Predictive value of plant traits to grazing along a climatic gradient in the Mediterranean

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Summary

1. In the face of large-scale environmental changes, predicting the consequences for species composition from species' traits can be a major step towards generalizing ecological patterns and management. Few studies, however, have explored the applicability of this tool in relation to different climatic conditions. Here, the changes in species composition along a gradient of sheep-grazing pressure (high, low, abandonment) were used to test whether a common set of plant functional traits (PFT) would provide consistent predictions of species' responses to grazing in different biogeographical regions.

2. Data were collected across an altitudinal and climatic gradient from Mediterranean rangelands to subalpine grasslands in north-eastern Spain. Species' responses were calculated using partial constrained ordination to account only for the effect of grazing intensity. Regression trees and general linear models were applied to identify traits that could predict species' responses.

3. Results were mostly consistent with the ruderal vs. competitive strategy (*sensu* Grime), in terms of life cycle, life form and plant height, and their expected responses to repeated disturbance.

4. However, the predictive capacity of the investigated traits changed with climatic conditions. Traits generally related to grazing did not show a strong repeatability across the climatic gradient. Convergent selection of climatic conditions and grazing indicated that plant features might reflect an adaptation to multiple selective forces. The climatic conditions acted as filters on the pool of PFT available and shifted the relevance of plant traits as potential predictors. Results were not substantially different after applying phylogenetically independent contrasts (PIC).

5. *Synthesis and applications*. At a local scale, plant functional traits are useful tools in predicting species' responses to grazing and, for conservation purposes, identifying species vulnerable to land-use changes. However, predictions cannot be extrapolated from one climatic region to another. The methodology proposed in this study to detect predicting traits can be applied more generally. Regression trees, in particular, appear to be a useful tool because they account for non-additive effects and allow visualizations of trait combinations.

Key-words: CCA, disturbance, land-use change, plant functional group, phylogeny, regression tree, sheep

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Introduction

Predicting the response of vegetation to environmental and land-use change has become a major concern in recent theoretical and applied ecological research (Smith, Shugart & Woodward 1997; McIntyre *et al*. 1999). Determining

individual species' responses at a given location is an essential step (Noy-Meir, Gutman & Kaplan 1989; Fensham, Holman & Cox 1999; Ackerly *et al.* 2002). However, in order to generalize the results beyond specific localities it is necessary to understand the adaptive significance of simple plant functional traits (PFT) and their predictive capacity of species' responses (McIntyre *et al.* 1999; Díaz, Noy-Meir & Cabido 2001; Lavorel & Garnier 2002; Vesk, Leishman & Westoby 2004). In this application of PFT, the behaviour of individual species is considered as a dependent variable and the traits, or combination of traits, as their potential predictors (as in Bullock *et al.* 2001 for grazing studies).

The main objective of this study was to test whether a common set of traits would provide robust predictions of species' responses to grazing across different climatic conditions and vegetation types. Changes in species' frequencies in areas under grazed (high and low pressure) and ungrazed conditions were related to plant traits. A representative range of vegetation types along a climatic and altitudinal gradient in north-eastern Spain, from lowland arid conditions to upland humid areas, was surveyed for this purpose. A large number of species (404) was considered, which included annuals, grasses, legumes, forbs, shrubs and trees species. The possible relevance of species' phylogeny on PFT predictive values was also evaluated.

However, there are several limitations to this use of PFT. The predictive capacity of a common set of traits must exhibit repeatability over a wide range of conditions (Smith, Shugart & Woodward 1997; McIntyre *et al.* 1999; Pausas, Rusch & Lepš 2003). Recent studies on grazing systems have attempted to overcome the limits of local studies. Díaz, Noy-Meir & Cabido (2001) compared responses to grazing of similar herbaceous communities from subhumid grasslands in two continents, showing how plant height could be the best single predictor, followed by life span, leaf dimension and leaf specific area. However, Vesk, Leishman & Westoby (2004) could not find robust proof that the same traits predicted species' responses in more arid conditions, giving the difference in the vertical structure of vegetation as a hypothetical explanation. Pakeman (2004), in a multi-site data analysis of British grasslands, found that the relationship between many traits and grazing was mediated by site productivity. A similar conclusion was reached by Osem, Perevolotsky & Kigel (2004) in Mediterranean annual plant communities, where the relevance of plant dimensions shifted across a small-scale productivity gradient.

Based on their response to grazing, species are traditionally categorized into 'increasers', 'decreasers' or 'neutrals' (Noy-Meir, Gutman & Kaplan 1989; Díaz, Noy-Meir & Cabido 2001; McIntyre & Lavorel 2001). Multivariate unconstrained ordination procedures have had a certain degree of success in calculating the optimal species distribution along putative gradients of grazing intensities, but may be distorted by other environmental variables interacting with grazing regimes (Fensham, Holman & Cox 1999). For this reason we proposed to constrain species' responses to grazing only by removing the effects of other variables (McIntyre & Lavorel 2001; Pakeman 2004) and using the species' scores obtained by the constrained axis as quantitative response variables.

Certain traits may also be ecologically relevant only within some growth forms (annuals, grasses, forbs, shrubs, etc.) or could affect species' responses differently in different groups. To avoid this problem, a hierarchical approach has been suggested, with the analysis traditionally carried out within separate groups (e.g. grasses and forbs; McIntyre & Lavorel 2001). An alternative is to use regression trees and to perform the analysis without any a priori division into groups. The regression tree approach, i.e. successive hierarchical splitting of the data set, allows for traits having a positive effect in one group but possibly no effect in other groups.

Methods

STUDY AREA

The study was conducted in five locations in north-east Spain, along an altitudinal and climatic gradient. The study locations encompassed the transition between the Mediterranean and Boreo-alpine biogeographical regions (de Bolòs 1987). Locations were selected according to the regional vegetation belts (Table 1). The driest and warmest location was in the semi-desert area of Monegros

Table 1. Climate and vegetation of the study locations. Mean annual values for climate parameters. PET, potential evapotranspiration

Location	Altitude (m a.s.l.)	Temperature (°C)			Rainfall (mm)	PET (mm)	Vegetation type	Biome
		Mean	Minimum	Maximum				
1. Monegros 0°3′W, 41°21′N	256	15·2	5·4	25·6	325·2	844·6	Mediterranean	Garrigue with *Pinus halepensis*
2. Castelldans 0°45′E, 41°29′N	312	14·7	4·8	24·5	392·3	777·2	Mediterranean	Transition from garrigue to evergreen *Quercus ilex* forest
3. Vilamajor 0°45′E, 41°53′N	709	12·8	3·6	22·3	461·1	642·6	Submediterranean	Transition from evergreen *Q. ilex* to deciduous *Quercus faginea* forest
4. Boixols 1°9′E, 42°10′N	1354	9·2	1·4	17·3	783·2	586·2	Montane	*Quercus humilis* and *Pinus sylvestris* forest
5. Alinyà 1°27′E, 42°12′N	2051	7·1	−1·1	14·6	952·8	463·8	Subalpine	*Pinus mugo* ssp. *uncinata* forest

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in the Ebro river basin, while at the opposite climatic extreme the most mesic location was in a subalpine area in the Eastern Pyrenees, approximately 390 km from the first location. Loamy soils had evolved from marl in the two driest locations and from limestone in the other three locations. Soils were generally carbonate-rich, with the exception of the subalpine site, where decarbonation processes are common (Sebastià 2004). Climatic parameters were obtained from the Atles Climàtic Digital de Catalunya (http://magno.uab.es/atles-climatic) and from the Confederación hidrográfica del Ebro database (http://www.chebro.es/).

There is a centuries-long history of livestock (mainly sheep and goat) grazing in the study area. In some cases transhumance is still a traditional practice: shepherds may behave as semi-nomads and flocks of animals are moved up or down the altitude gradient according to the state of the vegetation. As in most areas of the northern rim of the Mediterranean Basin, rural abandonment is causing successional changes (Lavorel, Rochete & Lebreton 1999). Modernization of livestock production has resulted in a decline of the use of extensive rangelands and semi-natural grasslands in the last few decades (Perevolotsky & Seligman 1998).

EXPERIMENTAL DESIGN

Local shepherds, farmers and technicians were interviewed to identify a gradient of grazing intensity: areas were (i) abandoned for more than 10 years; (ii) visited a few times a year; or (iii) frequently visited per year. It was not possible to quantify grazing pressure more precisely and thus broad categories were used (Jauffret & Lavorel 2003). Recently burnt land and areas with rock outcrops were avoided. Distances of areas from water points and corrals were also taken into account in determining grazing pressure. Further evidence of grazing intensity came from signs of animal tracks in the soil and fresh excrement (Landsberg *et al*. 2002). We excluded areas abandoned as a result of their low productivity and selected only those abandoned because of their long distance from local corrals. Only sloping lands were considered as, usually, flat areas are used for arable production.

In each location, four independent plots were established per grazing intensity (two replicates on south- and two on north-facing slopes). The experiment was established using a factorial design: 5 localities × 3 sheep grazing intensities × 2 aspects × 2 replicates = 60 plots. Slope inclination was fixed between 19° and 35°. The field study was performed during 2 years (in spring and summer 2001 for the first three locations; in summer 2002 for locations four and five). Plots were surveyed at the time of the expected peak of vegetation development in the corresponding location. Species' composition and frequency were sampled in each of the 60 plots in a 10×10-m plot, divided into 100 1-m^2 subplots. We recorded all vascular species whose vertical projection was included in each subplot. Each species was then

characterized by its frequency. Nomenclature follows de Bolòs *et al*. (1993).

QUANTIFICATION OF SPECIES' RESPONSES

Species' responses to grazing intensity were evaluated by canonical correspondence analysis (CCA) using CANOCO (ter Braak & Šmilauer 1998). Selection of significant environmental variables (grazing intensity, climatic conditions, slope aspect and inclination) was performed by forward selection. CCA analyses were executed for each location separately and also for the whole climatic gradient (combined data set). Species' frequencies in each plot were log(x + 1) transformed prior to analysis. The significance of the environmental variables was evaluated by the Monte Carlo permutation test (499 permutations). The 'downweighting of rare species' option was used (for details see Lepš & Šmilauer 2003).

In the second step, a partial constrained analysis (partial CCA) was carried out in which grazing was used as the only explanatory variable. Other variables that were significant in the forward selection (see the Results) were used as covariates. The score of a species on the ordination axis constrained by grazing was used as the species' response to grazing. We changed the polarity of all the scores obtained from CCA so that positive values indicated species whose frequency increased with grazing intensity.

PLANT TRAITS

We selected 23 basic plant life-history traits (see Table S1 in Supplementary material). Traits were chosen from information available for our species and according to existing trait databases (Kleyer 1995; Kuhn, Durka & Klotz 2004) and leaf manuals (Leaf Architecture Working Group 1999). Selected traits were compiled from existing local floras (de Bolòs *et al*. 1993) and field observations and included plant architecture, life form, life cycle, leaf form and dimensions, among others.

Traits, including quantitative traits, were all categorized by means of fuzzy coding, to account for species' variability for a given trait and to handle similar variables (Kleyer 1995; Pakeman 2004). This is the case when species show multiple membership to the different categories in which a trait is divided (e.g. height or life-form). The percentage membership of a species (0–100%) for each category of the trait was estimated. For example, species that can behave as annuals and biennials would have a score of 0·5 as therophytes and 0·5 as hemicryptophytes with a total of 1 for the life-form trait.

PREDICTION OF SPECIES' RESPONSES FROM PLANT TRAITS

A data-driven approach was implemented to investigate whether some of the traits (independent variables) could predict the response of species to grazing (quantitative dependent variable). Predictions were calculated for

each location separately and for the whole climatic gradient (combined data set). The responses of 404 species, from a total of 467 found in the field, were used. Infrequent species with no data for more than 25% of the traits were excluded. For each category in which the trait was divided (see Table S1 in Supplementary material) missing data were replaced with the mean of the category (for eight out of the 404 species).

Two different techniques were applied, a novel approach based on a pruned regression tree (R software; R Development Core Team 2004) and a stepwise linear regression in which the trait was included if $P < 0.05$. The regression tree is a non-parametric regression that displays a binary tree built through binary recursive partitioning. In our case, the trait that best distinguishes species' responses splits the species into two groups; then, within each subset, another trait splits the species further, and so on. The pruned regression tree is based on reducing a fully grown regression tree, with the extent of the reduction based on the minimal cost–complexity criterion. The cross-validation procedure provides an estimate of the pruning level needed to achieve the best tree size (Venables & Ripley 2002).

PFT were selected as having good predictive power if this was shown by both regression techniques and in different locations. Each of these PFT was tested as a single predictor for species' responses in all locations and for the combined data set. Separate univariate general linear model analyses were performed for each selected trait. Note that with the fuzzy coding each trait was coded as a series of indicator variables, each denoting a single category. Unlike ANOVA, the use of the general linear model allows for fuzzy coding. Also, in the stepwise procedure a single category can be selected, which is more informative in our case (i.e. therophytes can be selected as a functional trait and not the whole variable describing life form). SPSS for Mac 10·0 (SPSS Inc., Chicago, IL) was used for general linear models and stepwise linear regressions.

The capacity of most relevant PFT to predict the species' optima along the climatic gradient was also analysed. Species' optima along the climatic gradient (species' responses to climate) were calculated with partial CCA, with the potential evapotranspiration (Table 1) as the only explanatory variable (grazing and aspect were covariables). To test the possible relevance of species' phylogeny in the applicability of PFT, we carried out a phylogenetic analysis of the data with the Phylocom software (C. Webb, D. Ackerly & S. Kembel, unpublished data; www.phylodiversity.net/phylocom/), applying the general procedures of the analysis of traits (AOT) module developed by D. Ackerly (unpublished data). In our case, because of the lack of a resolved tree for Mediterranean species, the phylogeny represented genera as a polytomy within each family, and species as a polytomy within each genus. Family names were checked with the APweb list (P. Stevens, unpublished data; www.mobot.org/MOBOT/research/APweb/). The AOT was based on two types of calculations: (i) the phylogenetic conservatism of PFT and species' responses, conservatism (the tendency for related species to resemble each other; Blomberg & Garland 2002) being estimated by the mean value of trait and species' response divergences across the tree (relative to a null model with values randomly assigned to species); and (ii) the phylogenetically independent contrasts (PIC) calculated between the species' responses (as independent variables) and the traits (as dependent). Independent contrasts were calculated from internal node averages of the daughter nodes. For each polytomy a single degree of freedom was obtained.

Results

Grazing accounted for a similar amount of variance in floristic composition across the different locations (i.e. the cumulative percentage of variance explained by the constrained axis of grazing regime was within 21–27%). The effect of grazing regime and aspect was significant in all locations (see Table S2 in Supplementary material). Slope inclination had a secondary effect. Around 50% of the species present in at least three locations (29/62) showed a consistent response to grazing, a quarter of the species responded inconsistently to grazing and the response of the remaining species shifted along the climatic gradient.

The most predictive traits were similarly selected by the stepwise linear regression and regression tree analyses (Table 2 and Fig. 1). In each location, at least two traits were selected by both methods and showed similar relative importance. The first selected (i.e. the most important) traits were usually similar for both regressions; the methods differed in the results of less important traits (i.e. the last selected traits in linear regression when compared with those in the last branches of the tree). The non-additivity of regression trees was especially evident in the results for the Monegros locality: flowering period discriminated the response of annuals but was indifferent for perennials (i.e. late flowering was a disadvantage for growing in grazed habitats only for annuals; Fig. 1a).

Among all selected PFT, life cycle, plant height and life form were the traits showing the greatest effects on species' responses to grazing in most locations and in the combined data set (Tables 2 and 3 and Fig. 1). Small stature and short life span were confirmed as mechanisms for coping with disturbance, while the phanaerophyte life form was related to the increase in shrubs and trees after abandonment. However, their relevance as adaptations to grazing was different in the different locations (Table 3), with no effect in the subalpine location (Alinyà). The relevance of a short life span decreased from the most arid to the montane areas (Tables 2 and 3 and Fig. 1). In the subalpine area, most of the species possessed a small stature independent of grazing (90% of the species had heights between 10 and 30 cm; Table 3). Consequently, stature was relevant in this location only when the effect of other traits was taken into account (Table 3 and Fig. 1e).

Table 2. Stepwise linear regression results. Traits that predicted species' responses to grazing intensity for each location across the climatic gradient and for the combined data set are shown. Water stress decreases from left to right locations while altitude increases (see also Table 1). For each trait, standardized *B*-values indicate the sign and the magnitude of the predicted response to grazing. In each location the order in which stepwise regression included the traits into the model is respected. Th, Therophytes; H, Hemicryptophytes; G, Geophytes; WoodyCh, woody Chamaephytes; P, Phanaerophytes; > 3 M or < 3 M, flowering period longer or shorter than 3 months. See Table S1 in Supplementary material for detailed traits' definitions.

Monegros		Castelldans		Vilamajor		Boixols		Alinyà		Combined	
Traits	*B*	Traits	*B*	Traits	*B*	Traits	*B*	Traits	*B*	Traits	*B*
Th	0·41	Th	0·65	Th	0·29	P	−0·21	Rosette	0·24	Th	0·54
G	−0·25	P	−0·32	Stem erect	−0·19	G	−0·19	Pinnate leaf form	0·21	Deciduous	−0·11
FlowerSpring > 3M	0·23	Succulent	0·18	Semirosette	0·24	Strictly annual	0·29	Rhizome	−0·15	woodyCh	0·22
Lobed leaf	0·21	FlowerEarSum < 3M	−0·27	Elliptic leaf	0·18	woodyCh	0·22	Leaf Length 10–20 cm	0·10	Grass	0·12
		Rosette	0·11	Entire leaf	0·17	Oblong leaf	−0·18			Biennial	0·10
		Forb	−0·27			Toothy leaf margin	−0·15			Multipinnate leaf	−0·11
		Biennial	0·21			Stem prostrate	0·09			H	0·15
		No elongated stolons/rhizome	−0·12							Succulent	0·09
		Elliptic leaf	−0·11								
		Stem erect	−0·31								
		Leaf Length 0·5–3 cm	−0·14								
		Stem ascending	−0·19								
		Obovat leaf	0·12								

Discussion

Our results are consistent with previous descriptions of the distribution of ruderal and competitive strategists (i.e. related species from different biogeographical regions).

Overall, conservation of traits and species' responses were significant for every variable except for spring flowering (*P* > 0·05; one-tailed test for signal). Related species might consequently diverge in flowering options (i.e. related species from different biogeographical regions).

Overall, the significance of some traits (i.e. grasses and succulents) lowered the significance of others (i.e. grasses and succulents). The low number of contrasts lowered the significance of some traits (i.e. grasses and succulents). Overall, conservation of traits and species' responses to climate (Table 4). The low number of contrasts lowered the significance of the species' responses to grazing than to climate (Table 4). Differences were more marked for the calculations for the species' responses to grazing (Table 4). Differences were more marked for the obtained with phylogenetically independent contrasts (Table 4). The results were only slightly different from those obtained with phylogenetically independent contrasts the species level were only slightly different from those along the climatic gradient. The results obtained at stature species, in contrast, were equally present all along the climatic gradient. The results obtained at rhizome) increased at higher altitudes (Table 4). Small and species with vegetative spread (with stolons or rhizome) increased at higher altitudes (Table 4). Small increased towards low altitude, while perennial forbs and species with vegetative spread (with stolons or life span, phanaerophyte and spring-flowering species increased towards low altitude, while perennial forbs optima along the climatic gradient (Table 4). Short life span, phanaerophyte and spring-flowering species relevant PFT (Table 3) were also related to the species' optima along the climatic gradient (Table 4). Short short life span (Table 2 and Fig. 1f). Overall, the most relevant PFT (Table 3) were also related to the species' biennials or facultative annuals among species with a short life span (Table 2 and Fig. 1f). Overall, the most Fig. 1f). Strict annuals responded more markedly than biennials or facultative annuals among species with a decreased (combined data set in Tables 2 and 3 and Fig. 1f). Strict annuals responded more markedly than grasses increased with grazing while perennial forbs decreased (combined data set in Tables 2 and 3 and together, woody chamaephytes, succulent species and grasses increased with grazing while perennial forbs

When data from all the locations were analysed together, woody chamaephytes, succulent species and to grazing regime.

Flowering period was more relevant in dry conditions (Table 2 and Fig. 1) while thorniness was never related to grazing regime.

However, their marginal effect, i.e. their effect as sole predictors, was never significant (*P* > 0·05, results not shown in Table 3) except for the rosette habit illustrated above. Overall, these traits did not show a strong repeatability across the climatic gradient, being significant only in some locations (i.e. prostrate or erect habit). Flowering period was more relevant in dry conditions multiple regressions or in a hierarchical way within subgroups defined by other traits (Table 2 and Fig. 1). However, their marginal effect, i.e. their effect as sole predicted species' responses as a partial effect in multiple regressions or in a hierarchical way within habit, distribution of leaves and leaf characteristics) predicted species' responses as a partial effect in grazing in the literature (i.e. flowering period, plant habit, distribution of leaves and leaf characteristics)

Most of the traits generally reported as related to grazing in the literature (i.e. flowering period, plant semi-rosette.

characteristic distributed their leaves in a rosette or semi-rosette. with big leaves were primarily found in grazed habitats (Table 2 and Fig. 1e) but six of seven species with this characteristic distributed their leaves in a rosette or as single predictor only in subalpine area). Species with big leaves were primarily found in grazed habitats tion to grazing than in other locations (Table 2; *P* < 0·05 as single predictor only in subalpine area). Species Also, the rosette habit was more important as an adaptation to grazing than in other locations (Table 2; *P* < 0·05 Fig. 1e) and the presence of stolons slightly increased species' responses (*P* = 0·09; as a single predictor). No effect of these traits was found in the other locations. Also, the rosette habit was more important as an adaptaspecies increased with reduced grazing (Table 2 and Fig. 1e) and the presence of stolons slightly increased and the other locations. In the subalpine area, rhizomatous species increased with reduced grazing (Table 2 and

Overall, relevant traits differed between the subalpine and the other locations. In the subalpine area, rhizomatous
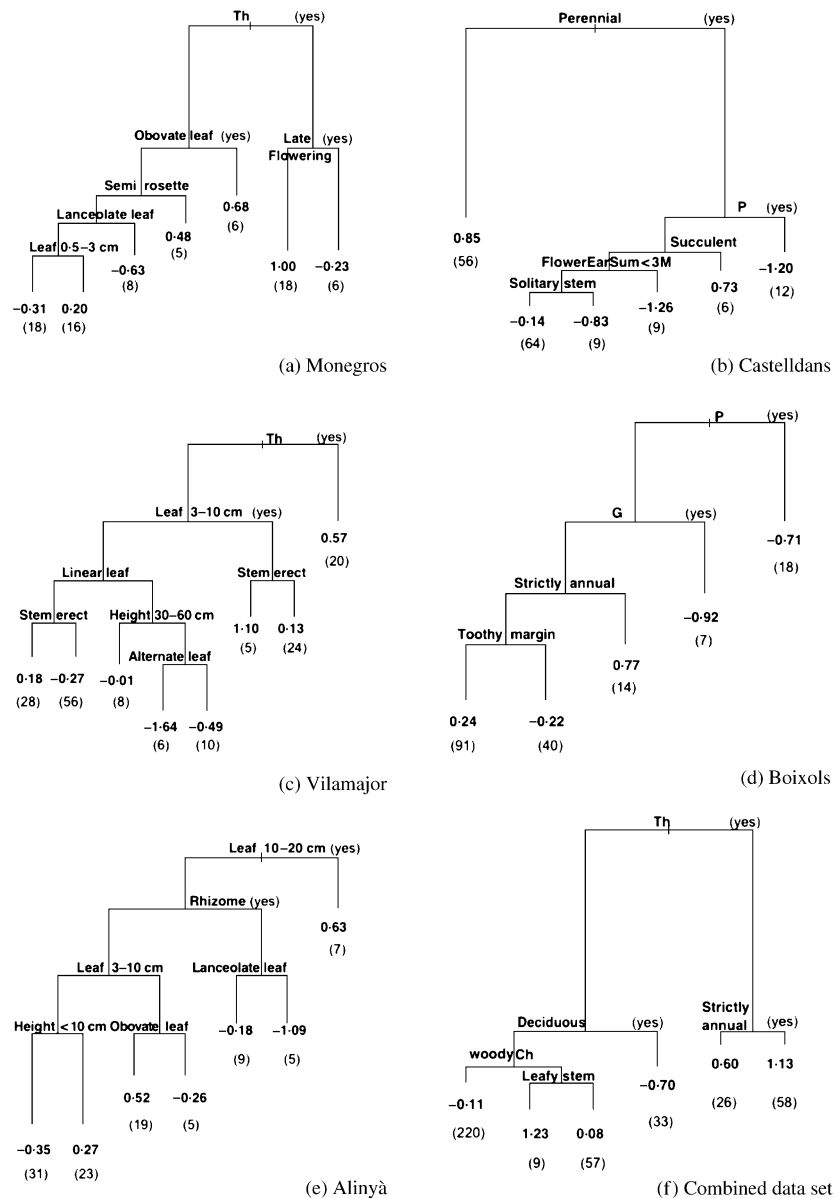
Fig. 1. Hierarchical predictions of species' responses to grazing from plant traits using regression trees. The corresponding pruned tree is showed for each location (a, b, c, d, e) and for the combined data set (f). In any graph, the right branch should be followed downwards if a species possesses the trait at any splitting node (indicated by 'yes' for the first nodes); positive values at the end of the branches indicate a positive (predicted) response of the species sharing the set of traits defined by the upper nodes; numbers between parentheses indicate the number of species sharing those characteristics. For example, in location 1, the right branch of the first split corresponds to Therophytes (Th); the flowering period further discriminates the response of this group of species (furthest right branch; six species are late flowering annuals with a negative response to grazing). Scaling in the figures reflects the original tree dimensions. With fuzzy coding of the PFT, the regression tree can handle traits as quantitative predictors (the method also provides a threshold value for each trait to produce a split, but for clarity the threshold values are not shown in the figures).

along successional and/or disturbance gradients (Prach, Pyšek & Šmilauer 1997; Díaz *et al.* 1999; Lavorel, Rochete & Lebreton 1999; Bullock *et al.* 2001; Grime 2001). Replacements of annuals and small species by perennials, tall shrubs and trees during post-disturbance succession have been reported (Belsky 1992; Prach, Pyšek & Šmilauer 1997) as has the general importance of life cycle, stature and life form in predicting species' distributions along grazing gradients (Belsky 1992; Díaz, Noy-Meir & Cabido 2001; McIntyre & Lavorel 2001). However, this study also demonstrates

how the relative importance of individual traits, and thus their predictive value, changes with climatic conditions.

The change in the predictive value of PFT highlighted a filtering effect of climatic conditions on the regional species pool. Environmental filters act by removing species that lack traits for persisting under a particular set of conditions (reviewed by Díaz, Cabido & Casanoves 1998). In this sense, climate and grazing (or disturbance in general) are both examples of such filters. The first acts at a broader scale, selecting out the species from the

Table 3. Results of the univariate general linear models for most relevant traits as single predictors. Adjusted R^2 (R^2 adj.), number of species possessing (at least partially) the trait (n) and P-values (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, $P > 0.05$) are shown for each location and combined data set. The number of species included in each analysis is given in the first row; + or –, positive or negative response to grazing; †significant among perennials

		Monegros	Castelldans	Vilamajor	Boixols	Alinyà	Combined
	n	77	152	157	170	100	404
Strictly annuals	R^2 adj.	0·153	0·383	0·039	0·044	0·00	0·207
	n	20	49	16	11	4	65
	P	***(+)	***(+)	*(+)	**(+)	NS	***(+)
Short life span (annuals and biennials)	R^2 adj.	0·149	0·380	0·034	0·038	0·00	0·204
	n	25	61	24	21	9	97
	P	***(+)	***(+)	*(+)	*(+)	NS	***(+)
Phanaerophytes (just species > 2 m)	R^2 adj.	0·00	0·145	0·00	0·066	0·005	0·052
	n	6	12	19	18	3	35
	P	NS	***(–)	NS	***(–)	NS	***(–)
All phanaerophytes (species > 40 cm)	R^2 adj.	0·105	0·157	0·013	0·069	0·025	0·080
	n	17	26	34	26	6	67
	P	**(–)	***(–)	0·082(–)	**(–)	NS	***(–)
Small stature (10–30 cm)	R^2 adj.	0·081	0·084	0·045	0·038	0·019	0·068
	n	57	113	109	123	90	404
	P	0·074(+)	**(+)	0·052(+)	*(+)	NS	***(+)
Grasses	R^2 adj.	0·00	0·045	0,00	0·002	0·00	0·018
	n	8	18	22	21	11	49
	P	NS	**(+)	NS	NS	NS	**(+)
Subshrubs (woodyCh)	R^2 adj.	0·00	0·00	0·00	0·030	0·00	0·00
	n	26	38	38	30	20	76
	P	NS	NS	NS	*(+)	NS	NS(+)†
Perennial forbs	R^2 adj.	0·00	0·089	0·00	0·016	0·00	0·042
	n	11	27	54	79	60	144
	P	NS	***(–)	NS	0·094(–)	NS	***(–)
Geophytes	R^2 adj.	0·067	0·005	0·00	0·038	0·00	0·010
	n	2	5	8	7	5	19
	P	*(–)	NS	NS	*(–)	NS	0·068(–)
Succulents	R^2 adj.	0·00	0·038	0·00	0·00	0·0	0·016
	n	4	8	5	4	2	16
	P	NS	*(+)	NS	NS	NS	*(+)

Table 4. PFT predictive value of species responses to grazing and climate (PET) at the species level and with phylogenetic independent contrasts (PIC). Results for the combined data set (see also Table 3). The number of positive contrasts (contrasts where the differences of species' responses was positive between clades) on the total number of contrasts is shown (nc+/nc). Significance for the independent contrasts is based on a sign test (nc+ vs. nc–). + or –, positive or negative response to grazing/PET

	Species' level		PIC			
	Grazing	PET	Grazing		PET	
PFT	P	P	P	nc+/nc	P	nc+/nc
Strictly annuals	***(+)	***(+)	***(+)	(29/32)	***(+)	(26/32)
Short life span	***(+)	***(+)	***(+)	(32/38)	***(+)	(29/38)
Phanaerophytes	***(–)	NS	NS	(5/16)	NS	(8/16)
All phanaerophytes	***(–)	***(+)	0·052	(6/22)	NS	(15/22)
Small stature	***(+)	NS	*	(29/44)	NS	(19/44)
Grasses	**(+)	NS	NS	(1/2)	NS	(2/2)
Perennial forbs	***(–)	***(–)	**(–)	(13/46)	***(–)	(10/46)
Succulents	*(+)	NS	NS	(7/9)	NS	(5/9)
Spring flowering	NS	**(+)	NS	(29/57)	**(+)	(39/57)
Rhizome	*(–)	**(–)	NS	(15/31)	*(–)	(9/31)
Stolons	NS	***(–)	NS	(14/29)	*(–)	(8/29)

regional species pool, and grazing acts as a successive filter. Díaz, Cabido & Casanoves (1998) showed how the relative frequency of a common set of PFT varied in different vegetation types with respect to the regional pool. This effect may explain why most traits in our study did not show a strong repeatability across the climatic gradient, being significant only in some locations (Tables 2 and 3 and Fig. 1).

The ability of plant height to predict species' responses to grazing, for example, often differs according to

local conditions (Díaz, Noy-Meir & Cabido 2001; Osem, Perevolotsky & Kigel 2004; Vesk, Leishman & Westoby 2004). In our study, the majority of the species in the subalpine area showed a small stature independent of grazing (Table 3) and, consequently, the effect of plant height was less pronounced there. The small number of annuals in the subalpine area (4% of the species pool; Table 3) revealed how short life span is a less successful adaptation under the colder conditions of high altitudes (Giménez *et al.* 2004). Results obtained by Sebastià, Canals & Gamarra (1998), in a regional subalpine grassland survey, showed a similar proportion of annual species (around 2%). Annuals need to complete their life cycle within a single growing season, and the length of favourable period for growth decreases with altitude (Giménez *et al.* 2004). Our study showed a decrease of annuals along the climatic gradient accompanied by an increase of perennial forbs and clonal species (Table 4). The presence of rhizomes and stolons in the subalpine location confirmed their importance, as found in similar grasslands by Pakeman (2004). Consequently, life-cycle and reproductive strategies can be considered as examples of adaptations to both climate and grazing. Grazing favoured ephemeral species more markedly in dry locations (Tables 2 and 3 and Fig. 1). In our case, adaptations to grazing and dry conditions were convergent (Table 4). Similar patterns have been illustrated by other authors (Milchunas, Sala & Lauenroth 1988) and also suggest that grazing results in more rapid moisture loss from soil (Landsberg *et al.* 2002; Osem, Perevolotsky & Kigel 2004; Pakeman 2004).

These results were not substantially different by applying PIC (Table 4), despite the trait and niche conservatism reported. Conservatism indicated here that related species resemble each other in traits and distribution, and consequently may adapt to similar environments through similar features. In this sense, conservatism has been interpreted as another evidence of habitat filtering (Webb *et al.* 2002). Related species tend to be similar as they share a similar niche and they are therefore subject to similar stabilizing selection pressures. General high concordance of correlations at species level and with independent contrasts (Table 4) has been reported (reviewed by Price 1997; as an example on PFT predictive capacity see Prinzing *et al.* 2002). The effect of third traits, not taken directly into account with independent contrasts (Price 1997), may also be the cause of different results (i.e. as leaf phenology selected by regression trees in Fig. 1f). The application of not fully resolved trees that distinguish species between each genus may also limit the power of the test. It has been argued that contrasts may be particularly useful as a means of investigating evolutionary diversification, rather than the current predictive value of traits and their present-day function (Westoby, Leishman & Lord 1995; Price 1997; Díaz, Cabido & Casanoves 1998). In our case, the application of PIC demonstrates that our results were not because of a small number of

evolutionary events followed by multiple diversifications of the resulting clades.

Overall, this study shows some explanatory mechanisms responsible of the change in the adaptive significance of PFT, demonstrating that a common set of PFT could not provide consistent predictions of species' responses to grazing in different climatic conditions. It is shown that plant features are subject to multiple selective filters that act on the local species pool, and that most general adaptations are not specific to a given environmental factor (Fonseca *et al.* 2000). Extrapolation based on plant height and life span related traits cannot be generalized to all conditions. Simple PFT sets could be applicable only within situations where vegetation type, productivity and grazing regimes are comparable (Díaz, Noy-Meir & Cabido 2001). Site productivity, season and evolutionary history of grazing may mediate PFT predictions by affecting vegetation composition (Milchunas, Sala & Lauenroth 1988; Bullock *et al.* 2001; Landsberg *et al.* 2002; Pavlů *et al.* 2003; Osem, Perevolotsky & Kigel 2004; Pakeman 2004; Vesk, Leishman & Westoby 2004).

There are two types of applied implication from these findings. The first implication is that the PFT can be a useful tool in predicting species' responses to grazing and, for conservation purposes, identifying species promoted by or vulnerable to land-use changes. However, these predictions cannot be extrapolated from one region to another and are applicable only at local scales. This means that we need more empirical studies in different situations. A network of long-term studies on land-use change effects could be implemented in different vegetation types, within a region, to allow monitoring and more realistic extrapolations. It remains unclear if the study of more functional traits specifically related to disturbance regimes, lacking at the moment for the Mediterranean flora, could provide more consistent predictions (Pausas, Rusch & Lepš 2003). However, even with these simple traits available, management implications could be derived. Grazing abandonment, for example, favoured the development of shrubs and trees in most conditions (Tables 2 and 3 and Fig. 1), indicating that animals limit these species in grazed zones. The prevalence of shrubs can lead to further deterioration of pasture quality and, in turn, to further abandonment (Perevolotsky & Seligman 1998). To conserve traditional grazing systems and their species, the proper agricultural subsidies should be applied in order to equilibrate economic benefits with environmental protection. Subsidies based on the numbers of animals are leading to big flocks, which in turn are more difficult to manage in remote or in dense vegetation areas, resulting in a greater abandonment (Rook *et al.* 2004). In contrast, subsidies based on the surface of land grazed encourage shepherds to exploit a larger part of the territory, if enough controls are provided (i.e. remote-sensing tools).

The second implication of this study is that the methodology proposed to detect predicting traits from a

data set can be applied in general. Regression trees in particular appear to be a useful tool because their effects are usually non-additive, allowing for visualization of trait combinations. The approach proposed here, with traits as predictors and species' responses as the dependent variable, appears to be useful for research on mechanisms that help a plant to cope with given environmental conditions (e.g. grazing, fire, climate change, gap colonization and restoration success). In this way, PFT might uncover the adaptations involved in making species respond similarly to environmental factors (i.e. functional response groups; Lavorel & Garnier 2002). In contrast, if the PFT averages are calculated at the community level, the possible different adaptive strategies among species could be obscured (Ackerly *et al.* 2002). A higher importance could also be given to dominant species and to dominant traits if species' relative abundance is taken into account (Díaz *et al.* 1999; Lavorel, Rochete & Lebreton 1999). Overall, techniques that consider PFT at a community level and as dependent variables appear conceptually related to the study of ecosystem functioning (as for functional effect groups; Díaz & Cabido 2001; Lavorel & Garnier 2002). Despite the distinction of these two approaches, they provide important tools to help generalize the effects of global change on vegetation and their consequences for ecosystem functioning.

Acknowledgements

Supplementary material

The following supplementary material is available for this article online.

Table S1. Life-history traits and their subdivision into the classes utilised in the analysis matrix.

Table S2. Example of species' responses to grazing in the different locations calculated with partial CCA.

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