

# Pyrenean meadows in Natura 2000 network: grass production and plant biodiversity conservation

Ramón Reiné<sup>1\*</sup>, Olivia Barrantes<sup>2</sup>, Cristina Chocarro<sup>3</sup>, Alejandro Juárez<sup>3</sup>,  
Alfonso Broca<sup>2</sup>, Melchor Maestro<sup>4</sup> and Carlos Ferrer<sup>2</sup>

<sup>1</sup> *Departamento de Ciencias Agrarias y del Medio Natural. Escuela Politécnica Superior. Universidad de Zaragoza. Carretera de Cuarte, s/n. 22071 Huesca, Spain.* <sup>2</sup> *Departamento de Ciencias Agrarias y del Medio Natural. Facultad de Veterinaria. Universidad de Zaragoza. c/ Miguel Servet, 147. 50013 Zaragoza, Spain.*

<sup>3</sup> *Departamento de Producción Agraria y Ciencia Forestal. ETSEA. Universidad de Lleida. Avda. Rovira Roure, 177. 25198 Lleida, Spain.* <sup>4</sup> *Instituto Pirenaico de Ecología. CSIC. Avda. Montañana, s/n. 50016 Zaragoza, Spain*

---

## Abstract

In semi-natural mountain meadows, yield and forage quality must be reconciled with plant biodiversity conservation. This study was performed to analyze the relationships between these three parameters. To quantify plant biodiversity and pastoral value (PV), phytosociological inventories were performed in 104 semi-natural meadows in the Central Spanish Pyrenees included in the Natura 2000 network. Forage yields were calculated and forage samples were analyzed for relative feed value (RFV). We identified two main types of meadows: (i) those that had “more intensive management,” relatively close to farm buildings, with little or no slope, dominated by grasses, with low plant biodiversity, high PV and yield, but low forage quality and (ii) those that had “less intensive management,” distant from farm buildings, on slopes, richer in “other forbs”, with high plant biodiversity and forage quality, but low PV and yield. Conservation policies should emphasize less intensive management practices to maintain plant diversity in the semi-natural meadows in the Pyrenees. The widespread view that “other forbs” have low nutritional value should be revised in future research. These species often are undervalued by the PV method, because their nutritional quality, digestibility and intake are poorly understood.

**Additional key words:** phytosociological inventories; botanical composition; Shannon index; pastoral value; Ellenberg indices; relative feed value; “other forbs”.

---

## Introduction

According to Kahmen & Poschlod (2008), one major objective in European nature conservation but also within agro-environmental schemes (cross compliance with national good farming practices) is to maintain the semi-natural grasslands by means of appropriate management. However, the transfer of conservation knowledge between sites or regions remains difficult due to different species sets. Semi-natural meadows provide many ecological services including biodiversity conservation, landscape maintenance, reception capacity of pollinators and other wildlife, protection against erosion, soil and water protection and carbon

sink (Paracchini *et al.*, 2008). In France (Piveteau, 1998) and the United Kingdom (MAFF, 2002), the amount of area covered by meadows is used as an indicator of sustainability in agriculture at the farm, landscape, and regional scales. In addition, the conservation of high nature value (HNV) farmland is central to achieving the biodiversity targets set in 2010 in the sixth Environment Action Programme of the European Union (EU), and alpine meadows are typical HNV (Paracchini *et al.*, 2008). Furthermore, high environmental performances of livestock systems contribute to the positive image and acceptability of their products (*e.g.*, meat, milk, cheese). The qualification as HNV farmland would lead to an added value.

---

Corresponding autor: [rreine@unizar.es](mailto:rreine@unizar.es)

Received: 13-06-13. Accepted: 05-02-14

Abbreviations used: ADF (acid detergent fiber); CP (crude protein); DDM (digestible dry matter); DM (dry matter); DMI (dry matter intake); ECA (ecological compensation areas); EU (European Union); HNV (high nature value); LU (livestock units); NDF (neutral detergent fiber); PCA (principal components analysis); PV (pastoral value); RFV (relative feed value); SC (specific contribution); SF (specific frequency); SI (specific index).

Meadows are reservoirs of plant and animal biodiversity and have agricultural and ecological benefits such as high persistence (Alard & Balent, 2007), high intake rates (Duncan *et al.*, 2003; Baumont *et al.*, 2008), positive effects on animal health, and high sensory and nutritional qualities of animal products (Farruggia *et al.*, 2008). These semi-natural areas are indicators of biodiversity in agricultural landscapes (Blackstock *et al.* 1999). Some countries have developed programs to conserve or restore the floristic biodiversity of meadows (Henle *et al.*, 2008), which include financial incentives for farmers.

To maintain or restore semi-natural meadows, mowing or grazing are integral aspects of the management to produce forage for livestock feed and to allow farmers to balance economic and environmental objectives; however, in Pyrenean meadows, the intensification of these practices can reduce plant biodiversity (Reiné *et al.*, 2004). Henle *et al.* (2008) suggested that the abandonment of marginally productive but HNV farmland is a major source of biodiversity-related conflicts and Hodgson *et al.* (2005) found that high biodiversity is associated with areas where the livestock carrying capacity and marginal returns are low. The economic benefits of intensification are associated with a reduction in biodiversity and the acceleration of the ecological processes that drive species losses in grassland ecosystems (Hodgson *et al.*, 2005).

The quantity and quality of the forage of a meadow depends on the floristic composition, which is influenced by the environmental, topographical and geographical features (*e.g.*, climate, soil, moisture, elevation, slope, distance to the main farm building), and the spatio-temporal aspects of plot management (*e.g.*, mowed or grazed, fertilization, time of year) (Kirkham & Tallowin, 1995; Wellstein *et al.*, 2007; Cop *et al.*, 2009; Andueza *et al.*, 2010). Thus, a combination of environmental, technical, socio-economic and historical aspects determines forage production. The valuation of the forage yield of a meadow is simple when it is mown only once (for hay or silage), but is more complex when it is used by grazing livestock. As a measure of quality, the chemical analysis of the total forage mass is valid only if the field is mowed; it underestimates quality when the field is grazed because livestock usually do not eat plants or parts of plants that are of low quality (selective herbivory). Following Daget & Poissonet

(1972), scientists began to assign each species an index of quality [specific index (SI)] that reflects its agronomic value (growth rate, productivity) and zootechnics (bromatological value, digestibility, attractiveness). The SI is advantageous because the toxic and unpalatable species that livestock do not consume when grazing are excluded from the calculation of pastoral value (PV); however, the SI used in the PV method does not consider differences that are associated with phenology (Ansquer *et al.*, 2004).

The objectives of this study were to analyze the relations between plant biodiversity, yield, and nutritive value of 104 semi-natural meadows, with different botanical composition and environmental conditions, all located within the Natura 2000 network (OJ, 1992) in the Central Spanish Pyrenees. Our hypothesis is that the most productive grasslands will also have a high nutritional value but lower plant diversity, so that in the future could decrease their environmental value.

## Material and methods

### Study area

The study was performed in 45 municipalities of the Pyrenees classified as LFAs (less favoured areas) in northern Aragon, north-eastern Spain, close to the French border (42°29'-42°46' N, 0°22'-0°32' W). The mean annual temperature is 8-10°C, with a maximum monthly mean in July (16-20°C) and minimum in January (0-4°C). Average yearly rainfall is 950-1600 mm, with the lowest values in the summer months. The mountainous region has alpine grasslands, semi-natural meadows (only 3.5% of the total area is arable land), and relatively high beef cattle stocking rates (Barrantes *et al.*, 2009). Those semi-natural meadows are floristically rich (Reiné *et al.*, 2004), cover 9609 ha (92% of the available cropland) (Barrantes *et al.*, 2009), and are included in the 6510 (Chocarro *et al.*, 2009) and 6520 (Reiné *et al.*, 2009) codes of the Natura 2000 network (OJ, 1992). In a previous study, analyzing 33 soils of these meadows, we obtained the following average values: 19% clay, 55% sand, and 26% silt, CaCO<sub>3</sub> = 4.3%, pH = 6.4, organic matter = 10.7%, nitrogen = 0.15%, C/N = 11.7, and P<sub>2</sub>O<sub>5</sub> (Olsen) = 41 ppm (Ferrer *et al.*, 1990).

A total of 104 meadows were chosen based on floristic and management characteristics: provided that

**Table 1.** Elevation, slope, species richness, Shannon index, cover and species grasses, legumes, and ‘other forbs’, life forms (Raunkiaer, 1934), Ellenberg indices (Ellenberg *et al.*, 1991; Hill *et al.*, 2004), plant height, yield, forage quality and pastoral value (Daget & Poissonet, 1972) in 104 plots in semi-natural meadows in the Spanish Pyrenees

	Mean	Standard deviation	Minimum	Maximum
Elevation (m a.s.l.)	1329.4	160.1	953	1657
Slope (%)	14.8	12.4	0	70
Species richness (species number inventory <sup>-1</sup> )	32.8	7.6	15.0	51.0
Grasses (% species)	27.2	6.6	15.8	43.5
Grasses (% cover)	44.3	12.6	16.3	80.3
Legumes (% species)	17.4	4.6	5.7	29.4
Legumes (% cover)	18.6	8.4	0.1	45.0
Forbs (% species)	55.4	8.7	29.4	70.8
Forbs (% cover)	37.2	13.7	4.1	68.7
Shannon index	2.55	0.28	1.66	3.24
Life form (% species)				
Therophytes	14.8	7.0	0	39.5
Geophytes	2.6	2.9	0	13.0
Hemicryptophytes	76.5	7.5	53.5	100.0
Chamaephytes	5.8	3.0	0	15.0
Phanerophytes	0.1	0.6	0	3.5
Ellenberg index				
Light (L)	6.63	0.33	5.55	7.26
Moisture (F)	4.28	0.36	3.52	5.35
pH (R)	6.05	0.34	4.98	6.67
Nitrogen (N)	4.21	0.46	3.16	5.67
Mean plant height (m)	0.84	0.31	0.30	1.60
Yield (kg DM ha <sup>-1</sup> )	4316	1305	1919	8880
CP (% DM)	10.87	2.16	6.87	17.09
NDF (% DM)	55.92	5.96	42.75	70.07
ADF (% DM)	32.37	3.52	22.62	38.70
DDM (%)	63.69	2.74	58.76	71.28
DMI (% of kg BW)	2.17	0.25	1.71	2.81
RVF	107.66	16.65	78.58	153.27
PV	50.03	11.08	23.65	74.48

DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, DDM: digestible dry matter, DMI: dry matter intake, BW: body weight, RVF: relative feed value, PV: pastoral value.

their vegetation was included in the Habitat Directive (OJ, 1992) and their management practices could be known. Following these two premises a stratified preferential sampling was applied in four valleys. The average size of the meadow was 6,187 m<sup>2</sup> (465 m<sup>2</sup> minimum and 33,237 m<sup>2</sup> maximum). At the sampling sites, elevation ranged from 953 to 1,657 m.a.s.l. and slope ranged from 0% to 70% (Table 1).

## Management practices

Most of the meadows were cut once for hay in July and grazed twice a year (spring and autumn) by cows

or sheep at stocking rates less than 1.4 LU ha<sup>-1</sup> year<sup>-1</sup>. Farmers used NPK inorganic, cattle slurry, or manure like fertilizers. Accessibility of the plots and distance to farm buildings influenced agricultural management, a gradient being noticed from intensively managed meadows (one cut, two grazing, and inorganic or slurry fertilizer) close to the village, to more extensively managed fields (no cut, two grazing, no fertilizer) close to the forest (Reiné *et al.*, 2004).

Farmers were interviewed about management regimes of meadows (cutting, grazing and fertilization) and time taken to access the plots from the farms. Meadows were classified in three ways based on their management practices: (i) cutting once, or only

pastured, (ii) grazing by cows, by sheep or mixed, (iii) fertilizers every year with inorganic fertilizer (300-350 kg ha<sup>-1</sup>, N-P-K ratio of 8-15-15) or cattle slurry (35-40 t ha<sup>-1</sup>), with manure (20-25 t ha<sup>-1</sup>) every year, with manure (20-25 t ha<sup>-1</sup>) every two or three years, or no fertilized.

### Vegetation measurements

The vegetation was sampled on 1-15 July 2008, immediately before the harvest, at the peak of aboveground production. Phytosociological inventories were recorded in a 100 m<sup>2</sup> plot in the centre of each meadow. We used the Braun-Blanquet (1965) species abundance-dominance scale (from + to 5). The average plant height was calculated for each plot. Species nomenclature followed Tutin *et al.* (1964-1980). To estimate yield, one biomass sample was collected from within a 0.25 m<sup>2</sup> quadrat in each plot. Dry matter was calculated using samples that had been dried in a laboratory at 70°C for 48 h. Species richness was the number of species recorded in each inventory. The plant diversity of the grassland community was calculated using the Shannon information index, H' (Shannon & Weaver, 1949):  $H' = -\sum_{i=1}^s p_i \ln p_i$ , where  $p_i$  is the proportion of individuals belonging to the  $i^{\text{th}}$  species and  $s$  is the number of species in the plant community. Plant species were classified based on their functional traits, in: (i) as either grasses, legumes, or "other forbs"; (ii) as either therophytes, geophytes, hemicryptophytes, chamaephytes, or phanerophytes (following Raunkiaer, 1934); (iii) following Ellenberg *et al.* (1991), who devised a comprehensive system for describing the response of individual species of vascular plants to an array of ecological factors (*e.g.*, light, moisture, pH, nitrogen). The functional traits of each species were based on the *Plantatt* database (Hill *et al.*, 2004).

### Pastoral value

The pastoral value (PV) was calculated for each plot using the Daget & Poissonet (1972) method. The field-derived cover-abundance data were transformed into percentage cover values based on the Braun-Blanquet coefficients: + = 0.1%, 1 = 5%, 2 = 17.5%, 3 = 37.5%, 4 = 62.5, and 5 = 87.5% (Van der Maarel, 1979). Those values are denominated specific frequency (SF) and, after

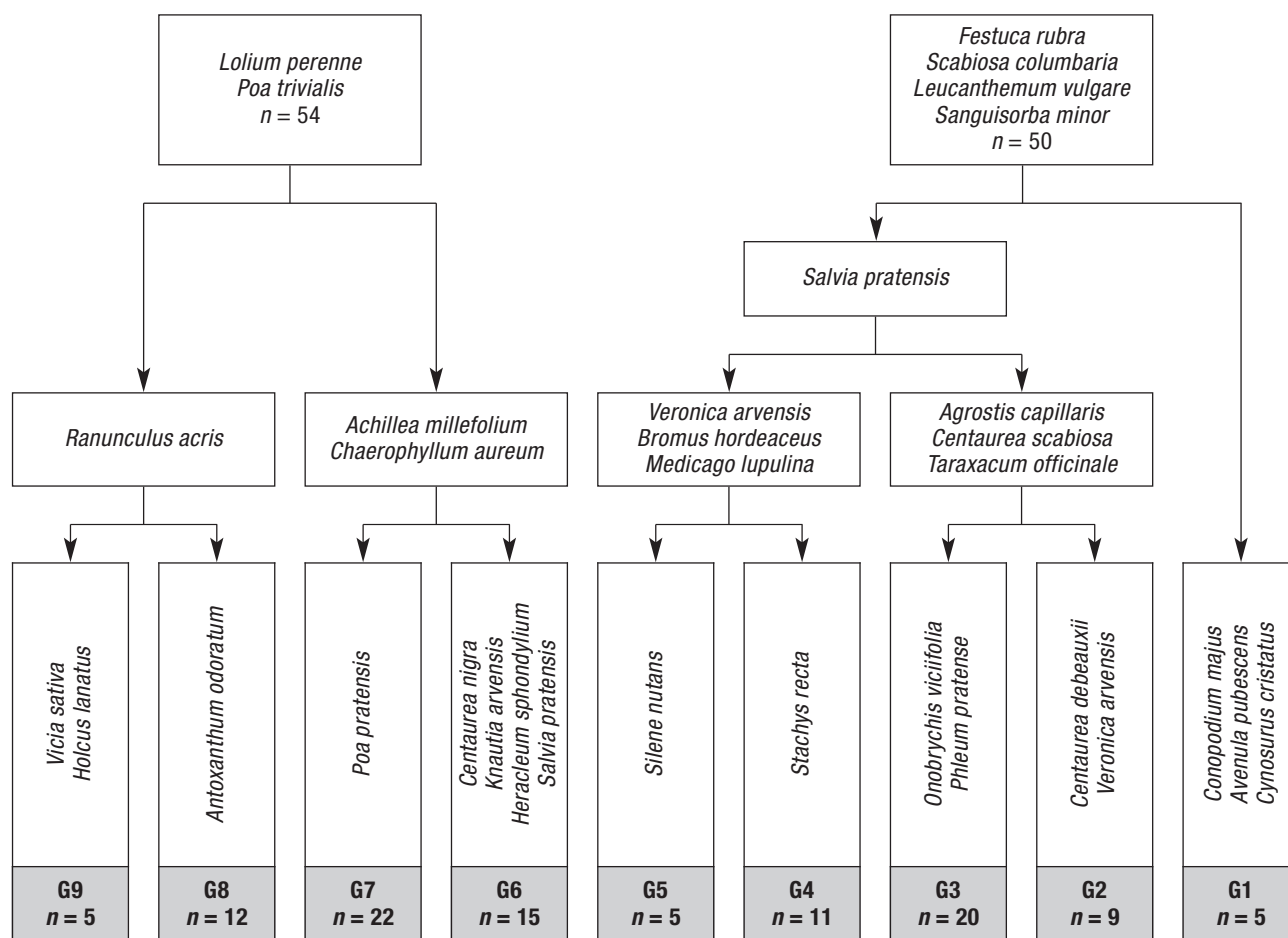
adjustment,  $\sum SF_i$  is typically >100. In all the inventories, vegetation cover was 100%. Specific frequency ( $SF_i$ ) values were converted to the specific contribution (SC) of each species using the following equation:  $SC_i = (SF_i / \sum SF_i)100$ . The specific index (SI) ranges from 0 to 5 and is based on the productivity, digestibility, and attractiveness of each grassland species (Daget & Poissonet, 1972; Gillet *et al.*, 2002). To assign a SI to each of the plant species identified in the inventories, we used the database of the Department of Agricultural and Environmental Sciences, University of Zaragoza, Spain, and Roggero *et al.* (2002). PV was calculated using the following equation:  $PV = 0.2 \sum_{i=1}^s (SC_i \cdot SI_i)$ .

### Chemical analysis

Forage samples were oven dried at 65°C for 2 days and ground in a mill (IKA MF10, IKA-Werke, Staufen, Denmark) to the point where the material could pass through a 1 mm screen. Crude protein (CP) concentrations ( $N \cdot 6.25$ ) were quantified using the combustion method in an elemental analyzer (Elementar Vario Max N/CN, Hanau, Germany). Concentrations of ash-free neutral detergent fiber (NDF) and acid detergent fiber (ADF) were quantified using an Ankom 200 fiber analyzer (Ankom Technol. Corp., Fairport, NY, USA). Relative feed value (RFV) is an index that combines important nutritional factors (potential intake and digestibility) into a single number, which provides a quick, effective method of evaluating feed value or quality. The RFV is calculated using the estimates of digestible dry matter (DDM %) and potential dry matter intake (DMI % of body weight) of the forage based on the ADF and the NDF fractions, respectively (Linn & Martin, 1999), as follows:  $DDM\% = 88.9 - [0.779 \cdot ADF (\% \text{ of DM})]$ ;  $DMI (\% \text{ of body weight}) = 120 / NDF (\% \text{ of DM})$ ;  $RFV = (DDM \cdot DMI) / 1.29$ ; forage quality standard =  $f(RFV)$ : prime (>151), 1<sup>st</sup> (151-125), 2<sup>nd</sup> (124-103), 3<sup>rd</sup> (102-87), 4<sup>th</sup> (86-75) and 5<sup>th</sup> (<75).

### Data analysis

Data analyses were performed using SPSS-Statistics (IBM, 2010). Pearson correlation matrices were used to assess the relationships between variables. To limit the number of variables included in the correlation matrix, we used principal components analysis (PCA). We have



**Figure 1.** Classification of 104 plots in semi-natural meadows in the Spanish Pyrenees based on a TWINSpan analysis of the presence-absence of plant species (in July) and the indicator species of each group (G1 to G9).  $n$ =number of plots in each group.

performed two PCA: (i) between variables of plant families, plant biodiversity, Ellenberg indices and topography, and (ii) between variables of plant families, plant biodiversity plant height, yield, forage quality and PV.

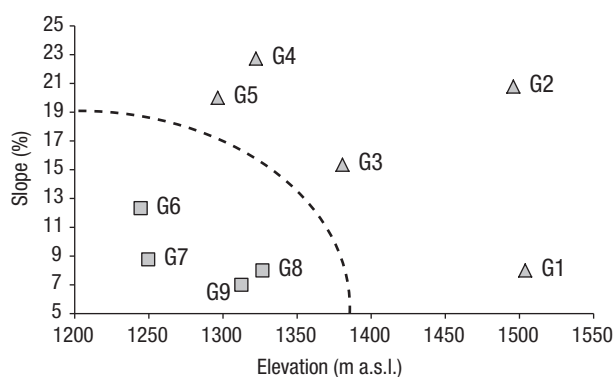
To create a classification of the meadows based on floristic composition, the presence-absence data of plant species were subjected to a TWINSpan analysis (Hill, 1979). Normality of the 16 variables (grasses, legumes, forbs, species richness, Shannon index, light, moisture, pH, nitrogen, mean plant height, yield, CP, NDF, ADF, RFV and PV) was tested using Kolmogorov-Smirnov test. Cover values of grasses, legumes and forbs, expressed as percentages were arcsine square root transformed following Van der Maarel (1979). Differences for these 16 variables among the TWINSpan groups of plots were assessed using one way ANOVA. When significant differences were detected, a HSD post hoc Tukey Test was used to compare means (Zar, 1984). The dispersion

of the nine TWINSpan groups was evaluated using linear, exponential, and logarithmic regressions.

## Results

### Topography, land management, floristic composition, and plant biodiversity

The 104 semi-natural meadows of this study fell into one of nine groups (hereafter, G) (Fig. 1). Each group was characterized by some 'indicator' species. G7 comprised the largest number of meadows (22), and G1, G5, and G9 had the fewest (5). The meadows had one of two general types of plant communities: 50 meadows that had *Festuca rubra*, *Scabiosa columbaria*, *Leucanthemum vulgare*, and *Sanguisorba minor* as indicator species (G1, G2, G3, G4, and G5), and 54 meadows that had *Lolium perenne* and *Poa trivialis* as



**Figure 2.** Mean elevation and slope of the nine groups (G) of meadows identified in a TWINSPAN analysis in 104 plots in semi-natural meadows in the Spanish Pyrenees. (□) Groups that had more intensive management. (△) Groups that had less intensive management.

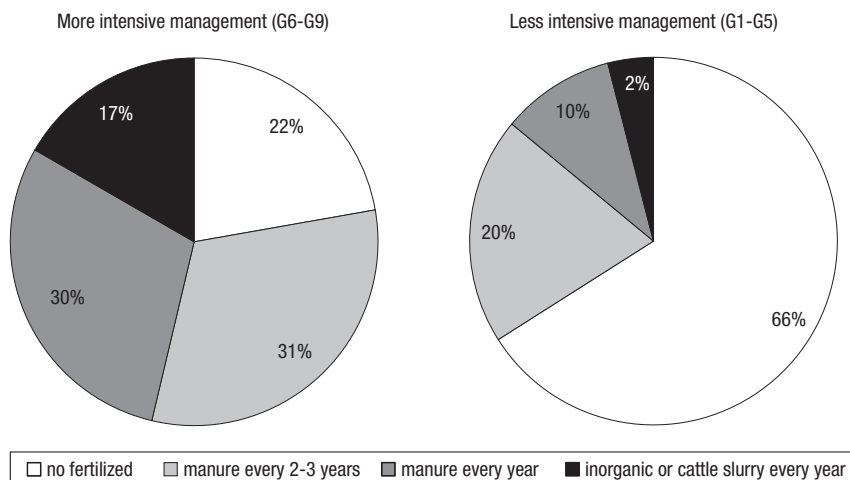
indicator species (G6, G7, G8, and G9) (Fig. 1). Furthermore, the meadows in the latter groups were at lower elevations and on shallower slopes than were the meadows in groups G1, G2, G3, G4, and G5 (Fig. 2). The management practices used on grasslands, which are largely influenced by topography (elevation and slope), determine the types of vegetation (*e.g.* Blackstock *et al.* 1999; Andrieu *et al.*, 2007). For simplicity, we refer to G6, G7, G8, and G9 as meadows under “more intensive management” and G1, G2, G3, G4, and G5 as meadows under “less intensive management”.

Most of the meadows sampled were cut once (91%) and grazed twice (100%) by cattle (72%), sheep (11%), or mixed cattle and sheep (17%). Fertilization practices were: yearly with inorganic or slurry (11%), yearly with manure (20%), every 2-3 years with manure

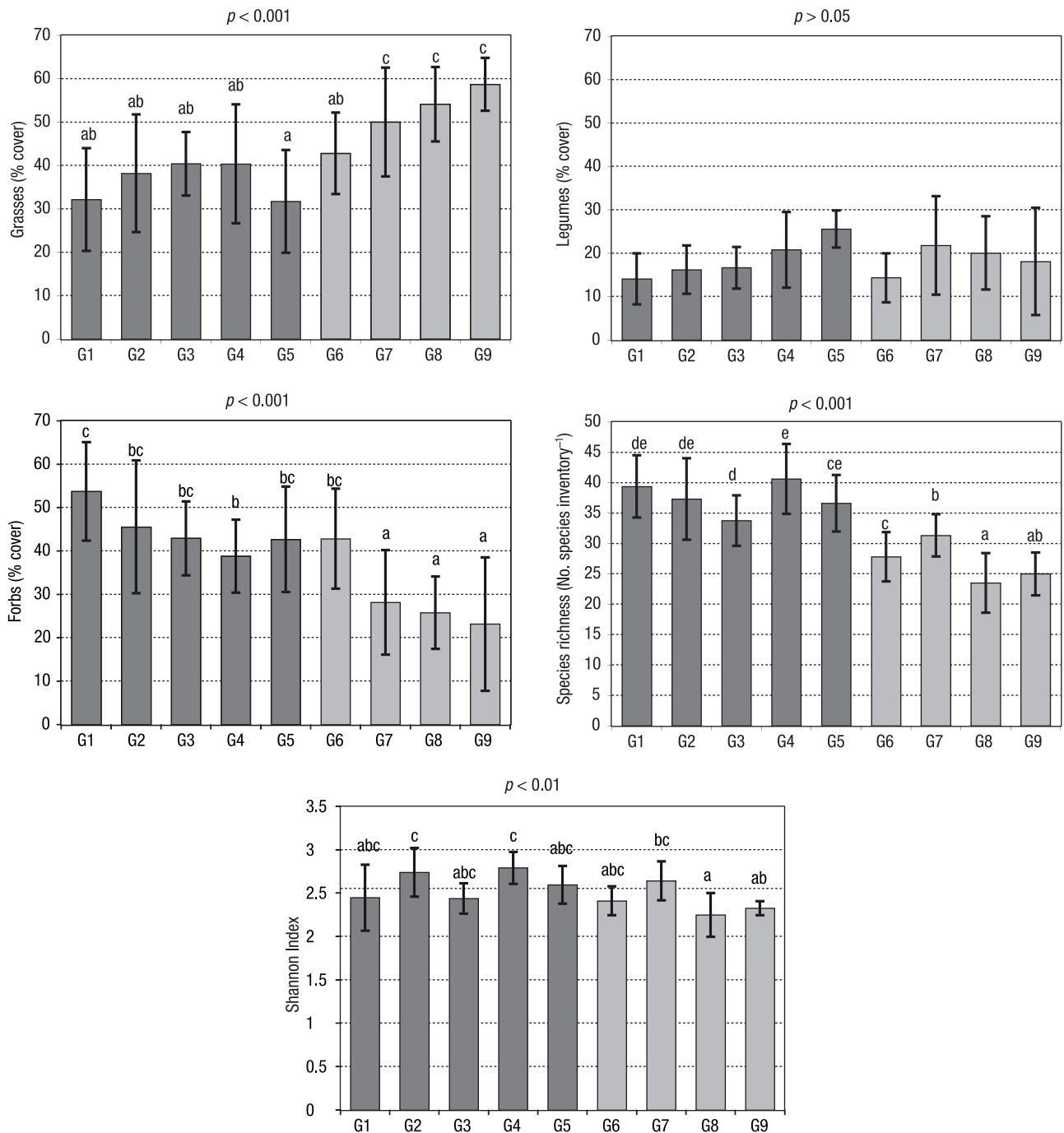
(26%) or not fertilized (43%). Meadows subjected to more intensive management (G6-G9) were all cut once and grazed twice (72% by cattle, 15% sheep, and 13% mixed cattle and sheep) and fertilized yearly with inorganic or slurry (19%), yearly with manure (30%), every 2-3 years with manure (31%) or not fertilized (22%) (Fig. 3). The mean time to reach them with agricultural machinery from the farm was 12 min. Meadows less intensive management (G1-G5) were cut once and grazing twice (80%), or only pastured twice (20%) with the same percentages of animals (73%, 14% and 13%) and less fertilized (Fig. 3). Mean distances from the farm were 21 minutes. Meadows located in better sites (low elevation and slope) are exploited more intensively.

In the 104 plots, 182 plant species (32 families) were identified, of which 29 were grasses, 23 were legumes, and 130 were ‘other forbs’. The average number of species (species richness) per plot was 33 and the highest was 51 species (Table 1). On average, 27.2% of the species were grasses, which covered 44.3% of the plots, and 55.4% were ‘other forbs’, which covered 37.2% of the plots. Percentage of species (17.4%) and coverage (18.6%) were lowest among legume species. The average Shannon index ( $H'$ ) was 2.55 (range = 1.66-3.24).

Three (G7, G8, and G9) of the “more intensive management” groups had high coverage of grasses and lower coverage by ‘other forbs’ (Fig. 4). The other “more intensive management” group (G6) was similar to the groups that were under “less intensive management.” The less intensively and the more intensively managed meadows did not differ significantly in the coverage by



**Figure 3.** Percentages of fertilization practices in the meadows. G1-G5 ( $n=50$ ): groups that had less intensive management; G6-G9 ( $n=54$ ): groups that had more intensive management.



**Figure 4.** Mean coverage of grasses, legumes, and ‘other forbs’, and the species richness and Shannon index of the nine groups (G) of meadow types in 104 plots in semi-natural meadows in the Spanish Pyrenees. G1 to G5: groups that had less intensive management. G6 to G9: groups that had more intensive management. ANOVA test. Columns with different letters differ significantly ( $p < 0.05$ , HSD Tukey test). Error bars correspond to standard deviation.

legumes. Groups that had more intensive management had markedly lower species richness; however, the Shannon index did not differ significantly between the two types of management, although the values tended

to be lower in the groups comprising meadows that had more intensive management.

Most (76.5%) of the plant species were hemicryptophytes (Table 1), and 94.9% of the plots contained

hemipterophytes, therophytes and chamaephytes only. The less intensively and the more intensively managed meadows did not differ significantly in life forms (*sensu* Raunkiaer, 1934). Meadows under more intensive management had significantly higher levels of moisture and nitrogen, and tended to have higher light indices and pH than did the meadows under less intensive management (Fig. 5).

### Yield, forage quality, and pastoral value

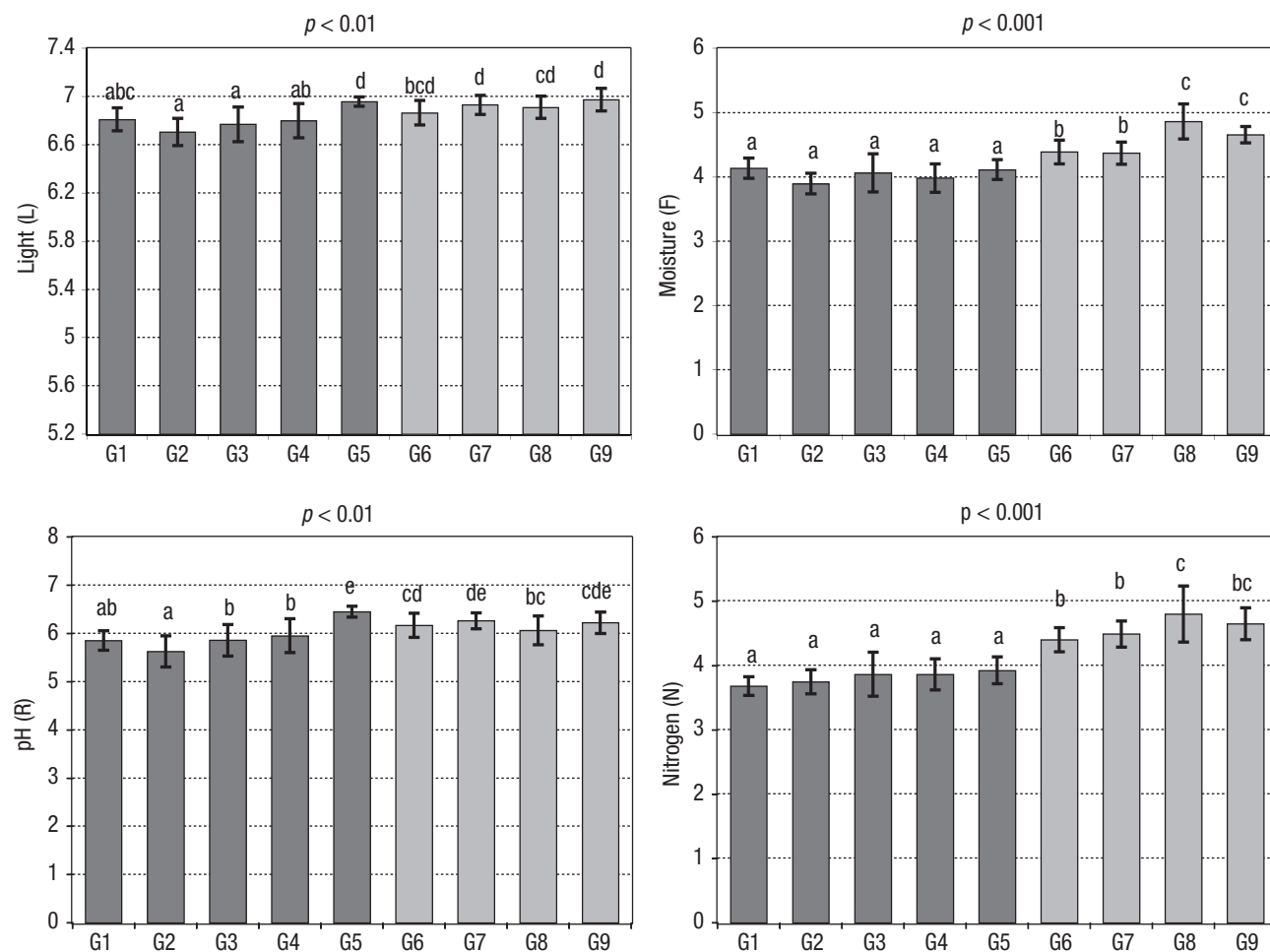
Yield, forage quality, and PV were highly variable among meadows (Table 1). In general, the meadows under more intensive management had higher average vegetation height, yield, NDF, ADF, and PV, and lower CP and RFV than did the meadows under less intensive management (Fig. 6). A matrix of Pearson correlations

( $n = 20$  variables) indicated that many of the variables were significantly correlated (Table 2). In the PCA, the first and second axes explained 44.27% and 13.42% (total = 57.69%) of the variance, respectively (Fig. 7a). In the second PCA, the first and second axes explained 43.58% and 16.90% (total=60.48%) of the variance, respectively (Fig. 7b). The most significant correlations obtained with data from the 104 plots (Table 2 and Fig. 7) are shown clustered into the nine groups, in Fig. 8.

### Discussion

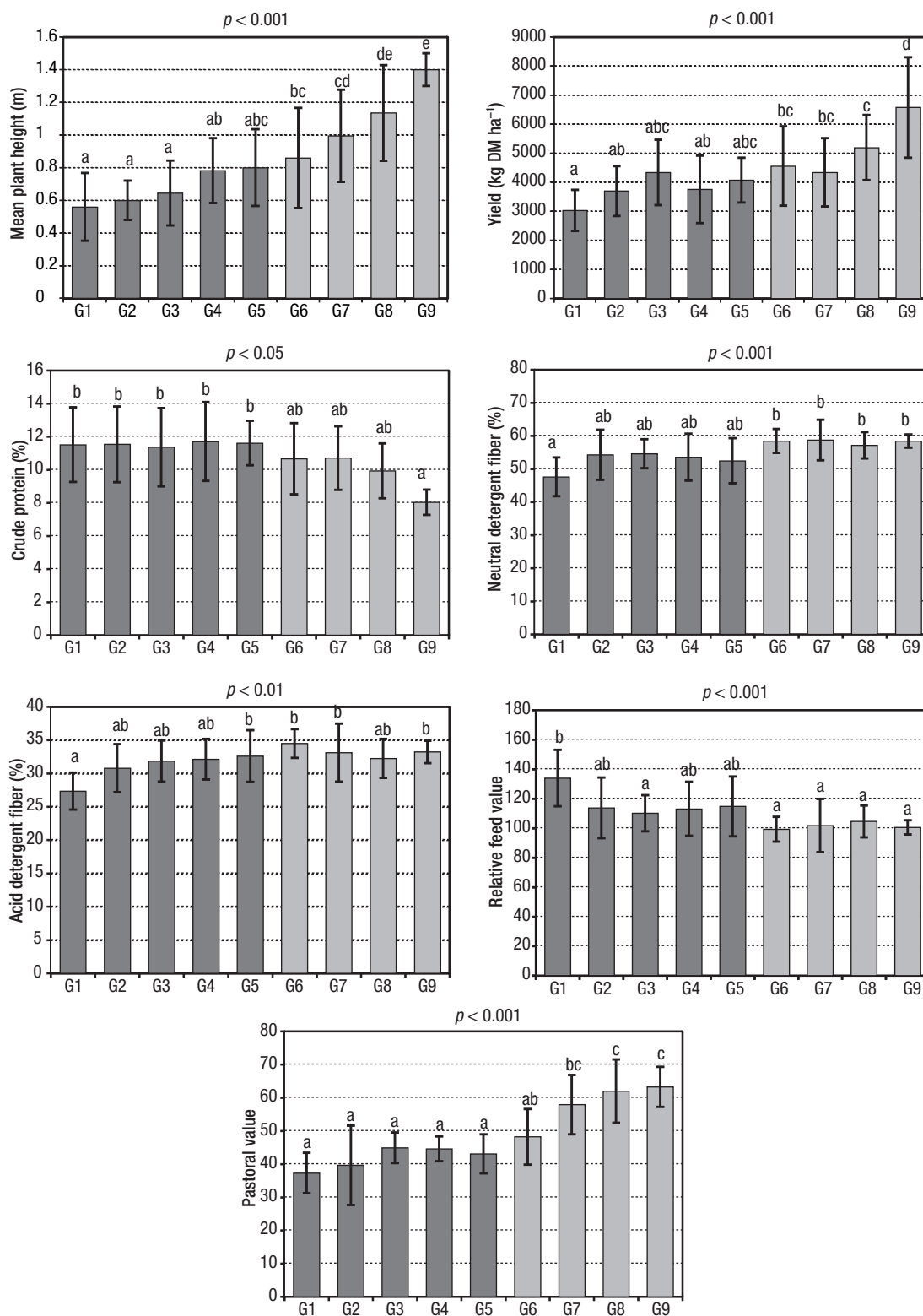
#### Geographical and topographical conditions, land management, and floristic composition

A TWINSpan analysis of the vegetation in 104 semi-natural meadows in the Spanish Pyrenees



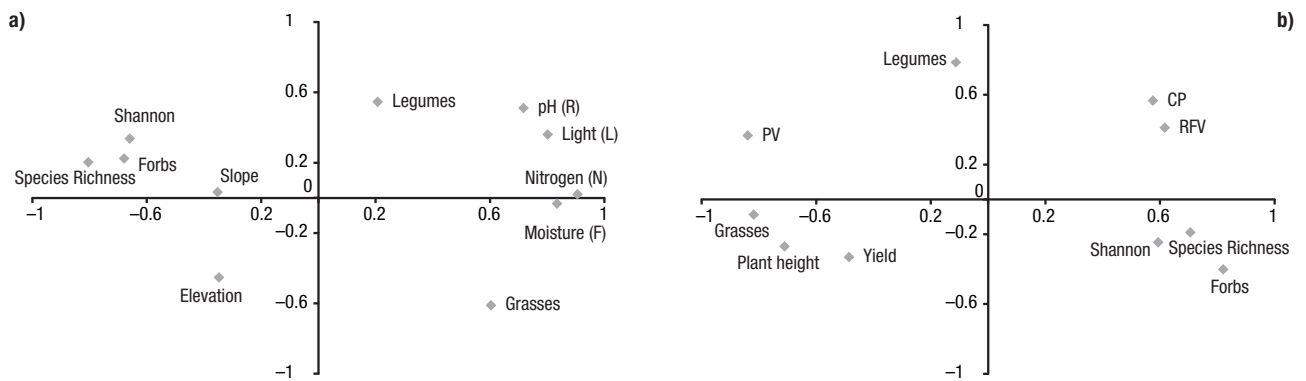
**Figure 5.** Mean Ellenberg indices of the nine groups (G) of meadow types in 104 plots in semi-natural meadows in the Spanish Pyrenees. G1 to G5: groups that had less intensive management. G6 to G9: groups that had more intensive management. ANOVA test. Columns with different letters differ significantly ( $p < 0.05$ , HSD Tukey test). Error bars correspond to standard deviation.





**Figure 6.** Mean plant height, yield, forage quality, and pastoral value of the nine groups (G) of meadow types in 104 plots in semi-natural meadows in the Spanish Pyrenees. G1 to G5: groups that had less intensive management. G6 to G9: groups that had more intensive management. ANOVA test. Columns with different letters differ significantly ( $p < 0.05$ , HSD Tukey test). Error bars correspond to standard deviation.





**Figure 7.** Principal component analysis (PCA) of a subset of the variables included in the matrix of correlations (see Table 2) in 104 plots in semi-natural meadows in the Spanish Pyrenees. a) Variables of topography, plant families, plant biodiversity and Ellenberg indices; b) Variables of plant families, plant biodiversity, plant height, yield, forage quality and pastoral value (CP: crude protein; RFV: relative feed value; PV: pastoral value).

and *A. capillaris* and *F. rubra* were associated with meadows under less intensive management.

In mesic semi-natural grasslands in SW Finland, *A. capillaris*, *F. rubra*, *L. vulgare*, *P. pratense*, *Taraxacum* sp. and *Veronica arvensis* were associated with grazed grassland (unmown and, therefore, under less intensive management) (Pykälä, 2005). In meadows in Switzerland, *Salvia pratensis* was an indicator species that differentiated less intensively managed meadows from more intensively managed meadows (Schwab *et al.*, 2002).

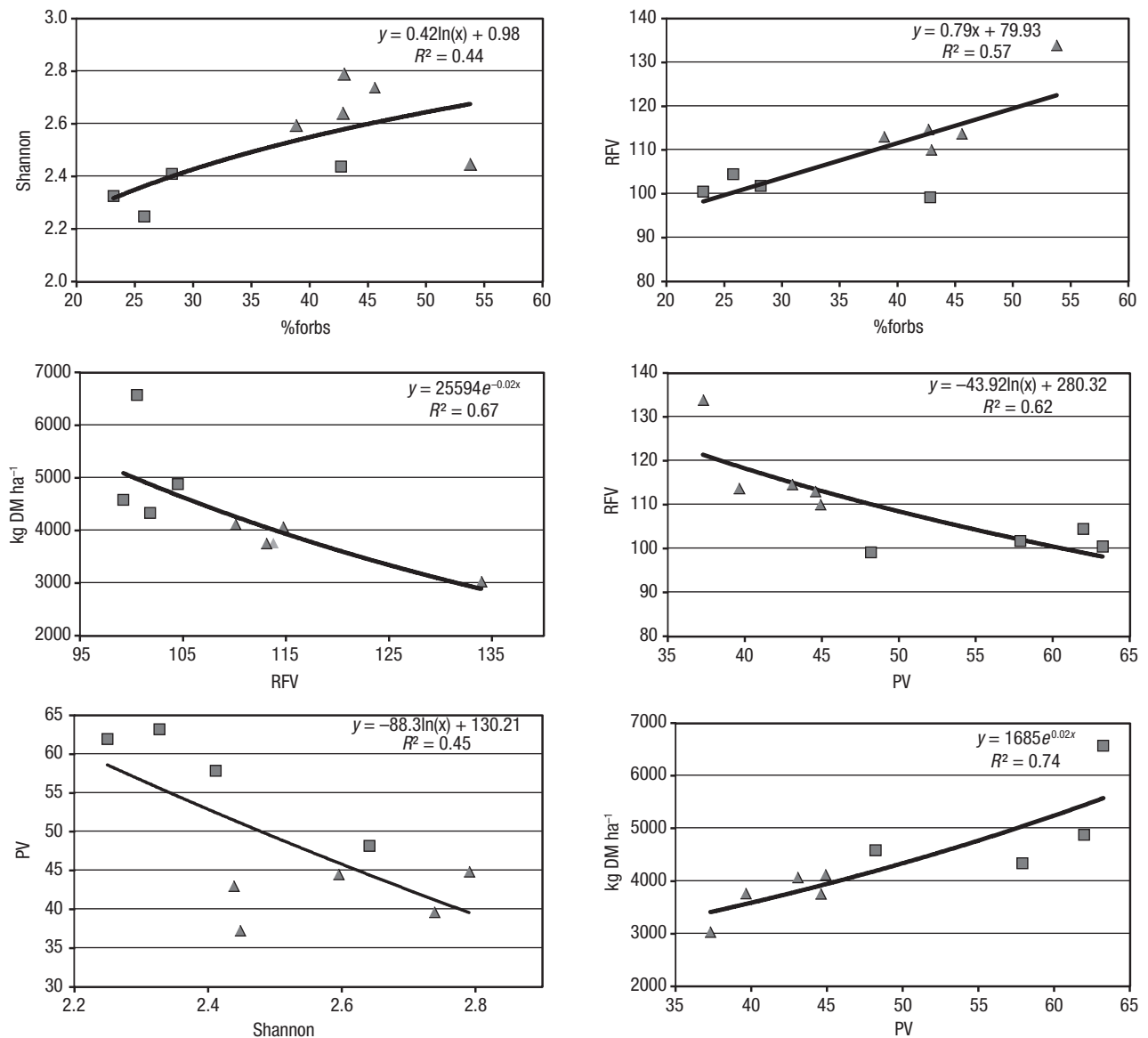
In our study, the groups that comprised meadows that were under more intensive management had more coverage by grasses (Fig. 4) and higher Ellenberg indices of moisture and nitrogen (Fig. 5) than did the groups that comprised meadows that were under less intensive management. In the former, the meadows were on level ground or shallow slopes and, therefore, could accumulate water and be fertilized mechanically more easily than could the meadows on steeper slopes. Intensive management tends to promote a plant community in which tall grasses predominate, which limits the light available to shorter plants (Marini *et al.*, 2008). In our study, coverage by grasses and Ellenberg light indices were significantly positively correlated (Table 2). Meadows in the G6-G9 groups were relatively close to the main farm building, had little or no slope, were fertilized using organic and inorganic fertilizers, had one harvest for hay or silage in summer, and were grazed by cattle in spring and autumn, when they move to or come back from the alpine grasslands (located between 1600 and 2500 m a.s.l.). Grassland management practices vary widely in their disturbance

impact, from low-intensity pasturing without fertilizers to mowing several times a year for fodder production (Wellstein *et al.*, 2007). In France, Andrieu *et al.* (2007) found that the soils of the meadows that had been cut for hay or silage were more N-rich than were the meadows that were grazed, only, because the cut meadows received more fertilizer. In our study, the meadows in groups G1-G5 tended to be distant from the main farm building, on slopes, had low moisture, less frequently fertilized, and were exploited more for grazing than for mowing. As in our study, in semi-natural grasslands in SW Finland that were grazed only, grazing increased the number of species associated with nitrogen-poor soils and low soil moisture (Pykälä, 2005).

## Plant biodiversity

In the 104 meadows of our study, the average number of plant species was 33 (Table 1). In mountain meadows in France, average specific richness ranged between 25 and 38 (Gibon *et al.*, 2004) and, in mountain pastures in Switzerland, the average was 30 (Meisser & Troxler, 2007). The mean SI of the meadows in the Pyrenees (2.55) was substantially lower than those observed in Switzerland (mean = 3.34), what can show a less balanced vegetation in our study.

Plant biodiversity (species richness and Shannon index) was highest in the meadows that had the least intensification (Fig. 4). Marini *et al.* (2008) suggested that, to preserve plant diversity in alpine meadows,



**Figure 8.** Relationship between some parameters in the nine groups (G) of meadow types obtained in TWINSPLAN analysis (see Fig. 1) in 104 plots in semi-natural meadows in the Spanish Pyrenees. (■) Groups that had more intensive management. (▲) Groups that had less intensive management (CP: crude protein; RFV: relative feed value; PV: pastoral value).

conservation policies should promote extensive management practices. In hay meadows in northeastern Switzerland, land management had a strong influence on species richness, and the sites under extensive management had the highest species richness and diversity (Schwab *et al.*, 2002). In our study, the meadows that were under less intensive management were more used for grazing than for mowing. Grazing increases species richness by increasing the diversity of microhabitats through selective consumption, trampling, urination, defecation, and by livestock acting as dispersal

agent (During & Willems, 1984; Wellstein *et al.*, 2007).

In the meadows of our study, high plant biodiversity was associated with plots on slopes and at the highest elevations (Fig 6a). The lower fertilization and the presence of microclimates in these topographical conditions interact to explain the biodiversity gain. In Finland, steep slopes that have poor soils experience extreme microclimatological conditions, which can increase species diversity by enabling less competitive plant species to persist (Pykälä *et al.*, 2005). Marini *et al.* (2008) found that

site features such as steep slopes were correlated with plant species richness in alpine meadows, and the effect was greatest in the meadows that had been mowed infrequently.

In the Pyrenean meadows, plant biodiversity was negatively correlated with the amount of grasses cover and each of the Ellenberg indices, and positively correlated with the amount of 'other forbs' (Fig. 7a and Table 2). Intensive land management, which entails high fertilization and heavy exploitation (mowing and grazing), favours the most competitive species; namely, those that have high growth rates and large size; *e.g.*, grasses. Moderately intense exploitation allows more of the less competitive and, typically, slower growing species to persist. The Ellenberg indices of nitrogen and pH indicated that plant biodiversity was lowest on fertilized soils (meadows under intensive management). In alpine areas in north-eastern Italy, species richness tended to be highest in the meadows that had low management inputs and the least fertilization, which reflected the detrimental effect of intensive management on plant diversity (Marini *et al.*, 2008). Increases in the use of fertilisers have been significant factors in the decline in grassland phytodiversity (Wellstein *et al.*, 2007). Fertilizers increase biomass production, but reduce diversity (Di Tommaso & Aarssen, 1989; Fischer & Wipf, 2002) because they favour grasses and legumes at the expense of characteristic species; *e.g.*, forbs (Fischer & Wipf, 2002). In our study, the amount of 'other forbs' and the Shannon index were positively correlated (Fig. 8a).

Intermediate levels of disturbance favour high species diversity (Grime, 1979; During & Willems, 1984; Wellstein *et al.*, 2007). High financial returns, the objective of agricultural production, are correlated with a decline in biodiversity at intermediate-to-high productivity; which is consistent with the 'hump-back' model of Grime (Hodgson *et al.*, 2005). In our study, we could consider that the meadows with 'less intensive management' are under these intermediate levels of disturbance.

The latest common agricultural policy (CAP) of the EU includes high nature value farming systems, and member countries will have to submit an agricultural systems catalogue that includes a reasoned explanation of the natural values of agricultural regions and the factors that limit their sustainability. Probably plant biodiversity indicators will be used to certify farmland as high nature value. In semi-natural

meadows, yield and forage quality must be balanced against the need to conserve biodiversity, even if farmers perceive this as an inconvenience, initially. The policy should insure that farmers recognize the many benefits of plant biodiversity including an increase in the persistence of meadows, an increase in food intake by grazing animals because they can forage selectively, nutritional advantages, the benefits to animal health and the sensory qualities of animal products (*e.g.*, milk, cheese, meat). In Spain, financial incentives should be used to encourage farmers to conserve biodiversity. In Switzerland, for example, each farmer is required to manage 7% of the farmland as ecological compensation areas (ECA), and the features that qualify as ECA include meadows, traditional orchards and hedgerows (Henle *et al.*, 2008).

### Yield and nutritive quality of forage

The forage yields of the meadows assessed in our study varied widely (1919-8880 kg DM ha<sup>-1</sup>), which paralleled the variability in plant height (0.3-1.6 m) (Table 1). The meadows that were under 'more intensive management' (G6-G9) had the tallest vegetation and the highest yields (Fig. 6). In addition, yields were positively correlated with the amount of coverage of grasses and negatively correlated with plant biodiversity (species richness and Shannon index) and with the amount of coverage by 'other forbs' (Fig. 7b). Fertilizers increase yield because grasses are favoured at the expense of many 'other forbs'.

Forage quality varied widely (Table 1). CP ranged from 6.87 to 17.09 and RFV ranged from 78.58 (quality standard = 4) to 153.27 (prime quality). CP and RFV were correlated with the amount of coverage by 'other forbs' (Fig. 7b, see also Fig. 8b) and with plant biodiversity (species richness and Shannon index) (see also Table 2). Thus, the meadows that had the least intensive management (G1-G5) had the highest quality forage (Fig. 6). Furthermore, forage quality (CP and RFV) was negatively correlated with the amount of coverage of grasses and with yield; *i.e.*, the forage of the meadows that were under intensive management had less quality (see Fig. 7b, and also Fig. 6), and was negatively correlated with forage quantity (Fig. 8c).

RFV is a function of digestible dry matter (DDM) and dry matter intake (DMI), which are inverse

functions of acid detergent fiber (ADF) and neutral detergent fiber (NDF), respectively. Thus, high NDF and ADF are negatively correlated with RFV, which was apparent in the most intensively managed meadows in the Pyrenees, where grasses predominated (Fig. 6, Table 2). At similar stages of growth, typically, grasses contain higher concentrations of NDF and ADF than do legumes and 'other forbs' (Vázquez de Aldana *et al.*, 2009). Concentrations of fiber are higher in the leaves and stems of grasses than they are in legumes (Buxton & Redfearn, 1997). In legume leaves, few tissues develop thick secondary walls; consequently, the total cell wall concentration of legume leaves does not increase with maturity as dramatically as it does in grass leaves (Jung & Engels, 2002). In grasses, the development of the stem at flowering involves a rapid accumulation of cell wall material; however, in legumes, material is added to leaves and stems, concurrently (Nordkvist & Aman, 1986). Typically, an increase in dietary NDF has a negative effect on the amount of DM consumed by cows (Allen, 2000).

The digestibility of 'other forbs' can be high (Table 2 and Fig. 8b) and varies relatively little over time (Bruinenberg *et al.*, 2002, Andueza *et al.*, 2010); consequently, the nutritional value of biologically diverse meadows remains stable throughout the growing season (Baumont *et al.*, 2008). Biodiversity allows grazing livestock to forage selectively, which favours high intake rates (Bruinenberg *et al.*, 2002; Duncan *et al.*, 2003; Baumont *et al.*, 2008).

### **Pastoral value (PV). The case of the 'other forbs'**

The average PV of the meadows in the Spanish Pyrenees was 50, which was 20% higher than the average in mountain meadows in Switzerland (Gillet *et al.*, 2002; Gillet, 2008), although PV varied widely (23.65-74.48) (Table 1). In other mountain pastures in Switzerland, PV ranged between 38 and 52 (Meisser & Troxler, 2007). In our study, PV was positively correlated with the grasses, plant height and yield, and was highest in the meadows that were under more intensive management (Fig. 7b, see also Fig. 6). PV can be a bromatological index of pasture quality (Daget & Poissonet, 1972); however, in our study, PV was negatively correlated with CP and RFV (Table 2, Fig. 7b, Fig. 8d). Furthermore, PV was negatively correlated with plant biodiversity and with 'other

forbs' (Table 2, Fig. 7b and Fig. 8e). In wet grasslands in West Africa, PV and biodiversity were negatively correlated (Botoni-Liehouin *et al.*, 2006). Grazing livestock can choose from dozens of plant species, but their preferences are not correlated with the SI (Agreil *et al.*, 2004). In our study, PV was positively correlated with yield, but negatively correlated with forage quality.

In our view, the criteria used in the PV method to assign each species an index of quality (SI) are 'agronomic' and many of the species traditionally considered 'weeds' (typically, designated 'other forbs') are often underestimated or assigned an  $SI = 0$  because their nutritional qualities, digestibility, and intake are poorly understood (Baumont *et al.*, 2008). Farruggia *et al.* (2012) used metabarcoding (sequencing plant DNA fragments) to identify in the faeces of grazing cattle many plant taxa that had an  $SI = 0$ . Some of those taxa can contribute to the stability of the feed value of multispecific meadows (Alard & Balent, 2007), provide the nutritional benefits of secondary compounds such as condensed tannins (Ramirez-Restrepo & Barry, 2005), which reduce the solubilization and degradation of proteins by rumen microorganisms (Min *et al.*, 2000), phenolic compounds (including tannins) that provide insecticidal and antiparasitic benefits, which confers resistance to diseases and encourages herbivory (Waterman & Mole, 1994), and chemical compounds such as terpenes and phenols, which provide positive sensory qualities to animal products such as meat and cheese (Farruggia *et al.*, 2008). The widespread view that 'other forbs' have low nutritional value should be evaluated in future research.

In semi-natural meadows in the Spanish Pyrenees, some geographical and topographical features (elevation, slope, distance to the main farm building) have influenced environmental conditions (soil moisture) and management systems (mechanization, fertilization, mowing, and grazing). Collectively, those factors have influenced the floristic composition of the meadows and, consequently, the yield and quality of the forage. In meadows close to the main farm building, which were in areas that had little or no slope, experienced 'more intensive management' (easy mechanization, fertilization with organic and inorganic fertilizers, one harvest for hay or silage in summer, and livestock grazing in spring and autumn) and grasses predominated, plant biodiversity was low, PV and yield were high, but forage quality (CP and RFV) was low.

The meadows that were on slopes, at relatively high elevations, or distant from the main farm building were under 'less intensive management' (little or no fertilization, exploited more for grazing than for harvest) and had a high proportion of 'other forbs', plant biodiversity and forage quality were high, but PV and yield were low. Management options of the meadows, compatible with environmental conservation, are possible with small adjustments to traditional systems in many cases, rather than intensive management or abandonment of their use by livestock.

## Acknowledgements

The Government of Aragón (Project PM076/2007) provided financial support for this study. We are grateful to farmers for meadows management information.

## References

- Agreil C, Meuret M, Vincent M, 2004. GRENOUILLE: une méthode pour gérer les ressources alimentaires pour des ovins sur milieux embroussaillés. *Fourrages* 180: 467-481.
- Alard D, Balent G, 2007. Sécheresse: quels impacts sur la biodiversité en systèmes prairiaux et pastoraux? *Fourrages* 190: 197-206.
- Allen MS, 2000. Effect of diet on short-term regulation of feed intake by lactation dairy cattle. *J Dairy Sci* 83: 1598-1624.
- Andrieu N, Josien E, Duru M, 2007. Relationships between diversity of grassland vegetation, field characteristics and land use management practices assessed at the farm level. *Agr Ecosyst Environ* 120: 359-369.
- Andueza D, Cruz P, Farruggia A, Baumont R, Picard F, Michalet-Doreau B, 2010. Nutritive value of two meadows and relationships with some vegetation traits. *Grass Forage Sci* 65: 325-334.
- Ansquer P, Theau JP, Cruz P, Viegas J, Al Haj Khaled R, Duru M, 2004. Caractérisation de la diversité fonctionnelle des prairies naturelles. Une étape vers la construction d'outils pour gérer les milieux à flore complexe. *Fourrages* 179: 353-368.
- Barrantes O, Ferrer C, Reiné R, Broca A, 2009. Categorization of grazing systems to aid the development of land use policy in Aragon, Spain. *Grass Forage Sci* 64: 26-41.
- Baumont R, Aufrère J, Niderkorn V, Andueza D, Surault F, Peccatte JR, Delaby L, Pelletier P, 2008. La diversité spécifique dans le fourrage: conséquences sur la valeur alimentaire. *Fourrages* 194: 189-206.
- Blackstock TH, Rimes CA, Stevens DP, Jefferson RG, Robertson HJ, Mackintosh J, Hopkins JJ, 1999. The extent of semi-natural grassland communities in lowland England and Wales: a review of conservation surveys 1978-96. *Grass Forage Sci* 54: 1-18.
- Botoni-Liehoun E, Daget P, Cesar J, 2006. Activités de pâturage, biodiversité et végétation pastorale dans la zone Ouest du Burkina Faso. *Revue d'Élevage et de Médecine Vétérinaire des Pays Tropicaux* 59: 31-38.
- Braun-Blanquet J, 1965. *Plant Sociology. The study of plant communities.* Hafner Publ Co, NY, USA. 439 pp.
- Bruinenberg MH, Valk H, Korevaar H, Struik PC, 2002. Factors affecting digestibility of temperate forages from seminatural grasslands: a review. *Grass Forage Sci* 57: 292-301.
- Buxton DR, Redfearn D, 1997. Plant limitations to fiber digestion and utilization. *J Nutr* 127: 814-818.
- Chocarro C, Reiné R, Ascaso J, Yera J, Ferrer C, 2009. 6520 Prados de siega de montaña (*Trisetum-Polygonum bistortae*). In: Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España (VV.AA, eds.) Dirección General de Medio Natural y Política Forestal, Ministerio de Medio Ambiente, y Medio Rural y Marino, Madrid (Spain), 48 pp.
- Čop J, Vidrih M, Hacin J, 2009. Influence of cutting regime and fertilizer application on the botanical composition, yield and nutritive value of herbage of wet grasslands in Central Europe. *Grass Forage Sci* 64: 454-465.
- Daget P, Poissonet J, 1972. Un procédé d'estimation de la valeur pastorale des pâturages. *Fourrages* 49: 31-39.
- Di Tommaso A, Aarssen LW, 1989. Resource manipulation in natural vegetation: a review. *Vegetatio* 84: 9-29.
- Duncan AJ, Ginane C, Gordon IJ, Orskov ER, 2003. Why do herbivores select mixed diets? *Proc VI<sup>th</sup> Int Symp on the Nutrition of Herbivores, Mérida (México)*, Oct 19-24. pp: 195-209.
- During HJ, Willems JH, 1984. Diversity models applied to a chalk grassland. *Vegetatio* 57: 103-114.
- Ellenberg H, Weber HE, Dull R, Wirt HV, Werner W, Paulissen D, 1991. *Zeigerwerte von Pflanzen in Mitteleuropa.* Scripta Geobotanica 18: 1-248.
- Farruggia A, Martin B, Baumont R, Prache S, Doreau M, Hoste H, Durand D, 2008. Quels intérêts de la diversité floristique des prairies permanentes pour les ruminants et les produits animaux? *INRA Prod Anim* 21(2): 181-200.
- Farruggia A, Pompanon F, Ginane C, Vazeille K, Niderkorn V, Hulin S, 2012. Reconstituer la composition du régime alimentaire des herbivores domestiques au pâturage: l'approche par métabarcoding. *Fourrages* 209: 43-51.
- Ferrer C, Amella A, Maestro M, Broca A, Ascaso J, 1990. Praderas naturales de los fondos de valle del Pirineo Central: suelo, manejo, flora, producción y calidad. *Proc XXX Reunión Científica de la Sociedad Española para el Estudio de los Pastos, San Sebastian (Spain)*, pp: 168-183.
- Fischer M, Wipf S, 2002. Effect of low-intensity grazing on the species-rich vegetation of traditionally mown subalpine meadows. *Biol Conserv* 104: 1-11.

- Gibon A, Balent G, Alard D, Muntane J, Ladet S, Mottet A, Julien MP, 2004. L'usage de l'espace par les exploitations d'élevage de montagne et la gestion de la biodiversité. *Fourrages* 178: 245-263.
- Gillet F, 2008. Modelling vegetation dynamics in heterogeneous pasture-woodland landscapes. *Ecol Model* 217: 1-18.
- Gillet F, Besson O, Gobat JM, 2002. PATUMOD: a compartment model of vegetation dynamics in wooded pastures. *Ecol Model* 147: 267-290.
- Grime JP, 1979. Plant strategies and vegetations processes. John Wiley & Sons, Chichester, UK. 222 pp.
- Henle K, Alard D, Clitherow J, Coob P, Firbank L, Kull T, Mccracken D, Moritz RFA, Niemelä J *et al.*, 2008. Identifying and managing the conflicts between agriculture and biodiversity conservation in Europe. A review. *Agr Ecosyst Environ* 124: 60-71.
- Hill MO, 1979. Twinspan. A Fortran program for arranging multivariate data in an ordered two-way table by classification of the individuals and attributes. Cornell Univ, NY, USA. 90 pp.
- Hill MO, Preston CD, Roy DB, 2004. PLANTATT - Attributes of British and Irish plants: status, size, life history, geography and habitats. Centre for Ecology & Hydrology, Cambridge, UK. 73 pp.
- Hodgson JG, Montserrat-Martí G, Tallowin J, Thompson K, Díaz S, Cabido M, Grime JP, Wilson PJ, Band SR *et al.*, 2005. How much will it cost to save grassland diversity? *Biol Conserv* 122: 263-273.
- IBM, 2010. SPSS -Statistics v.19.0. IBM Company, Armonk, NY, USA.
- Jung HG, Engels FM, 2002. Alfalfa stem tissues: cell-wall deposition, composition and degradability. *Crop Sci* 42: 524-534.
- Kahmen S, Poschlod P, 2008. Effects of grassland management on plant functional trait composition. *Agr Ecosyst Environ* 128: 137-145.
- Kirkham FW, Tallowin JRB, 1995. The influence of cutting date and previous fertilizer treatment on the productivity and botanical composition of species-rich hay meadows on the Somerset levels. *Grass Forage Sci* 50: 365-377.
- Linn JG, Martin NP, 1999. Forage quality tests and interpretations. Univ. Minnesota, Extension WW-02637. Available in <http://www.extension.umn.edu/distribution/livestocksystems/DI2637.html> [4 June 2013]
- MAFF, 2002. Towards sustainable agriculture (a pilot set of indicators). Ministry of Agriculture, Fisheries and Food Publications, London, UK. 72 pp.
- Marini L, Scotton M, Klimek S, Pecile A, 2008. Patterns of plant species richness in Alpine hay meadows: Local vs. landscape controls. *Basic Appl Ecol* 9: 365-372.
- Meisser M, Troxler J, 2007. Effet du niveau de chargement sur la composition botanique d'un estivage jurassien. *Revue Suisse d'Agriculture* 39: 67-71.
- Min BR, McNabb WC, Barry TN, Peters JS, 2000. Solubilization and degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39; Rubisco) protein from white clover (*Trifolium repens*) and *Lotus corniculatus* by rumen microorganisms and the effect of condensed tannins on these processes. *J Agr Sci* 134: 305-317.
- Nordkvist E, Aman, P, 1986. Changes during growth in anatomical and chemical composition and in-vitro degradability of lucerne. *J Sci Food Agr* 37: 1-7.
- OJ, 1992. Directive 92/43/EEC of the Council of May 21. Official Journal of the European Union L 206 22/07/1992. p. 7.
- Paracchini ML, Petersen JE, Hoogeveen Y, Bamps C, Burfield I, Van Swaay C, 2008. High nature value farmland in Europe. An estimate of the distribution patterns on the basis of land cover and biodiversity data. Institute for Environment and Sustainability, European Commission, Luxembourg. 87 pp.
- Piveteau V, 1998. Agriculture et environnement: les indicateurs. Diffusion Lavoisier, Cachan Cedex, France. 72 pp.
- Pykälä J, 2005. Plant species responses to cattle grazing in mesic semi-natural grassland. *Agr Ecosyst Environ* 108: 109-117.
- Pykälä J, Luoto M, Heikkinen RK, Kontula T, 2005. Plant species richness and persistence of rare plants in abandoned semi-natural grasslands in northern Europe. *Basic Appl Ecol* 6: 25-33.
- Ramírez-Restrepo CA, Barry TN, 2005. Alternative temperate forages containing secondary compounds for improving sustainable productivity in grazing ruminants. *Anim Feed Sci Technol* 120: 179-201.
- Raunkiaer C, 1934. The life forms of plants and statistical plant geography. Clarendon Press, Oxford, UK. 632 pp.
- Reiné R, Chocarro C, Fillat F, 2004. Soil seed bank and management regimes of semi-natural mountain meadow communities. *Agr Ecosyst Environ* 104: 567-575.
- Reiné R, Ascaso J, Ferrer C, Yera J, Chocarro C, 2009. 6510 Prados de siega de montaña (*Arrhenatherion*). In: Bases ecológicas preliminares para la conservación de los tipos de hábitat de interés comunitario en España (VV.AA, eds.). Dirección General de Medio Natural y Política Forestal, Ministerio de Medio Ambiente, y Medio Rural y Marino, Madrid (Spain). 60 pp.
- Roggero PP, Bagella S, Farina R, 2002. Un archivio dati di indici specifici per la valutazione integrata del valore pastorale. *Rivista di Agronomia* 36: 149-156.
- Schwab A, Dubois D, Padruot MF, Edwards PJ, 2002. Estimating the biodiversity of hay meadows in north-eastern Switzerland on the basis of vegetation structure. *Agr Ecosyst Environ* 93: 197-209.
- Shannon CE, Weaver W, 1949. The mathematical theory of communication. Univ. Illinois Press, Illinois, USA. 144 pp.
- Tutin TG, Chater NA, Burges AO, Heywood JR, Edmonson VH, Moore DH, Valentine DH, Waters SM, Webb DA, 1964-1980. Flora europaea. Cambridge Univ. Press, Cambridge, UK. 2591 pp.
- Van Der Maarel E, 1979. Transformation of cover-abundance values in phytosociology and its effects on community similarity. *Vegetatio* 39: 97-114.
- Vázquez De Aldana BR, García-Ciudad A, García-Criado B, 2009. Relación entre compuestos fenólicos y calidad nutritiva en especies pratenses. In: La multifuncionalidad



- de los pastos: producción ganadera sostenible y gestión de los ecosistemas (Reiné R, Barrantes O, Broca A, Ferrer C, eds.). SEEP, Huesca (Spain), pp 273-278.
- Waterman PG, Mole S, 1994. Analysis of phenolic plant metabolites. Blackwell Sci. Publ., Oxford, UK. 238 pp.
- Wellstein C, Otte A, Waldhardt R, 2007. Impact of site and management on the diversity of central European mesic grassland. *Agr Ecosyst Environ* 122: 203-210.
- Zar JH, 1984. Biostatistical analysis. Prentice Hall, NJ, USA. 944 pp.