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

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Abstract

BACKGROUND: 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.

RESULTS: 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.

CONCLUSION: 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.

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 bewteen 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.

EXPERIMENTAL

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 (Supporting Information 1), 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 (Supporting information 2).[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. 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 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. 1) and plant phenology (Supporting Information 1), 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); 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. 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. 3a), confirming the presence of transitional stages underlined by the hierarchical cluster analysis. Overlapping was also observed between 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. 3a). According to Landolt's indicator values, the ecological conditions were significantly different between mesophilic and dry grasslands (Fig. 3a; Table 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 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 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. 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). 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 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.

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, characterized by a higher soil moisture content and more intensive pastoral management and the dry grasslands, characterized 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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REFERENCES

1 Grunert KG, Trends in food choice and nutrition, in *Consumer attitudes to food quality products* ed. by Klopčič M, Kuipers A and Hocquette J-F. Wageningen Academic Publishers, Netherlands, pp. 23 – 30 (2013).

2 Elgersma A, Søegaard K and Jensen SK, Fatty acids, α-tocopherol, β-carotene, and lutein contents in forage legumes, forbs, and a grass – clover mixture. *J Agric Food Chem* **61**:11913 – 11920 (2013).

3 Jutzeler van Wijlen RP and Colombani PC, Grass-based ruminant production methods and human bioconversion of vaccenic acid with estimations of maximal dietary intake of conjugated linoleic acids. *Int Dairy J* **20**:433 – 448 (2010).

4 Renna M, Cornale P, Lussiana C, Malfatto V, Mimosi A and Battaglini LM, Fatty acid profile of milk from goats fed diets with different levels of conserved and fresh forages. *Int J Dairy Technol* **65**:201 – 207 (2012).

5 Howes NL, Bekhit AE-DA, Burritt DJ and Campbell AW, Opportunities and implications of pasture-based lamb fattening to enhance the long-chain fatty acid composition in meat. *Compr Rev Food Sci Food Saf* **14**:22 – 36 (2015).

6 Renna M, Lussiana C, Cornale P, Fortina R and Mimosi A, Changes in goat milk fatty acids during abrupt transition from indoor to pasture diet. *Small Rumin Res* **108**:12 – 21 (2012).

7 Willems H, Kreuzer M and Leiber F, Alpha-linolenic and linoleic acid in meat and adipose tissue of grazing lambs differ among alpine pasture types with contrasting plant species and phenolic compound composition. *Small Rumin Res* **116**:153 – 164 (2014).

8 Szumacher-Strabel M, El-Sherbiny M, Cieslak A, Szczechowiak J and Winiarska H, Bioactive lipid components from ruminant milk and meat: The new face of human health, in *Biotechnology of Bioactive Compounds: Sources and applications* ed. by Gupta VK and Tuohy M. John Wiley & Sons, UK, pp. 599 – 629 (2015).

9 Iussig G, Renna M, Gorlier A, Lonati M, Lussiana C, Battaglini LM and Lombardi G, Browsing ratio,

species intake, and milk fatty acid composition of goats foraging on alpine open grassland and grazable forestland. *Small Rumin Res* DOI: 10.1016/j.smallrumres.2015.09.013 (2015).

10 Lourenço M, Moloney AP and Fievez V, Botanically diverse forages: implications for ruminant product composition and quality. *Pastagens e Forragens* **30**:119 – 137 (2009).

11 Falchero L, Lombardi G, Gorlier A, Lonati M, Odoardi M, and Cavallero A, Variation in fatty acid composition of milk and cheese from cows grazed on two alpine pastures. *Dairy Sci Technol* **90**:657 – 672 (2010).

12 Gorlier A, Lonati M, Renna M, Lussiana C, Lombardi G, and Battaglini LM, Changes in pasture and cow milk compositions during a summer transhumance in the western Italian Alps. *J Appl Bot Food Qual* **85**:216 – 223 (2013).

13 Povolo M, Pelizzola V, Passolungo L, Biazzi E, Tava A, and Contarini G, Characterization of two Agrostis–Festuca alpine pastures and their influence on cheese composition. *J Agric Food Chem* **61**:447 – 455 (2013).

14 Clapham WM, Foster JG, Neel JPS and Fedders JM, Fatty acid composition of traditional and novel forages. *J Agric Food Chem* **53**:10068 – 10073 (2005).

15 Mir PS, Bittman S, Hunt D, Entz T and Yip B, Lipid content and fatty acid composition of grasses sampled on different dates through the early part of the growing season. *Can J Anim Sci* **86**:279 – 290 (2006).

16 Bovolenta S, Spanghero M, Dovier S, Orlandi D and Clementel F, Chemical composition and net energy content of alpine pasture species during the grazing season. *Anim Feed Sci Technol* **146**:178 – 191 (2008).

17 Van Ranst G, Fievez V, Vandewalle M, De Riek J and Van Bockstaele E, Influence of herbage species, cultivar and cutting date on fatty acid composition of herbage and lipid metabolism during ensiling. *Grass Forage Sci* **64**:196 – 207 (2009).

18 Wyss U and Collomb M, Fatty acid composition of different grassland species. *Grassl Sci Eur* **15**:631 – 633 (2010).

19 Garcia PT, Pordomingo A, Perez CD, Rios MD, Sancho AM, Volpi Lagreca G and Casal JJ, Influence of cultivar and cutting date on the fatty acid composition of forage crops for grazing beef production in Argentina. *Grass Forage Sci* DOI: 10.1111/gfs.12167 (2015).

20 MacDonald D, Crabtree JR, Wiesinger G, Dax T, Stamou N, Fleury P, Gutierrez Lazpita J and Gibon A, Agricultural abandonment in mountain areas of Europe: Environmental consequences and policy response. *J Environ Manage* **59**:47 – 69 (2000).

21 Cavallero A, Aceto P, Gorlier A, Lombardi G, Lonati M, Martinasso B and Tagliatori C, *I tipi pastorali delle Alpi piemontesi: vegetazione e gestione dei pascoli delle Alpi occidentali*. Alberto Perdisa Editore, Bologna, 467 pp. (2007).

22 Revello-Chion A, Tabacco E, Peiretti PG and Borreani G, Variation in the fatty acid composition of alpine grassland during spring and summer. *Agron J* **103**:1072 (2011).

23 Peiretti PG, Gai F, Alonzi S, Battelli G and Tassone S, Characterisation of alpine highland pastures located at different altitudes: forage evaluation, chemical composition, *in vitro* digestibility, fatty acid and terpene contents. *Plant Biosyst* DOI: 10.1080/11263504.2015.1064044 (2015).

24 Reynaud A, Fraisse D, Cornu A, Farruggia A, Pujos-Guillot E, Besle J-M, Martin B, Lamaison J-L, Paquet D, Doreau M and Graulet B, Variation in content and composition of phenolic compounds in permanent pastures according to botanical variation. *J Agric Food Chem* **58**:5485 – 5494 (2010).

25 Ozenda P, *La vegetation de la chaine alpine dans l'espace montagnard europeen.* Masson, Paris, 344 pp. (1985).

26 *Banca Dati Meteorologica* **-** *ARPA Piemonte.* [Online]. Available: www.regione.piemonte.it/ambiente/aria/rilev/ariaday/annali/meteorologici [28 November 2014]

27 Theurillat JP, Aeschimann D, Küpfer P and Spichiger R, The higher vegetation units of the Alps. *Colloq Phytosociol* **23**:190 – 239 (1995).

28 Rodwell JS, Schaminée JHJ, Mucina L, Pignatti S, Dring J and Moss D, *The diversity of European vegetation: an overview of phytosociological alliances and their relationships to EUNIS habitats*. LC-EMV 2002/54, Wageningen, Netherlands, 168 pp. (2002).

29 Lombardi G, Probo M, Renna M, Astegiano S, Bellio A, Ravetto Enri S, Lussiana C, Cornale P, Malfatto V, Mimosi A, Gariano GR, Gramaglia M, Decastelli L and Battaglini L, The Piedmont Noble Milk as a tool to improve the competitiveness of mountain farms. *J Nutr Ecol Food Res* **2**:232 – 236 (2014).

30 Daget P and Poissonet J, Une méthode d'analyse phytosociologique des prairies. *Ann Agron* **22**:5 – 41 (1971).

31 Probo M, Lonati M, Pittarello M, Bailey DW, Garbarino M, Gorlier A and Lombardi G, Implementation of a rotational grazing system with large paddocks changes the distribution of grazing cattle in the south-western Italian Alps. *Rangel J* **36**:445 – 458 (2014).

32 Lambertin M, *Calcul de la charge d'un alpage par un approche phyto-écologique: synthèse de deux années d'exploitation par des vaches laitières*. Institut Agricole Régional Aoste, Aosta, Italy, (1990).

33 Iussig G, Lonati M, Probo M, Hodge S and Lombardi G, Plant species selection by goats foraging on montane semi-natural grasslands and grazable forestlands in the Italian Alps. *Ital J Anim Sci* **14**:484 – 494 (2015).

34 Landolt E, Bäumler B, Erhardt A, Hegg O, Klotzli F, Lammler W, Nobis M, Rudmann-Maurer K, Schweingruber FH, Theurillat J-P, Urmi E, Vust M and Wohllgemuth T, *Flora indicativa: ökologische Zeigerwerte und biologische Kennzeichen zur Flora der Schweiz und der Alpen*. Haupt Verlag, Bern, 378 pp. (2010).

35 Magurran AE, *Ecological diversity and its measurement*. Princeton University Press, Princeton, 198 pp. (1988).

36 Association of Official Analytical Chemists, *Official methods of analysis of AOAC International*, 17th edition. AOAC, Gaithersburg (2000).

37 Van Soest PJ, Robertson JB and Lewis BA, Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* **74**:3583 – 3597 (1991).

38 Alves SP, Cabrita ARJ, Fonseca AJM and Bessa RJB, Improved method for fatty acid analysis in herbage based on direct transesterification followed by solid-phase extraction. *J Chromatogr A* **1209**:212 – 219 (2008).

39 Renna M, Gasmi-Boubaker A, Lussiana C, Battaglini LM, Belfayez K and Fortina R, Fatty acid composition of the seed oils of selected *Vicia* L. taxa from Tunisia. *Ital J Anim Sci* **13** (2014).

40 Studer-Ehrensberger K, Synthesis of semi-natural grassland vegetation of a biogeographically heterogeneous area: Mesophilous species-rich meadows in Switzerland. *Folia Geobot* **35**:289 – 313 (2000).

41 Dengler J, Rusina S, Boch S, Bruun HH, Diekmann M, Dierßen K, Dolnik C, Dupré C, Golub VB, Grytnes J-A, Helm A, Ingerpuu N, Löbel S, Pärtel M, Rašomavičius V, Tyler G, Znamenskiy SR and Zobel M, Working group on dry grasslands in the nordic and baltic region - Outline of the project and first results for the

class Festuco-Brometea. *Ann Bot* **6**:1 – 28 (2006).

42 Bornard A, Bassignana M, Bernard Brunet C, Labonne S and Cozic P, *Les végétations d'alpage de la Vanoise: description agro-écologique et gestion pastorale*. Editions Quae, Versailles, 237 pp. (2007).

43 Grabherr G and Mucina L, Die Pflanzengesellschaften Teil II: Natürliche waldfreie Vegetation. *Feddes Repert* **107**:286 – 288 (1993).

44 Catorci A, Cesaretti S and Gatti R, Effect of long-term abandonment and spring grazing on floristic and functional composition of dry grasslands in a central Apennine farmland. *Pol J Ecol* **61**:505 – 517 (2013).

45 Šilc U, Aćić S, Škvorc Ž, Krstonošić D, Franjić J and Dajić Stevanović Z, Grassland vegetation of the Molinio-Arrhenatheretea class in the NW Balkan Peninsula. *Appl Veg Sci* **17**:591 – 603 (2014).

46 Hatfield RD, Jung H-JG, Broderick G and Jenkins TC, Nutritional chemistry of forages, in *Forages: The Science of Grassland Agriculture* volume II, 6th edition ed. by Barnes RF, Nelson CJ, Moore KJ, and Collins, M. Blackwell Publishing, Ames, USA pp. 467 – 485 (2007).

47 Khan NA, Cone JW, Fievez V and Hendriks WH, Causes of variation in fatty acid content and composition in grass and maize silages. *Anim Feed Sci Technol* **174**:36 – 45 (2012).

48 Körner C, *Alpine Plant Life: Functional Plant Ecology of High Mountain Ecosystems*. Springer Berlin Heidelberg, Berlin, 323 pp. (2003).

49 Scotton M, Sicher L and Kasal A, Semi-natural grasslands of the Non Valley (Eastern Italian Alps): Agronomic and environmental value of traditional and new Alpine hay-meadow types. *Agric Ecosyst Environ* **197**:243 – 254 (2014).

50 Glasser F, Doreau M, Maxin G and Baumont R, Fat and fatty acid content and composition of forages: A meta-analysis. *Anim Feed Sci Technol* **185**:19 – 34 (2013).

51 Alarcón R, Ortiz LT and García P, Nutrient and fatty acid composition of wild edible bladder campion populations [*Silene vulgaris* (Moench.) Garcke]. *Int J Food Sci Technol* **41**:1239 – 1242 (2006).

52 Pavlů V, Hejcman M, Pavlů L, Gaisler J and Nežerková P, Effect of continuous grazing on forage quality, quantity and animal performance. *Agric Ecosyst Environ* **113**:349 – 355 (2006).

53 Pontes LS, Carrère P, Andueza D, Louault F and Soussana JF, Seasonal productivity and nutritive value of temperate grasses found in semi-natural pastures in Europe: responses to cutting frequency and N supply. *Grass Forage Sci* **62**:485 – 496 (2007).

54 Dewhurst RJ and Moloney AP, Modification of animal diets for the enrichment of dairy and meat products with omega-3 fatty acids. *Food Sci Technol Nutr* **252**:257 – 287 (2013).

55 Bele B, Østerlie M and Norderhaug A, Quality of food products from species-rich alpine pastures, in *Proceedings of the 23rd General Meeting of the European Grassland Federation*. Mecke Druck und Verlag, Kiel, Germany, pp. 773 – 775 (2010).

Table 1. Botanical families (% of relative abundance) and plant community variables for the two main ecological groups (mesophilic and dry grasslands)

	Mesophilic grasslands	Dry grasslands	Independent sample *t*-test	
	Mean ± SE[a]	Mean ± SE	*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 ($P > 0.05$).

Table 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.

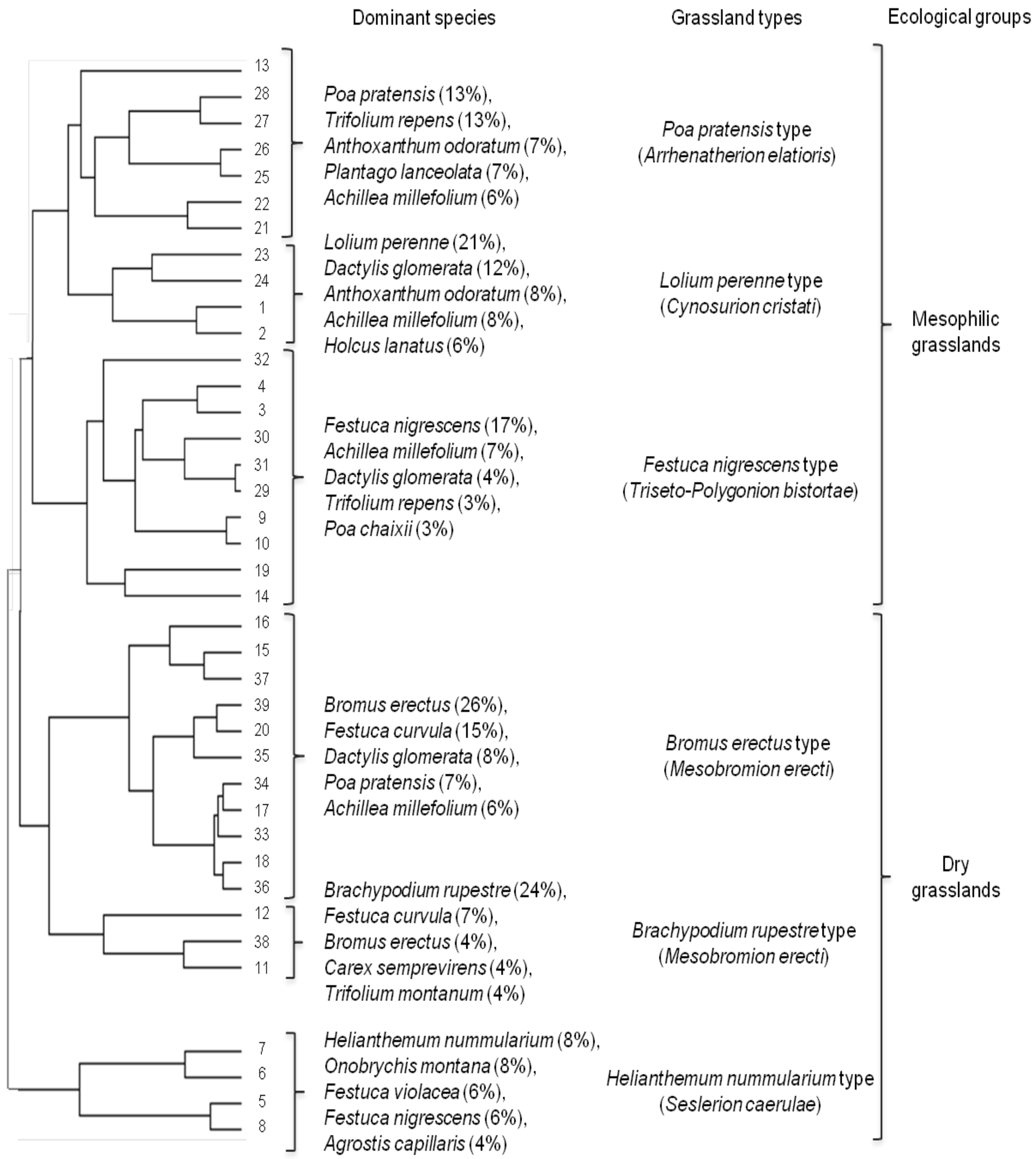

Figure 1. Dendrogram with the classification of the vegetation surveys obtained by cluster analysis, with the identification of ecological groups, grassland types (with the corresponding phytosociological alliances in brackets) and their dominant species. Numbers indicate sample codes (see Supporting Information 1).

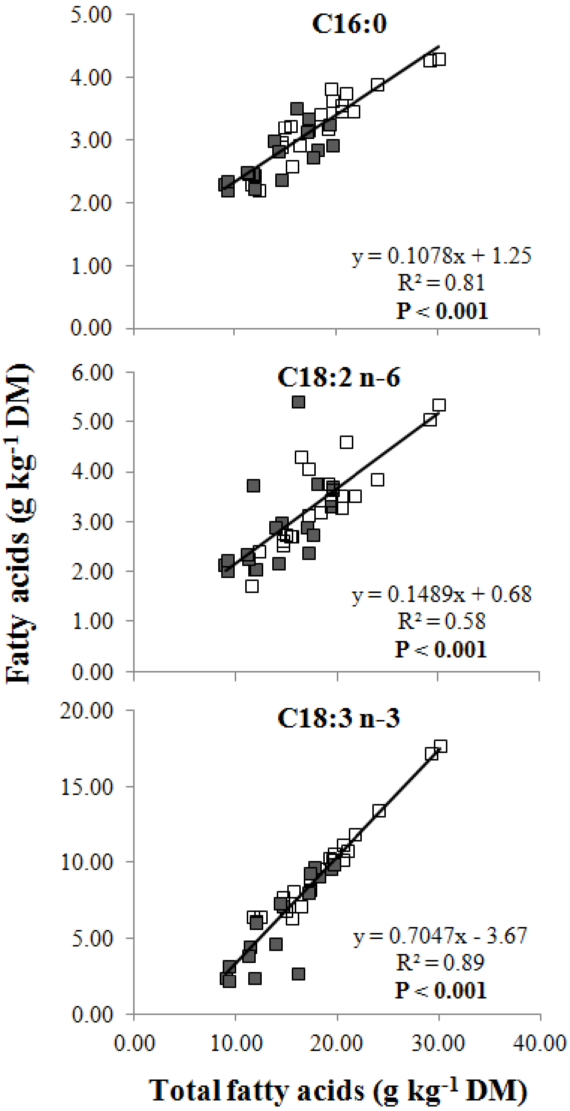

Figure 2. Changes in the concentrations of palmitic (C16:0), linoleic (C18:2 n-6) and alpha-linolenic (C18:3 n-3) acids in relation to changes in the total fatty acid content of grass samples. Grey squares represent dry grassland samples, while white squares indicate mesophilic grassland samples.

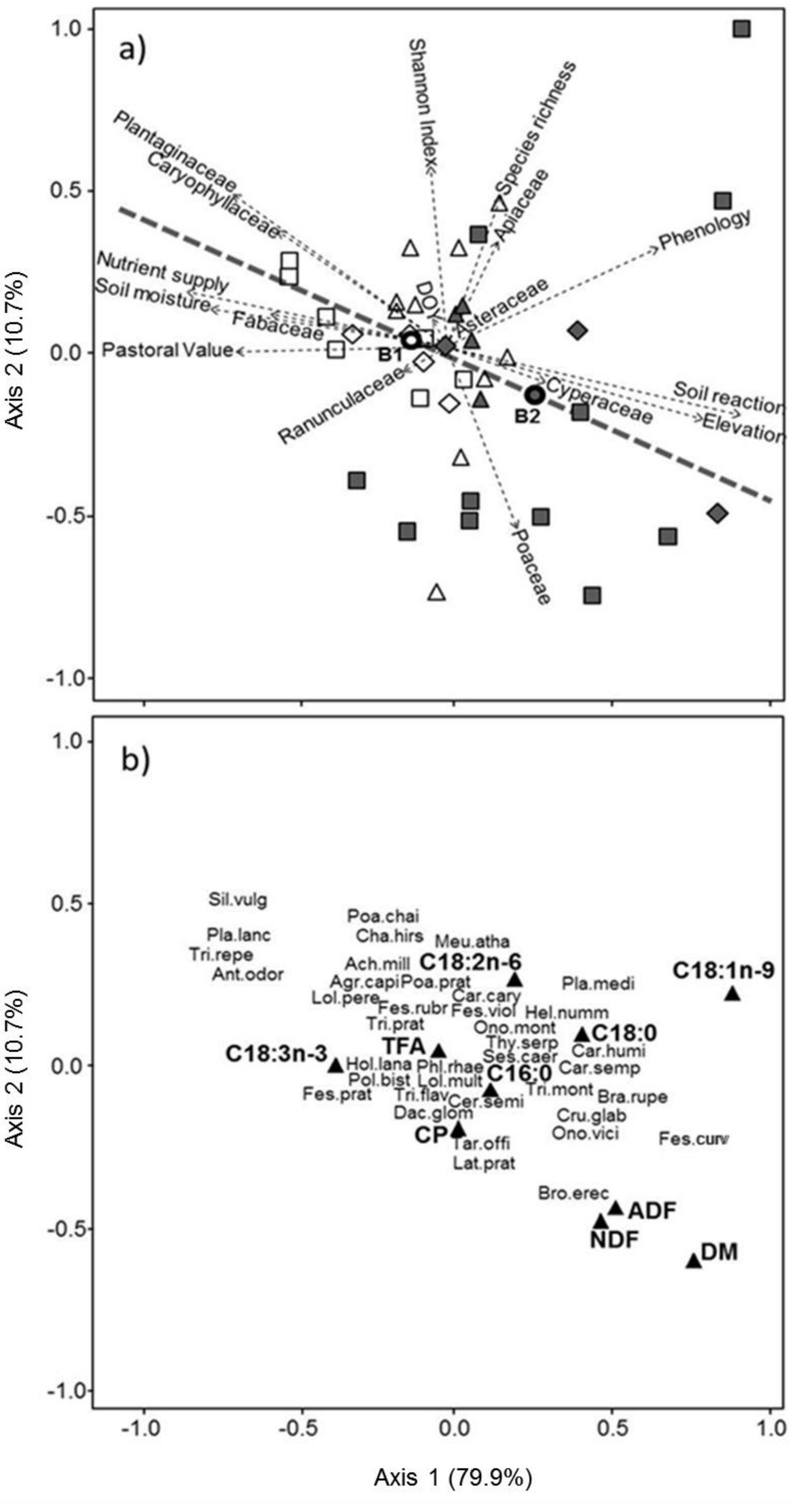
a)
b)
Axis 2 (10.7%)
Axis 1 (79.9%)
Shannon Index
Species richness
Plantaginaceae
Caryophyllaceae
Apiaceae
Phenology
Nutrient supply
Soil moisture
Fabaceae
DOY
Asteraceae
Pastoral Value
B1
B2
Ranunculaceae
Cyperaceae
Soil reaction
Elevation
Poaceae
Sil.vulg
Poa.chai
Pla.lanc
Cha.hirs
Meu.atha
Tri.repe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

Figure 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*.

Supporting information 1

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

Sample code	Ecological Group	Grassland type	Elevation (m a.s.l.)	Sampling date	DOY	Lambertin's Phenology	Exploitation	Latitude	Longitude
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

Supporting information 2

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	