. Treatment was considered as a fixed factor, whereas vegetation transect was considered as a random factor nested within area. A Poisson distribution was specified for count variables which were not overdispersed, whereas a negative binomial distribution was used for overdispersed count data. When the normality of the distribution was met a normal distribution was used for continuous data, otherwise a gamma distribution was specified.

Results and discussion

Four years after the implementation of TNCA, the percentage of shrub cover was reduced, while the herbaceous cover increased (Table C1). Most of the reduction of shrub cover occurred due to the intense trampling damages caused by cattle and the occurring bare ground gaps have been progressively recolonized by herbaceous vegetation. The average herbaceous height constantly increased for four years after treatments, mainly due to the intense fertilization effect by dung and urine deposition within TNCA. Both herbaceous species belonging to meso-eutrophic grassland and fringe and tall-herb grassland vegetation units increased over time, while boreal shrubland and woodland species were reduced. Indeed, the cover of meso-eutrophic species was more than four times higher compared to the pre-treatment state. Enhanced availability of nitrogen in the soil deriving from intense fecal deposition favored the recolonization of the bare ground gaps by meso-eutrophic plant species, such as *Poa pratensis*, *Agrostis tenuis*, and *Poa alpina*. These species have also a high index of specific quality, so a significant improvement of forage quality of about 80% has been assessed four years after treatment. Even though species richness significantly increased in 2014, there was not difference between TNCA and paired control areas in the following year. This result shows the importance of inter-annual fluctuations in plant diversity patterns and the need of long-term vegetation monitoring to understand the overall effectiveness of grassland restoration practices

Conclusions

In conclusion, the implementation of temporary night camp areas was an effective pastoral practice to reverse shrub-encroachment, restore meso-eutrophic grassland vegetation and increase herbage mass and forage quality.

Table C1. Effects of temporary night camp areas (TNCA) on vegetation structure, vegetation units, number of species and forage pastoral value, with respect to paired control sites. Values shown are the mean and the standard error (SE) of the mean, and in 2011 they refer to pre-treatment. Asterisks represent the statistical significance level of differences between treatment and control sites: *** = P < 0.001; ** = P < 0.01; * = P < 0.05; . = P < 0.1; n.s. = not significant (P > 0.05).

		Treatment	Control	
		mean ± SE	mean ± SE	P
Vegetation structure variables				
Shrub cover (%)				
	2011	56 ± 4	57 ± 4	n.s.
	2012	29 ± 5	57 ± 5	***
	2013	29 ± 5	58 ± 5	***
	2014	21 ± 5	59 ± 5	***
	2015	27 ± 6	55 ± 7	**
Herbaceous cover (%)				
	2011	33 ± 3	32 ± 5	n.s.
	2012	40 ± 5	33 ± 5	n.s.
	2013	52 ± 6	33 ± 4	*
	2014	64 ± 5	33 ± 4	***
	2015	64 ± 6	41 ± 7	.
Average herbaceous height (cm)				
	2011	10 ± 1	10 ± 1	n.s.
	2012	13 ± 1	11 ± 1	*
	2013	16 ± 2	10 ± 1	**
	2014	19 ± 1	13 ± 1	***
	2015	27 ± 4	15 ± 2	***
Vegetation units				
Meso-eutrophic grassland species cover (%)				
	2011	8 ± 2.42	4.5 ± 0.99	n.s.
	2012	14.8 ± 4.33	8.5 ± 2.42	n.s.
	2013	17.3 ± 4.1	6.5 ± 1.87	*
	2014	25 ± 4.6	8.3 ± 2.61	**
	2015	33.5 ± 6.03	12.5 ± 3.66	*
Fringe and tall herb grassland species cover (%)				
	2011	14.5 ± 3.43	12.3 ± 3.4	n.s.
	2012	23.8 ± 5.13	14.8 ± 3.7	n.s.
	2013	32.5 ± 6.22	13.8 ± 3.2	***
	2014	36.8 ± 7.43	18.3 ± 4.64	**
	2015	41 ± 8.123	20.5 ± 5.37	***
Boreal shrublands and woodland species cover (%)				
	2011	71.8 ± 8.13	73.3 ± 5.5	n.s.
	2012	48 ± 8.89	71.5 ± 5.85	n.s.
	2013	44 ± 7.85	75.3 ± 5.8	***
	2014	44.8 ± 8.24	76 ± 6.81	***
	2015	53.3 ± 11.5	86.5 ± 8.9	*
Number of species				
	2011	26.6 ± 2.97	27.6 ± 1.9	n.s.
	2012	31.9 ± 3.22	31.6 ± 2.17	n.s.
	2013	31.6 ± 3.08	28.2 ± 1.86	n.s.
	2014	34.8 ± 3.42	26.9 ± 1.66	**
	2015	33.6 ± 2.94	31.4 ± 2.61	n.s.
Forage Pastoral Value				
	2011	9.1 ± 0.93	9.3 ± 0.79	n.s.
	2012	12 ± 1.49	9.8 ± 1	n.s.
	2013	12.6 ± 1.25	9.3 ± 1.07	**
	2014	13.9 ± 1.4	9.9 ± 0.9	*
	2015	16.4 ± 1.73	10.1 ± 1.05	***

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Annex C

Other publications and participation to congresses

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