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Plant traits and functional types in response to reduced disturbance in a semi-natural grassland

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Abstract

Question: How do functional types respond to contrasting levels of herbage use in temperate and fertile grasslands?

Location: Central France (3°1' E, 45°43' N), 870 m a.s.l.

Methods: Community structure and the traits of dominant plant species were evaluated after 12 years of contrasted grazing and mowing regimes in a grazing trial, comparing three levels of herbage use (high, medium and low).

Results and Conclusions: Of 22 measured traits (including leaf traits, shoot morphology and composition, phenology), seven were significantly affected by the herbage use treatment. A decline in herbage use reduced individual leaf mass, specific leaf area and shoot digestibility, but increased leaf C and dry matter contents. Plants were taller, produced larger seeds and flowered later under low than high herbage use. Nine plant functional response types were identified by multivariate optimization analysis; they were based on four optimal traits: leaf dry matter content, individual leaf area, mature plant height and time of flowering. In the high-use plots, two short and early flowering types were co-dominant, one competitive, grazing-tolerant and moderately grazing-avoiding, and one grazing-avoiding but not -tolerant. Low-use plots were dominated by one type, neither hardly grazing-avoiding nor grazing-tolerant, but strongly competitive for light.

Keywords: Extensification; Grazing; Leaf trait; Plant attribute.

Nomenclature: Tutin et al. (1993).

Abbreviations: BE = Beginning of flowering period; DI = Digestibility; IT = Height at top of inflorescence; LA = Individual leaf area; LCC = Leaf carbon concentration; LDM = Leaf dry mass; LDMC = Leaf dry matter content; LFM = Leaf lamina fresh mass; LNC = Leaf nitrogen concentration; ME = Flowering plant height, highest leaf elongated; MH = Flowering plant height, highest leaf not elongated; NG = Number of growing green leaves; NM = Number of mature green leaves; PRT = Plant functional response type; RA = Leaf:Shoot dry matter ratio; SLA_F = Specific leaf area (fresh mass based); SLA_D = Specific leaf area (dry mass based); SM = Seed mass.

Introduction

In semi-natural grasslands, nutrient availability and disturbance regime are two major factors influencing vegetation dynamics that are constrained by agricultural management. At present, part of the permanent grasslands in Europe's mountain areas are used more extensively, i.e. lower or no mineral fertilizer supply and a low degree of disturbance resulting from reduced stocking rates. While the impacts of fertilizer use and of grazing and mowing regimes on vegetation dynamics have been widely studied in the past, current European studies tend to focus more on grasslands which have recently been taken out of intensive use (e.g. Watt et al. 1996). In such studies, changes in species number and composition are reported, with sometimes dramatic changes in dominance (Marriott et al. 1996; Laser 2002). However, the plant species involved are mostly site specific and, therefore, general trends in the effects of extensive management of formerly fertile grasslands are still difficult to identify.

A functional analysis of vegetation may help to understand and predict the impact of this extensification in a more general way. Screening for plant traits allows two questions to be addressed: 1. Which traits are associated with a given set of plant populations in a community? 2. How do the traits of the selected plant populations control ecosystem functioning and productivity? (Lavorel & Garnier 2002). In this paper, we focus on the first question.

We assume that plant species selected in response to a given factor (e.g. herbage use level) exhibit some converging traits that condition their performance in the community. Recent studies (Díaz et al. 2001) have demonstrated that similar plant traits, in different floras, were associated with a specific response to grazing. In addition, plant response generally involved functional trade-offs, implying association between plant traits

(Elberse & Berendse 1993; Reich 1993; Poorter & Garnier 1999). The analysis of these relationships helps in the understanding of plant responses to environmental factors and is mostly used to identify plant functional types (PFTs) (Lavorel et al. 1998). The identification of PFTs, based on functional traits or on easy to measure ('soft') traits related to function (Weiher et al. 1999), could provide a powerful approach to the understanding of vegetation response to environmental factors, but this method has only recently been used for agriculturally managed grasslands (Cruz et al. 2002).

We analyse the results of an extensification experiment conducted over 12 years in a fertile temperate grassland under three levels of herbage use. We address the following specific questions: 1. How are plant traits affected by reduced disturbance? 2. Which plant functional response types (PRT) can be identified to describe vegetation changes under extensive management? We adopt the definition that a PRT is a group of plants similar in a set of traits and similar in their response to given environmental factors (Díaz & Cabido 2001; Lavorel & Garnier 2002).

Material and Methods

Site description and experimental design

The experiment was established in 1989 on permanent grassland located at Theix (Puy de Dôme, France; 3°1' E, 45°43' N), 870 m a.s.l., on a fertile brown and slightly acidic sandy soil (53% sand, 22% loam, 25% clay, 5.5% organic matter, pH 5.6). Over the period 1989-2001, the mean air temperature was 8.8 °C and the mean annual precipitation was 803 $mm.a^{-1}$, ranging from 507 to 1114 mm.

The site had been maintained as a grassland at least since the 1960s, and most of it since the 1940s. Before the start of the experiment, it was fully exploited by grazing, cutting and with regular organic and inorganic fertilizer supply. The grassland was species-poor, belonging to the class of *Arrhenatheretea* (Guinochet 1970).

Three experimental treatments have been applied since 1989, in a randomized complete block design with two replicates. The plots, each 460 to 480 m^2, were subjected to management regimes defined by three levels of herbage use: high (H), medium (M) and low (L). The H-plots were first grazed in mid-April, cut for hay in mid-June and the regrowth grazed three times in July, September and November. At each of the four grazing periods, eight ewes per plot were left grazing for a few days until the sward height, measured daily with an 'HFRO's sward stick' (Barthram 1986), reached 5.5 - 6.5 cm; thus grass availability in the H-plots determined grazing duration. The M- and L-plots were not cut for hay, and were grazed at the same time as the H-plots, in the four grazing periods (M-plots) or in only one grazing period in mid-April (L-plots). For treatments M and L, the number of ewes per plot and the duration of each grazing period were similar to those used in the H-plots. No fertilization was applied after 1989.

Nutrient status and herbage use

Annual measurements of number of ewes grazing days (number of grazing days × eight ewes) and herbage yield at cutting in H-plots allowed us to assess herbage dry matter used per plot. Once a year, at the end of May, sampling units were cut at ground level on two to four 0.35-m^2 quadrats per plot. These were dried, weighed, measured for N (Kjeldahl's method), P (Dyer's method) and K (after extraction by ammonium acetate) contents on green and dead material. Herbage availability and nutrient content were used to assess the N-nutritional status according to Lemaire & Gastal (1997) and the nutritional status of P and K according to Duru (1992) and Duru & Calvière (1996).

Vegetation surveys

In 2000, after 12 years of management treatments, the plant species composition was determined in all the plots, and species traits were measured. For these measurements each plot was divided in two subplots; there was thus a total of 12 subplots in the experiment.

In each subplot species composition was estimated by a point quadrat method in May 2000. Presence/absence of species was recorded at 40 points, 0.5 m apart on a transect, using a pin which was moved downward and which could hit more than one species. For each species presence was calculated as the number of hits per 100 points (Grant 1993). This was standardized to the sum of presences for all species as % presence. This allowed us to filter out differences in total cover arising from variation in productivity and herbage use, emphasizing differences in species and functional types composition.

Plant traits

Plant traits of the most abundant species in each subplot were measured. These species were those accounting for at least 85% of the total species presence in the subplot. For each species, a set of 22 traits adapted from Weiher et al. (1999) was measured (Table 1). These involve leaf morphology and composition, plant morphology and reproductive characteristics. In May 2000, three weeks after grazing, ten undamaged and unshaded

Table 1. Aggregated values at the community scale of plant traits measured in plots managed at three contrasted levels of herbage use. A cross symbol denotes traits that are included in the common core list of plant traits (Weiher et al. 1999). Values with different letters indicate a significant (l.s.d. method, $p < 0.05$) herbage use treatment effect in an ANOVA ($n = 6$) for a block design. Data were collected on V = vegetative and R = reproductive shoots, or through O = visual observation at the community scale. Values are means (± SE) of two replicate plots. The block factor is significant only for ME. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

	Traits	Data collection method	High	Medium	Low	Treatment
LL	Leaf length (mm)	V	149	169	150	n.s.
LA	Individual leaf lamina area (cm^2)	V	8.0	6.7	6.2	n.s.
LFM	Individual water saturated leaf lamina fresh mass (g)	V	0.19	0.11	0.12	(*p*<0.10)
LDM	Individual leaf lamina dry mass (g DM)	V	0.029	0.025	0.027	n.s.
SLA_F^X	Specific leaf lamina area on an FM basis ($cm^2.g^{-1}$ FM)	V	61.8	67.9	57.2	n.s.
SLA_D^X	Specific leaf lamina area on a DM basis ($cm^2.g^{-1}$ DM)	V	297	298	245	(*p*<0.10)
$LDMC^X$	Leaf lamina DM content (mg-DM g^{-1}FM)	V	210^a	230^b	230^b	**
LNC	Leaf lamina N content (%)	V	3.0	3.0	3.5	n.s.
LCC	Leaf lamina C content (%)	V	41.1^a	39.7^b	42.0^c	*
DI	*In vitro* cellulase shoot digestibility (%)	V	76	70	70	(*p*<0.10)
NG	Number of growing leaves per shoot axis	V	1.3	1.1	1.8	n.s.
NM	Number of mature leaves per shoot axis	V	2.6	2.5	3.2	n.s.
RA	Leaf to shoot DM ratio	V	57	54	59	n.s.
VE^X	Vegetative plant height (mm) elongated	V	288	343	306	n.s.
VH^X	Vegetative plant height (mm) not elongated	V	317	425	334	n.s.
ME^X	Flowering plant height (mm), highest leaf elongated	R	517^a	711^b	723^b	*
MH^X	Flowering plant height (mm), highest leaf not elongated	R	455^a	671^b	684^b	*
IB^X	Height of inflorescence base (mm)	R	616	863	764	(*p*<0.10)
IT^X	Height of inflorescence top (mm)	R	721^a	1000^b	941^b	*
SM^X	Seed mass (g per 100 seeds)	R	0.075^a	0.138^b	0.178^b	*
BE^X	Beginning of flowering period (Julian day)	O	156^a	165^b	178^c	*
DU	Duration of flowering period (Days)	O	28	30	40	n.s.

plants were selected for each species and subplot. Plant heights, elongated or not, were recorded in the field. For leaf traits, the vegetative tiller, shoot or the whole plant, respectively for grasses, clover and other dicots, was collected. In the laboratory, the tiller or shoot base was cut in de-ionized water and the plant tissue allowed to rehydrate at 4 °C for at least 6 h in the dark (Garnier et al. 2001). After rehydration, leaf lamina fresh mass (LFM), leaf length (LL) and leaf area (LA) were measured with an electronic planimeter (LI 3100, Li-Cor, Lincoln, NE, USA), on the youngest fully expanded leaves of each of the ten individuals. The leaves were then oven dried at 60 °C for 48 h and weighed (LDM). Specific leaf area, based on fresh water saturated mass (SLA_F) or on dry mass (SLA_D) and leaf dry matter content (LDMC) were calculated according to Garnier et al. (2001) for the leaf laminae. For these traits, the value was estimated as the mean of the ten measurements. Three or four leaves were then pooled forming three sub-samples. Total N-concentration and C-content were determined for each with an elemental analyser (Carlo Erba Instruments, CNS NA 1500 ThermoFinnigan, Milan, Italy). The leaf N (LNC) and C-(LCC) concentration for each species in each subplot was calculated as the mean of the three subsamples.

A second sample consisting of ten individuals, vegetative tillers or shoot axes, was cut at ground level and collected. The number of growing (NG) and mature (NM) green leaves per shoot axis was recorded. The material was then sorted into leaf and stem or sheath, dried at 60 °C for 48 h and weighed. For each species, the material (leaf, sheath or stem of the ten replicates) was then pooled and the determination of the *in vitro* pepsin cellulase digestibility (DI) was performed according to Aufrère & Michalet-Doreau (1988).

From April to October, once a week, the developmental stage of each species in each subplot was visually assessed on undefoliated plants (i.e. on quadrats which were excluded from the hay cut in H plots and on ungrazed plants in the other two treatments), which allowed us to determine the times of beginning (BE) and end of flowering (visible anthers). A sample of ten mature individuals per species was measured for height at the top (IT) and the base (IB) of the inflorescences, highest lamina elongated or not – ME and MH respectively – and the inflorescences were collected and pooled. A subsample of 100 seeds per species was sorted, oven dried (60 °C, 48 h) and weighed (SM).

Aggregated plant traits

The data set consisted of trait values measured on the main species in each of the 12 subplots. Aggregated trait values were calculated in each of the 12 subplots, at the community level, as means weighted by the % presence of the species. In each plot, ($n = 6$), the mean value of the aggregated traits was then calculated as the mean of the two subplots.

Plant functional response types identification

For the identification of PRTs the multivariate method of Pillar & Sosinski (2003) implemented in the application SYNCSA (http://ecoqua.ecologia.ufrgs.br) was used. Data were organized in three matrices describing the plant populations by traits, the subplots by the % presence of plant populations and the subplots by treatment level, i.e. the index of the herbage dry matter used per subplot. The data were subjected to a recursive algorithm that searched for a trait subset and PRTs maximally associated to herbage use level, as indicated by an objective function (congruence measure, Pillar & Sosinski 2003). The algorithm was similar to that described in Pillar (1999), but at any given recursive iteration a cluster analysis was involved as an additional step to define PRTs by a polythetic classification based on the matrix of populations by traits. For choosing the number of PRTs (partition level), the algorithm additionally searched, for each trait subset, a partition level maximizing the objective function. The % presence values of the PRTs were thus obtained in each of the 12 subplots. In each plot (n = 6), the mean value of the PRT % presence values was then calculated as the mean of the two subplots.

Data analysis

Effects of the herbage use treatments and of the block were tested by ANOVA on herbage use index and nutrition indices (Table 2), species composition (Table 3), aggregated plant traits (Table 1) and on the PRTs identified (Table 4), considering a complete randomized blocks design, each consisting of the three plots ($n = 6$).

A PCA (Statgraphics Plus, Manugistics) was run on the optimal traits × populations matrix to summarize the correlations among traits within the data set.

Results

Herbage use level and nitrogen status

From the beginning of the experiment in 1989, the annual amount of herbage used (cut or grazed) was lower in the M- and L-treatments than the H-treatment by ca. 60 and 90%, respectively (Table 2a); 12 years after the beginning of the experiment, N-nutrition levels were higher in the L-plots, compared to M- and H-plots (Table 2B). This effect was significant for 2001 ($p <$ 0.02) and close to significance ($p < 0.06$) in 2000. The P- and K-nutrition levels were non limiting (close to or above 100) in all plots and were not significantly affected by herbage use treatments, except for a higher K-level in M compared to L and H in 2001 (Table 2B).

Plant community structure

The grassland communities consisted of perennial C_3 species (grasses, legumes and forbs). Marked differences in species composition were observed in 2000, as a result of 12 years of contrasted management treatments (Table 3). Considering the two replicate plots pooled per treatment, species numbers reached 19, 20 and 27 in H, M and L, respectively, and dicots accounted for 37, 30 and 52% of these totals. There were 6, 2 and 10 treatment specific species in H, M and L, respectively. The number of dicot species per plot was significantly lower ($p < 0.05$) in M (17%) compared to H and L (32 and 42%, respectively) (Table 3). Among the most abundant species of each subplot, there were only five grasses common to the three treatments (*Holcus lanatus*, *Poa trivialis, Elytrigia repens*, *Agrostis capillaris* and *Poa pratensis)*.

Table 2a. Mean herbage use index in the treatments with High (H), Medium (M) and Low (L) levels of herbage use. Annual number of ewes grazing days, total intake (assuming a daily intake of 2 kg DM per ewe), DM yield of herbage cut for hay and total herbage DM used, mean from 1989 to 2000. The herbage use index is calculated as the total amount of herbage used in the M and L treatments relative to that in the H treatment (index value of 100). **b.** Nutrition index for N, P and K in may 2000 and 2001. Different letters on the same row indicate significant differences between treatments (ANOVA, $p < 0.05$). Values are means (± s.e.) of two replicate plots. The block effect is not significant.

a. Herbage use index		H	M	L	Treatment
Ewes grazing days (d.ha^{-1}.a^{-1})		2159 ^{a} ± 134	2148 ^{a} ± 141	583 ^{b} ± 49	**
Total intake (t-DM.ha^{-1}.a^{-1})		4.32 ^{a}	4.30 ^{a}	1.17 ^{b}	**
Cut herbage (t-DM.ha^{-1}.a^{-1})		6.07 ± 0.51	-	-	
Total herbage use (t-DM.ha^{-1}.a^{-1})		10.39 ^{a}	4.30 ^{b}	1.17 ^{c}	**
Herbage use index		100 ^{a}	41 ^{b}	11 ^{c}	**
b. Nutrition indices (%)					
N	2000	55 ± 2	66 ± 1	76 ± 3	NS
	2001	51^a ± 2	58^a ± 2	80^b ± 7	**
P	2000	122 ± 2	116 ± 1	113 ± 6	NS
	2001	112 ± 2	119 ± 3	109 ± 6	NS
K	2000	109 ± 4	112 ± 4	110 ± 2	NS
	2001	98^a ± 1	113^b ± 2	103^a ± 5	*

There were between five and nine species accounting for at least 85% of the % presence values per subplot. Therefore, trait values which have been measured on the dominant species of each of the 12 subplots were measured on a total of 93 populations. Nevertheless, because of some missing values for a few traits, 86 populations were fully described and used for the trait's analysis and PRT identification. Excluded populations were three of *Poa angustifolia*, two of *P. trivialis*, one of *Bromus erectus*, one of *Cirsium arvense* and one of *Festuca rubra*.

Aggregated plant traits

The level of herbage use significantly affected ($p < 0.05$) seven out of the 22 traits measured at the community scale (Table 1). The same trend was also found for four other traits, but less significantly ($p < 0.10$).

A decline in herbage use reduced ($p < 0.10$) the LFM, the SLA_D and the DI. Both LDMC and leaf lamina C content of LDM increased ($p < 0.05$) from low to high herbage use. At reproductive stage, M and L treatment plant communities were taller ($p < 0.05$), produced larger seeds ($p < 0.05$) and flowered later ($p < 0.05$) than H communities. The duration of the flowering period was longer but this effect was not significant.

Identification of plant functional response types: the optimal solution

The method to identify PFTs, applied to the matrix of 22 traits measured on 86 plant populations, considering the level of herbage use as the environmental factor, resulted in the identification of nine plant functional response types (PRTs) based on four traits: mature plant height, individual leaf lamina area, leaf dry matter content and beginning of flowering period (Table 4). This optimal solution imparted a vegetation description with a very high (0.92) level of congruence with herbage use.

The number of plant populations in each of the nine PRTs varied from one to 29 (Table 4). Three major PRTs accounted for 73 populations. The classification in different plant types of plant populations belonging to the same species is related to within species trait variation (phenotypic or genotypic plasticity). *Holcus lanatus*, *Dactylis glomerata*, *Elytrigia repens* and *Trifolium repens* were plastic enough to be allocated to two different plant types in different treatments (Table 4).

PRT 1 contains the highest plant population number (29) and is characterized by short plants, early flowering, with very small laminae of medium LDMC. This abundant type (ca. 24% of the total species presence in May 2000) was still present in all subplots after 12 years of contrasted management (Table 4). PRT 3 (ca. 18% of

Table 3. Mean relative cover of plant species in May 2000 in the H-, M- and L-plots (%). For each species, ANOVA was performed on transformed data (by arcsine of square root) to test the effect of the level of herbage utilisation and the block. When there was a significant treatment effect ($p < 0.05$), the means were compared by the l.s.d. method and different letters within one row indicate significant herbage use effects. In bold type, the most abundant species, which accounted for at least 85 % of the % presence values and were selected for the plant trait measurements. Values are means of two replicate plots per treatment. The block effect is not significant.

Species	H	M	L
Monocotyledons			
Agrostis capillaris	**11.6**	**6.7**	**3.6**
Alopecurus pratensis	1.2	1.5	0
Anthoxanthum odoratum	0.2	0	0
Arrhenatherum elatius	0	**16.0**	**8.9**
Avena pubescens	0	0	0.6
Bromus hordeaceus	**1.9**	0	0
Dactylis glomerata	1.9	**9.7**	**4.9**
Elytrigia repens	**4.0**a	**18.9**b	**36.7**c
Festuca arundinacea	**3.0**	**4.3**	0.3
Festuca rubra	0	**1.8**	0.3
Holcus lanatus	**11.9**	**14.8**	**4.9**
Holcus mollis	0	0	0.4
Lolium perenne	**16.6**	0.4	0
Poa angustifolia	0	**1.3**	**5.4**
Poa pratensis	**5.8**	**6.2**	**7.8**
Poa trivialis	**14.9**	**13.2**	**3.6**
Trisetum flavescens	**2.7**	1.6	**2.6**
Dicotyledons			
Achillea millefolium	0	0.3	0
Artemisia vulgaris	0	0	**1.3**
Cerastium spec.	1.7	0	0
Chaerophyllum aureum	0a	0a	1.9b
Cirsium arvense	0	0.2	**3.1**
Cirsium vulgare	0.2	0	0
Cruciata laevipes	0	0.4	0.3
Fraxinus excelsior	0	0	0.6
Galium aparine	0	0.5	1.9
Galium mollugo	0	0	0.6
Lathyrus pratensis	0	0	0.6
Malva moschata	0	0	0.3
Potentilla reptans	0	0	0.3
Ranunculus repens	0.7a	0b	0b
Stellaria graminea	0	0	0.3
Taraxacum officinale	**13.9**a	0b	0.3b
Trifolium repens	**8.2**a	0b	0b
Urtica dioica	0	0.9	**8.4**
Veronica chamaedrys	0.4	0	0.3
Vicia sativa	0.2	0.3	0
Cryptogams	0	0.2	0

Table 4. PRTs corresponding to the optimal solution maximizing correlation with level of herbage use and ANOVA results for herbage use. The optimal solution defined PRTs by four traits including mature plant height (MH), individual leaf area (LA), leaf dry matter content (LDMC) and beginning of the flowering period (BE). PRTs are sorted by the number of plant populations involved. The plant species involved, the mean value in each trait and the mean % presence per treatment are given for each PRT. Plant populations are labelled for each trait according to percentile values (very low, low, medium, high, very high). Data are means of the two replicate plots per treatment. Different letters in the same row indicate significant differences among herbage use treatments (l.s.d. method, ANOVA, $n = 6$). Note that some plant species contribute to more than one PRT and that the sum of the relative cover of the nine groups is < 100. This is because of missing values for a small number of traits that led to PRT analysis on 86 plant populations (see Material and Methods).

PRT	Population nr	Traits				Description	Plant species involved	Mean %presence per treatment (%)			Effect
		MH (cm)	LA (cm^2)	LDMC ($mg.g^{-1}$)	BE (d)			H	M	L	
1	29	47	3	240	160	Short plants, with very small laminae, medium LDMC, early flowering	*Poa pratensis, P. trivialis, Agrostis capillaris, Trisetum flavescens*	33a	24b	15c	**
2	27	71	7	240	170	Tall plants, with medium size laminae, medium LDMC, mid-season flowering date	*Holcus lanatus, Arrhenatherum elatius, Elytrigia repens, Dactylis glomerata, Festuca arundinacea*	7a	37b	53b	*
3	16	50	7	200	160	Short plants, with medium size laminae, very low LDMC, early flowering	*H. lanatus, Trifolium repens, Lolium perenne*	35a	15ab	1b	*
4	4	20	28	120	110	Very short plants, with very large laminae, very low LDMC, very early flowering	*Taraxcum officinale*	14 a	0b	0b	***
5	3	96	13	230	160	Very tall plants, with large laminae, medium LDMC, early flowering	*Urtica dioica, D. glomerata*	0	6	8	NS
6	3	83	13	130	200	Tall plants, large size laminae, very low LDMC, very late flowering	*Cirsium arvense*	0a	0a	2b	***
7	2	146	25	200	220	Very tall plants, very large laminae, very low LDMC, very late flowering	*Artemisia vulgaris*	0	0	1	NS
8	1	78	15	270	180	Tall plants, very large size laminae, very high LDMC, late flowering	*Elytrigia repens*	0	5	0	NS
9	1	22	7	300	150	Very short plants, with medium size laminae, very high LDMC, early flowering	*T. repens*	2	0	0	NS

the % presence values) also consisted of short and early flowering plants but with medium size laminae of very low LDMC. A reduction of herbage use significantly reduced the contribution of PRTs 1 and 3 (Table 4). By contrast, a reduction in herbage use favoured tall plants, with mid-season beginning of flowering, medium size laminae and medium LDMC (PRT 2, ca. 32% of the % presence values).

The other PRTs generally accounted for less than 5% of the % presence. PRT 4 (*Taraxacum officinale*) was specific to the high herbage use treatment ($p < 0.001$), whereas PRT 6 (*Cirsium arvense*) was specific to the low herbage use treatment ($p < 0.05$). These PRTs also displayed contrasting trait values, with very short plants, very early flowering, with very large laminae and very low LDMC (PRT 4), contrasting with tall to very tall plants, very late flowering, with large to very large laminae and very low LDMC (PRT 6). PRT 5 was present only in M- and L-plots, but because of a high variability in its % presence values, the treatment effect was not significant. The three remaining PRTs (7 to 9) were not significantly affected by the herbage use treatments and consisted of one or two plant populations.

Correlation structure in the populations × traits matrix

The PCA carried out on the four optimal traits by 86 populations matrix revealed two principal components accounting for 47 and 36% of the variance (Fig. 1). BE and LDMC, which were optimal traits for the PRT definition, have on the first axis opposed trends to DI, RA, LA (also an optimal trait) and LFM. Variation on LDM, NG and plant height traits at the reproductive stage (MH, ME, IT) were mostly reflected on the second axis. The remaining traits were poorly correlated with the first two components of the PCA.

Some regressions between traits are significant:

- between shoot digestibility and leaf dry matter content: $DI = 499\ LDMC^{-0.36}$; $r^2 = 0.58$; $p < 0.001$;
- between beginning of flowering period and mature plant height: $BE = 0.052\ MH + 134.7$; $r^2 = 0.40$; $p < 0.001$;
- between specific leaf area and leaf:shoot ratio: $SLA_F = -0.83\ RA + 109$; $r^2 = 0.50$; $p < 0.001$;
- between specific leaf area and individual leaf dry mass: $SLA_F = 26\ LDM^{-0.22}$; $r^2 = 0.60$: $p < 0.001$.

These statistical models were selected to maximize r^2.

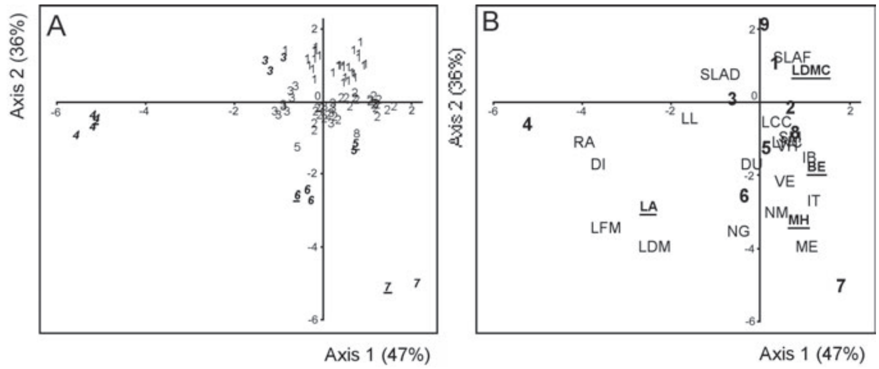

Fig. 1. Ordination plots of the first two principal components of a PCA carried out on the 86 populations × 4 optimal traits matrix. **A.** Populations identified by the corresponding PRT (1-9; Table 4 and the main text); dicots are in italics and underlined. **B.** PRTs plotted by their mean. Optimal traits (underlined) and non-optimal traits were plotted based on their correlations with the ordination axes after rescaling.

Discussion

Extensification impacts on grassland dynamics

The extensification experiment reduced herbage use by altering the disturbance pattern of the grassland plots. During most of the growing season, the under-used plots had taller canopies, increased herbage mass and increased accumulation of dead material and shoot litter (Louault 1999). Hence, the light microclimate was altered and relatively less light was presumably transmitted to the ground level in response to the reduction in disturbance (Aerts 1999). New species of forbs and grasses appeared in the single grazing treatment (L), despite a high level of competition for light that is generally assumed to prevent the recruitment and germination of new plants (Pärtel et al. 2000). The large amount of dead shoot material remaining above the soil (Louault 1999) contributed to create bare soil patches that may have favoured plant recruitment (Lavorel et al. 1998).

The experimental treatments also affected the nutrient balance. Nutrients were removed from the mown plots (H) by harvesting. From Table 2, it can be calculated that ca. 80 - 90 kg-N.ha^{-1} were removed from the H-plots by the spring cut for haymaking. Nevertheless, the spring cut did not significantly affect the N-nutrition index (comparison of M and H), possibly because of the presence of N_2-fixing legumes (mainly *Trifolium repens*, Table 3) in the high use plots. At the same time, a significantly higher level of N-nutrition was measured in the L- compared to the M-treatment (Table 2). Vegetation responses in this extensification experiment were therefore directly affected by the disturbance regime without a major change in N-availability to the plants.

Plant response traits aggregated at the community scale.

Which traits filter the admission of a given set of species to a given community? This question has been addressed by measuring plant traits for the dominant species after 12 years of contrasted management. As leaf and vegetative height traits were measured at the same regrowth stage, i.e. three weeks after the early spring grazing, the trait values were not affected by differences in terms of tissue or shoot ages (Sultan 1995). However, reproductive traits and the height at maturity were measured at dates that were dependent on the phenology of each species.

Leaf traits. Most of the leaf traits aggregated at community scale were significantly affected by the treatments. In response to a decline in the disturbance regime, the vegetation displayed leaves with a higher LDMC and smaller SLA_D. However, the shoot architecture was not significantly modified, since neither the number of leaves per shoot axis, nor the leaf:shoot ratio were affected. These results suggest that significant changes occurred, possibly leading to a reduction in the potential relative growth rate. Indeed, with three ecologically contrasting grass species, high relative growth rates were correlated with short life spans of the roots and shoots and with low dry matter content (Schläpfer & Ryser 1996). Low tissue density has also been found to be an important component of the high specific leaf area and high root length ratio of grass species (Garnier & Laurent 1994; Ryser & Lambers 1995). The relationship between specific leaf area and relative growth rate appears a robust one for grasses, but not for dicot species (van der Werf et al. 1998). Since dicots accounted for a minor part of the total % species presence in the community, differences in specific leaf area values at community scale may reveal differences in potential growth rate.

Under our experimental conditions, the individual size of leaves, in terms of fresh mass was reduced under low herbage use regime. This finding contrasts with the report by Díaz et al. (2001) who observed a decline in the leaf size of plants responding positively to grazing pressure, although the costs and benefits of large vs small leaf size are poorly understood (Westoby et al. 2002).

Height. Plant height has been shown to be a good predictor of competitive ability when competition is primarily for light (see Bullock et al. 2001). In our results, there was no significant difference in plant height among treatments when measured in spring on vegetative plants at the same regrowth stage, but mature plant height (measured on undefoliated plants) increased in low use plots compared to the H-treatment. Thus, plant height differences developed gradually over the growing season to become significant towards maturity, at a time period when competition for light became greater in the under-used compared to the well-used plots. Since there was a positive correlation between the mature plant height (ME, MH) and BE (Fig. 1), generally, tall plants matured later than short plants.

Regeneration traits. Seedlings from large seed species tend to survive better in a closed canopy (Grime et al. 1997) and an increase in the mean individual seed mass has been reported (reviewed by Westoby et al. 2002) to be a response trait associated with an increase in canopy height. A decline in the mean seed size in response to increase in grazing intensity has also been reported by McIntyre & Lavorel (2001), for both perennial grasses and forbs. After 25 years, a grazed community also produced fewer seeds of large mass than the fallow (Kahmen et al. 2002). The increased delay in flowering in the low use plots is consistent with the higher contribution of late flowering herbs at a low compared to a high grazing pressure (Hellström et al. 2003).

Functional linkages and trade-offs among traits

The negative relationship between specific leaf area (SLA_F and SLA_D) and LDMC reported by Garnier et al. (2001) for a large range of species and of habitats was not observed here. This discrepancy may be due to a relatively small range in the values of these leaf traits under our experimental conditions and/or to the co-occurrence of contrasted plant types in the same community. The individual LDM and the RA ratio were negatively correlated with the SLA_F (Fig. 1). Therefore, the size of leaf laminae, their thickness (inversely related to the SLA_F) and their contribution to the total shoot were all positively correlated.

A strong negative relationship was observed between pepsin cellulase digestibility (DI) and LDMC (Fig. 1). Therefore, in agreement with previous observations on the chemical composition of grass leaves (van Arendonk & Poorter 1994), digestible energy content of the herbage was reduced when the LDMC of leaves increased. Since, under grazing conditions, domestic herbivores tend to maximize the digestibility of their diet (Garcia et al. 2003), the decline in the digestibility and quality of the plant tissues under a low disturbance regime, tended to further reduce the probability of defoliation by herbivores. This effect may, however, be partly compensated by an increased life span of leaves, as a result of an increased tissue density (Ryser & Urbas 2000), which would increase the probability for defoliation to occur before the start of leaf senescence.

Plant functional types

Individual plant species occurring at a particular location along an environmental gradient do not necessarily display similar trait values (see Westoby et al. 2002), i.e. different plant types may coexist. Thus, the weighted mean of a trait may fail to reveal differences between communities (van der Werf et al. 1998).

In our experiment, the multivariate optimization algorithm identified nine PRTs, based on four optimal traits. The vegetation appears as a mixture of three major types, which together account for 74% of the % presence (Table 4): a common type (PRT 1), is associated with either PRT 3 within the high use plots or PRT 2 in the low use plots.

PRT 3 appears to correspond to a 'pasture' type, since its % presence declines strongly when the herbage production is under-utilized (Fig. 2). This type displays some grazing avoidance characteristics (i.e. a short plant height), but is nevertheless likely to be preferentially selected by grazing herbivores because of a high prehensibility (i.e. large leaf size) and tissue quality (i.e. high digestibility and N content). It has been suggested by Díaz et al. (2001) that leaf traits which favour a high growth rate may compensate for the negative effect of animal defoliation on plant growth. PRT 3 would show some grazing tolerance, because of leaf trait values (high SLA, high leaf N and low LDMC) favouring a fast regrowth.

PRT 1 is an intermediate type, which is less sensitive than PRT 3 to a decline in herbage utilization (Fig. 2). It displays grazing-avoidance characteristics, both in terms of shoot morphology (short plants with small leaves) and of tissue composition (low digestibility and N-content of leaves). In contrast to PRT 3 it does not display leaf traits values which would favour a fast regrowth and hence grazing tolerance.

The relative abundance of PRT 2 increases markedly when the disturbance by grazing and mowing is reduced. This 'low disturbance' type shares some common leaf characteristics with the PRT 1 (high leaf tissue density, low specific leaf area, leaf N-content and digestibility), but is taller, with larger leaves and also flowers later (Fig. 2). PRT 2 has morphological characteristics that neither favour grazing avoidance (tall plants with large leaves) nor grazing tolerance (low specific leaf area, low leaf N content, high tissue density). The low quality of the leaf tissue (low digestibility, low N content) of this type is likely to reduce its selection by grazing herbivores. However, as mentioned by Díaz et al. (2001), rather than serving to deter grazers, these tissue traits may be related to the advantage of having stiff and tall leaves to capture light in the closed canopy

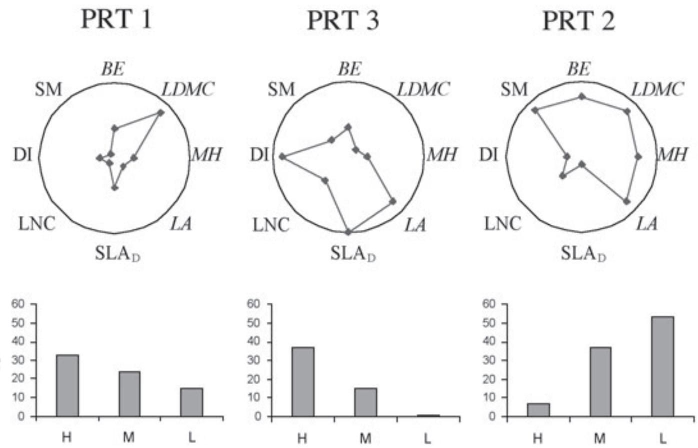

Fig. 2. Selected plant trait values of the three most abundant plant functional response types (1, 2 and 3) which account for 77, 76 and 69% of presences in the high (H), medium (M) and low (L) herbage use treatments. Scales on the radar plot are adapted for each trait : BE: 150-175 ; LDMC: 180-250 ; MH: 400-800 ; LA: 2-8 ; SLA_D: 250-350 ; LNC: 3.0-3.5 ; DI: 65-80; SM: 0.0-0.250, see Table 1 for units and abbreviations). Traits in italics were used by the multivariate optimization analysis for the identification of the plant types. Contribution of plant types to the % presence at the H-, M- and L- levels of herbage use is plotted as a bargraph.

that develops in the absence of grazing. These results suggest a trade-off between tissue composition traits that favour grazing avoidance (i.e. low digestibility and hence high fibre content and high LDMC, low leaf N content) and traits that favour grazing tolerance (i.e. low LDMC and high LNC). The co-dominance in the grazed plots of PRTs 3 and 1 may result from such a trade-off. In contrast, a single 'low disturbance' plant type was able to dominate the L-plots by displaying traits that favour a high ability to compete for light. PRT 2 was apparently able to exclude competitively PRT 3 from the low use plots, but not fully the 'intermediate' type 1. It may be hypothesized that the fast-growing pasture type PRT 3 is more competitive, whereas the 'intermediate' slow-growing type PRT 1 would display a conservative strategy that would favour an efficient nutrient recycling in the under-used plots.

Conclusion

Management of permanent pastures promotes plant types with some common attributes. The traits involved in the response to the level of herbage use relate to different strategies. Three major plant functional types have been identified in response to the degree of disturbance by grazing and mowing of a fertile temperate grassland. The trade-off between grazing avoidance and grazing tolerance may account for the co-dominance in high-use plots of a 'pasture' plant type, which is competitive, grazing-tolerant and moderately grazing-avoiding, and of an 'intermediate' type, which is grazing-avoiding but not grazing-tolerant. The low use plots are dominated by a single plant type with high ability to compete for light but little grazing avoidance and no grazing tolerance. The possible effects of these plant functional response types to disturbance by grazing and mowing on ecosystem processes need to be further investigated.

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References

Aerts, R. 1999. Interspecific competition in natural plant communities: mechanisms, trade-offs and plant-soil feedbacks. *J. Exp. Bot.* 50: 29-37.

Aufrère, J. & Michalet-Doreau, B. 1988. Comparison of methods for predicting digestibility of feeds. *Anim. Feed. Sci. Technol.* 20: 203-218.

Barthram, G.T. 1986. *Experimental techniques: the HFRO sward stick.* Biennial Report 1984-1985, 29-30. HFRO, Edinburgh, UK.

Bullock, J., Franklin, J., Stevenson, M.J., Silvertown, J., Coulson, S., Gregory, S.J. & Tofts, R. 2001. A plant trait analysis of responses to grazing in a long term experiment. *J. Appl. Ecol.* 38: 253-267.

Cruz, P., Duru, M., Therond, O., Theau, J.P., Ducourtieux, C., Jouany, C., Al Haj Khaled, R. & Ansquer, P. 2002. Une nouvelle approche pour caractériser les prairies naturelles et leur valeur d'usage. *Fourrages* 172: 335-354.

Díaz, S. & Cabido, M. 2001. Vive la différence: plant functional diversity matters to ecosystem processes. *Trends Ecol. Evol.* 16: 646-655.

Díaz, S., Noy-Meir, I. & Cabido, M. 2001. Can grazing response of herbaceous plants be predicted from simple vegetative traits? *J. Appl. Ecol.* 38: 497-508.

Duru, M. 1992. Diagnostic de la nutrition minérale de prairies permanentes de printemps. Etablissement de références. *Agronomie* 12: 219-233.

Duru, M. & Calvière, I. 1996. Effects of phosphorus and nitrogen nutrition status and of botanical composition of permanent pastures on their growth in spring. *Agronomie* 16: 201-268.

Elberse, W.T. & Berendse, F. 1993. A comparative study of the growth and morphology of 8 grass species from habitats with different nutrient availabilities. *Funct. Ecol.* 7: 223-229.

Garcia, F., Carrère, P., Soussana, J.-F. & Baumont, R. 2003. The ability of sheep at different stocking rates to maintain the quality and quantity of their diet during the grazing season. *J. Agric. Sci.* 140: 113-124.

Garnier, E. & Laurent, G. 1994. Leaf anatomy, specific mass and water content in congeneric annual and perennial grass species. *New Phytol.* 128: 725-736.

Garnier, E., Shipley, B., Roumet, C. & Laurent, G. 2001. A standardized protocol for the determination of specific leaf area and leaf dry matter content. *Funct. Ecol.* 15: 688-695.

Grant, S.A. 1993. Resource description: vegetation and sward components. In: Davies, A., Baker, R.D., Grant, S.A. & Laidlaw, A.S. (eds.) *Sward measurement handbook*. 2nd ed., pp. 69-97. British Grassland Society, University of Reading, UK.

Grime, J.P., Hodgson, J.G., Hunt, R., Thompson, K., Hendry, J.A.F., Campbell, B.D., Jalili, A., Hillier, S.H., Díaz, S. Burke, M.J.W. 1997. Functional types: testing the concept in Northern England. In: Smith, T.M., Shugart, H.H. & Woodward, F.I. (eds.) *Plant Functional Types, their relevance to ecosystem properties and global change,* pp. 122-150. Cambridge University Press, Cambridge, UK.

Guinochet, M. 1970. Clé des classes, ordres et alliances phytosociologiques de la France. *Nat. Monspel. Sér. Bot.* 21: 79-119.

Hellström, K., Huhta, A.P., Rautio, P., Tuomi, J., Oksanen, J. & Laine, K. 2003. Use of sheep grazing in the restoration of semi-natural meadows in northern Finland. *Appl. Veg. Sci.* 6: 45-52.

Kahmen, S., Poschlod, P. & Schreiber, K.-F. 2002. Conservation management of calcareous grasslands. Changes in plant species composition and response of functional traits during 25 years. *Biol. Conserv.* 104: 319-328.

Laser, H. 2002. Long-term and short term effects of undisturbed plant succession, mulching and meadow utilisation on the botanical diversity in a moist *Arrhenatherion elatioris*. Proc. 19th EGF Meeting, la Rochelle, 27-30 May 2002, *Grassl. Sci. Eur.* 7: 806-807.

Lavorel, S. & Garnier, E. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Funct. Ecol.* 16: 545-556.

Lavorel, S., Touzard, B., Lebreton, J.D. & Clément, B. 1998. Identifying functional groups for response to disturbance in an abandoned pasture. *Acta Oecol.* 19: 227-240.

Lemaire, G & Gastal, F. 1997. N Uptake and distribution in plant canopies. In: Lemaire, G. (ed.) *Diagnosis of the nitrogen status in crops*, pp. 3-43. Springer-Verlag, Berlin, DE.

Louault, F. 1999. *Dynamics of dead and living plant material in grasslands under non-intensive use*. Proc. 6th Int. Rangeland Congress, Townville, 19-23 july 1999, pp. 274-275.

Marriott, C.A., Bolton, G.R., Common, T.G., Small, J.L. & Barthram, G.T. 1996. Effects of extensification of sheep grazing systems on animal production and species composition of the sward. Proc. 17th EGF meeting, *Grassl. Sci. Eur.* 4: 465-468.

McIntyre, S. & Lavorel, S. 2001. Livestock grazing in subtropical pastures: steps in the analysis of attribute response and plant functional types. *J. Ecol.* 89: 209-226.

Pärtel, M., Zobel, M., Lura, J. & Zobel, K. 2000. Species richness limitations in productive and oligotrophic plant communities. *Oikos* 90: 191-193.

Pillar, V.D. 1999. On the identification of optimal plant functional types. *J. Veg. Sci.* 10: 631-640.

Pillar, V.D. & Sosinski, E. 2003. An improved method for searching plant functional types by numerical analysis. *J. Veg. Sci.* 14: 323-332.

Poorter, H. & Garnier, E. 1999. Ecological significance of inherent variation in relative growth rate and its components. In: Pugnaire, F.I. & Valladares, F. (eds.) *Handbook of functional plant ecology*, pp. 81-122. Marcel Dekker, New York, NY, US.

Reich, P.B. 1993. Reconciling apparent discrepancies among studies relating life span, structure and function of leaves in contrasting plant life forms and climates: 'the blind men and the elephant retold'. *Funct. Ecol.* 7: 721-725.

Ryser, P. & Lambers, H. 1995. Root and leaf attributes accounting for the performance of fast and slow-growing grasses at different nutrient supply. *Plant Soil* 170: 251-265.

Ryser, P. & Urbas, P. 2000. Ecological significance of leaf life span among Central European grass species. *Oikos* 91: 41-50.

Schläpfer, B. & Ryser, P. 1996. Leaf and root turnover of three ecologically contrasting grass species in relation to their performance along a productivity gradient. *Oikos* 75: 398-406.

Sultan, S.E. 1995. Phenotypic plasticity and plant adaptation. *Acta Bot. Neerl.* 44: 363-383.

Tutin, T.G., Burges, N.A., Chater, A.O., Edmondson, J.R., Heywood, V.H., Valentine, D.H., Walters, S.M. & Webb, D.A. (eds.) 1993. *Flora Europaea*. Vols. 1-5. Cambridge University Press, Cambridge, UK.

van Arendonk, J.J.C.M. & Poorter, H. 1994. The chemical composition and anatomical structure of leaves of grass species differing in relative growth rate. *Plant Cell Environ.* 17: 963-970.

van der Werf, A., Geerts, R.H.E.M., Jacobs, F.H.H., Korevaar, H., Oomes, M.J.M. & de Visser, W. 1998. The importance of relative growth rate and associated traits for competition between species during vegetation succession. In: Lambers, H., Poorter, H. & van Vuuren, M.M.I. (eds.) *Inherent variation in plant growth. Physiological mechanism and ecological consequences*, pp. 489-502. Backhuys, Leiden, NL.

Watt, T.A., Treweek, J.R. & Woolmer, F.S. 1996. An experimental study of the impact of seasonal sheep grazing on formerly fertilized grassland. *J. Veg. Sci.* 7: 535-542.

Weiher, E., Van der Werf, A., Thompson, K., Roderick, M., Garnier, E. & Eriksson, O. 1999. Challenging Theophrastus: a common core list of plant traits for functional ecology. *J. Veg. Sci.* 10: 609-620.

Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A. & Wright, I.G. 2002. Plant ecological strategies: some leading dimensions of variation between species. *Annu. Rev. Ecol. Syst.* 33: 125-159.

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