

Variability in Mediterranean annual grassland diversity driven by small-scale changes in fertility and radiation

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Abstract Using the small-scale gradients in light and fertility imposed by the presence of trees on Mediterranean open oak woodlands, we studied differences in the ecology, floristic diversity and species composition of an annual grassland, and modeled plant relationships with soil properties and radiation using generalized linear models. The strong intra-annual variability in the annual grassland greatly affected overall floristic diversity estimates. Therefore, sampling at least twice during the annual growing period was important to avoid underestimating floristic composition. As has been found in previous studies, different groups of grasses were clearly shaped as a response to the ecological gradient created by the influence of the tree. However, when analyzing

specific relationships, only a few individual species directly responded to the studied soil and light availability factors analyzed. Among them, those species considered ‘ruderal’ such as *Carduus tenuiflorus*, *Echium plantagineum*, *Lolium rigidum*, *Rumex bucephalophorus*, and *Tolpis barbata* showed a clear response to some nutrients and to light availability but displayed no relationship to N soil content. Our results suggest that individual species are responding to a combination of ecological factors. Therefore, some of the generally accepted assumptions regarding the ecology of specific taxa may not be as straightforward as traditionally considered.

Keywords Dehesa · Soil heterogeneity · Tree–grass interaction · Facilitation · Competition

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Introduction

The structure and composition of grasslands are determined by climate and meteorological patterns. In the Mediterranean, the most characteristic factors shaping grasslands are the existence of an intense summer drought and very strong intra- and inter-annual fluctuations in precipitation. However, they are also affected by human activities such as ploughing or husbandry as well as by other factors including soil variability related to micro-topography and plant–plant interactions (e.g., Peco 1989; San Miguel-Ayanz 2001; Brooker et al. 2008). Many

studies have shown how plant interactions play a dominant although variable role in determining plant composition, and that explaining this phenomenon is more complex than was previously thought (for a review, see Brooker et al. 2008). In ecosystems with an open tree overstory, the general effect of trees on the grassland understory is determined by spatio-temporal variability between facilitation and competition (e.g., Callaway and Walker 1997; Scholes and Archer 1997; Gea-Izquierdo et al. 2010). The trees increase the micro-scale spatial environmental heterogeneity surrounding them, modifying the micro-climatic conditions and creating a soil fertility and light availability gradient, which is also a consequence of the indirect effect of the tree as an attraction for animals. As a result soils below canopies have higher levels of soil organic matter, N, K, Ca, and Mg; present a higher cation exchange capacity and a lower bulk density (e.g., Belsky 1994; Rhoades 1997; Gallardo 2003). Herbaceous species adapt individually to the influence of the tree, either by specializing, i.e., establishing themselves in a specific microhabitat, or by displaying a more ubiquitous behavior (e.g., Reynolds et al. 1997; Scholes and Archer 1997). However, it is not easy to clearly identify which ecological factors (i.e., light availability, climate dynamics, soil fertility) or interactions determine the distribution and niche of individual species or group of species. Hence, groups of grassland species and their associated ecology have been actively described, although few studies have directly addressed the response of individual species to specific environmental factors. The species diversity is an important descriptor with a functional significance that helps to determine the performance and composition of a community as a result of the action of such a variety of environmental factors and plant-plant interactions (Montalvo et al. 1993; Hacker and Gaines 1997). Any fluctuation of the species diversity alters the nature of the species interactions, modifying the community function (Hacker and Gaines 1997).

The high grassland diversity supported by Mediterranean open oak woodland ecosystems as well as the influence of the trees on this diversity, has been identified and analyzed in many studies (e.g., González Bernáldez et al. 1969; Marañón 1985, 1986). However, previous research has not generally focused on the direct relationship between species

abundance and the changes in ecological variables. Understanding the ecology of these species is of importance not only in their native ecosystems (biodiversity, grassland ecology, and livestock management) but also in other ecosystems where they are considered invasive exotics (e.g., Austin et al. 1985; Huenneke and Mooney 1989; Gea-Izquierdo et al. 2007). In this study, using the small-scale changes in light and fertility gradients imposed by the presence of trees in open Mediterranean oak woodlands in the Iberian Peninsula, we attempt to address three issues related to the ecology of annual grasslands: (1) How does annual grassland composition vary over the course of the year and how does this affect sampling when studying plant diversity? (2) Do any soil nutrient or light availability factors directly explain small-scale floristic diversity in annual grasslands? (3) Which ecological factor or combination of factors determines the abundance of the most conspicuous grassland species?

Materials and methods

Study area

The study area was located in Central Spain (39°59'N, 5°8'W), at an approximate altitude of 350 m asl. The climate of the region is considered Mediterranean pluviseasonal oceanic with a mean temperature of 15.3°C and a mean annual precipitation of 572 mm ("Talavera de la Reina" meteorological station, Rivas-Martínez and Rivas-Saenz 2010). Soils in the area are sandy, acidic, and poor in organic matter. The study site was located in a flat zone with an approximate tree density of 10 trees/ha; a more detailed description of the study area can be found in Gea-Izquierdo et al. (2009, 2010). The site has been traditionally grazed by livestock (sheep and cattle) but today is only occasionally grazed by wild animals.

The landscape is representative of the *dehesa* ecosystem [agrosilvopastoral system (e.g., Olea and San Miguel-Ayánz 2006) very common in the south west of the Iberian Peninsula] which comprises an open oak woodland, with an understory formed mainly by grassland. The low nutrient content of soils in these systems is a key factor determining grassland communities (Gallardo 2003; Olea and San Miguel-Ayánz 2006; Gea-Izquierdo et al. 2009).

Because of the conditions which predominate in this dry Mediterranean climate, the grasslands tend to be composed of annual species, which avoid the stress summer period as seeds in the soil, then germinate with fall rains. Annual grasslands are characterized by their dynamism, adapting to the diverse environmental conditions and micro-gradients—niche diversification—through competition for light, nutrients, and water (Mamolos et al. 1995; Reynolds et al. 1997; Whittaker et al. 2001).

Field sampling design

Six isolated holm oaks (*Quercus ilex* L. subsp. *ballota* (Desf.) Samp. = *Quercus rotundifolia* Lam.) were selected for the study. Twelve 1 m² plots were established around each tree (72 plots in total), according to the design shown in Fig. 1. Grazing was avoided by using cages (0.5 × 2 m). During the spring of 2007, the 72 plots were examined once per month (March, April, May, June) to check for the presence/absence of every herbaceous species and to quantify their abundance using the scale recommended by Westhoff and van der Maarel (1978) (Table 1). Those species that could not be identified in the field were collected and identified in the laboratory. Species were only determined if flowers or fruits (or both) were present. Hence it was ensured that these key anatomical features were present in order to correctly identify to the species level.

Soil data from the top 20 cm of soil from 72 plots were collected in May 2006 and October 2007 and analyzed for organic matter (OM), total nitrogen (N), phosphorus—Bray method (P), potassium (K), calcium (Ca), magnesium (Mg), cation exchange capacity (CEC), pH, carbon nitrogen ratio (C/N), particle size (% sand, % silt, and % clay), and bulk density

Table 1 Ecological meaning and corresponding percentage of area covered relate to the abundance values used, following Westhoff and Van der Maarel (1978)

Value	Plot area cover (%)	Ecological meaning of abundance values
0	0	Not present
1	<1	Present but scarce
3	<5	Isolated individuals
5	5–25	Few groups of few individuals
7	25–50	Moderate size groups of many individuals
8	50–75	Big groups
9	75–100	Almost complete coverage

(hereafter called density); more details of these analyses can be found in Gea-Izquierdo et al. (2010) and Table 2. Soil properties are considered to be homogeneous in each 1 m² plot. In addition, hemispherical photographs were taken from the sampling plots in February 2006 and the light transmitted was estimated using GLA 2.0 (Frazer et al. 1999). Two periods of radiation were studied: that considered the vegetative period—“light (S-J)” (from 21st September to 21st June) and that considered the spring period—“light (M-J)” (from 1st March to 21st June) (Gea-Izquierdo et al. 2009).

Biodiversity indices and variables analyzed

The presence/absence/abundance data were used to create two common indices to study the floristic diversity and composition: (i) *Species Richness* (Whittaker et al. 2001) is a count of the number of species recorded at each study plot over the whole spring period, i.e., a species recorded at several

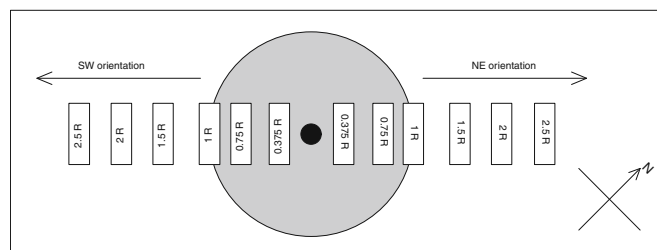


Fig. 1 Sampling design. Six plots with South West (SW) and North East (NE) orientations, maximum and minimum solar radiation directions, at proportional distances to the tree canopy

radius (R), hereafter, $0.375R$, $0.75R$, $1R$, $1.5R$, $2R$, and $2.5R$. These two factors indirectly study variations in insolation, water and nutrient availability

Table 2 Summary of soil and radiation data

Variable	Within canopy		Canopy edge		Beyond canopy	
	S–W (<i>n</i> = 12)	N–E (<i>n</i> = 12)	S–W (<i>n</i> = 6)	N–E (<i>n</i> = 6)	S–W (<i>n</i> = 18)	N–E (<i>n</i> = 18)
pH	5.80 (8.3)	5.67 (7.4)	5.41 (7)	5.37 (9.1)	5.10 (6.9)	4.97 (6.2)
OM (%)	1.63 (24.1)	1.47 (22.7)	1.30 (15.0)	1.18 (33.2)	1.01 (22.1)	0.76 (26)
CEC (meq/100 g)	9.66 (30.4)	8.69 (26.7)	7.84 (22.6)	7.37 (44.0)	7.02 (33.3)	6.39 (32.3)
N (%)	0.08 (51.9)	0.07 (35.1)	0.07 (49.3)	0.04 (34.7)	0.05 (32.7)	0.04 (32.3)
P (ppm)	31.10 (50.1)	26.79 (63.6)	25.43 (54.4)	29.47 (60.3)	22.02 (63.2)	28.75 (50.9)
K (ppm)	150.59 (26.8)	102.48 (33.9)	114.16 (22.8)	77.01 (37.7)	69.30 (31.1)	56.43 (18.4)
Ca (meq/100 g)	3.25 (42.8)	2.57 (39.6)	1.95 (32.2)	1.73 (40.4)	1.47 (32.7)	0.86 (29.8)
Mg (meq/100 g)	0.48 (29.8)	0.35 (33.2)	0.30 (20.4)	0.24 (29.3)	0.23 (39.1)	0.14 (29)
C/N	16.04 (64.9)	15.17 (16.2)	11.47 (15.5)	17.99 (23.3)	18.02 (60.5)	17.29 (32.3)
Sand (%)	84.41 (3.4)	85.02 (4.5)	85.10 (5.3)	83.80 (3.3)	82.04 (6.7)	86.25 (3.6)
Silt (%)	14.23 (19.1)	13.76 (26.2)	13.56 (31.5)	14.97 (16.5)	16.56 (30.9)	12.63 (23.8)
Clay (%)	1.36 (18.8)	1.22 (21.9)	1.33 (19.7)	1.23 (24.0)	1.40 (33.1)	1.11 (16.4)
Density (g/cm ²)	1.38 (11.2)	1.40 (9.9)	1.45 (7.7)	1.47 (7.1)	1.54 (10.4)	1.64 (9.1)
Light (S–J) (%)	53.71 (19.8)	48.51 (18.4)	67.80 (8.3)	60.31 (7.8)	86.91 (13.4)	84.34 (10.3)
Light (M–J) (%)	45.78 (27.3)	46.71 (18.9)	65.17 (12.4)	60.17 (11.3)	87.94 (13.7)	88.43 (10.6)

Mean and coefficient of variation (in brackets) is provided for each soil and radiation variable

“Within canopy” represents plots from 0.375R and 0.75R, “Canopy edge” 1R plots and “Beyond canopy” 1.5R, 2R, and 2.5R. “*n*” represents sample size

sampling times in one same plot is counted as one for the whole season; (ii) The *Shannon index* (H') was calculated for each plot taking into account the data accumulated over the whole spring period and using the maximum abundance value in case a species has been recorded several times in a plot.

Statistical analysis

Floristic diversity

The variations in the annual total Species Richness and the Shannon index were analyzed in two ways: (a) using a qualitative approach; analyzing the relationship to orientation, relative distance to the tree and the interactions between them; (b) using a quantitative approach in two regression models, studying the relationship of the 14 environmental covariates included in the study, OM, N, P, K, Ca, Mg, CEC, pH, C/N, % silt, % clay, density, light (S–J), and light (M–J)) with the Species Richness and the Shannon index as response variables.

The Shannon index approached a normal distribution and Species Richness followed a Poisson distribution. Generalized linear mixed models (GLMMs,

Diggle et al. 2002; Molenberghs and Verbeke 2005) were used to study the previously described relationships. Differences between the least-square means of all of them were compared using the Wald Chi-square test. The number of parameters included in the model was selected backwards from the full model using log-likelihood ratio tests (McCullagh and Nelder 1989). The 12 sampling points established around each tree were spatially correlated. The spatial correlation was taken into account by including a random intercept for the tree, modeling the variance–covariance matrix with a first order autoregressive structure. All the statistical analyses were done using SAS 9.0 (SAS Institute Inc. 2002). The goodness-of-fit of the models was estimated by measuring the difference in percentage between the deviance of the model and the deviance of a model with no covariates (hereafter efficiency, EF). The individual importance of covariates in the model was evaluated by calculating the difference in efficiency of the models, as covariates were included in the model, starting from a reference model with no covariates, to the full model incorporating the significant covariates (McCullagh and Nelder 1989; Guisan et al. 2002).

Species distribution

Only 41 of the 110 species (Appendix in Supplementary Material) identified during the field sampling were used in the analyses: those recorded more than 8 times in the 72 plots on the 4 sampling dates. Non-metric multidimensional scaling (NMS) was used to study the similarity in the plot species composition (Legendre and Legendre 1998). The 72 plots were used as rows and the relative abundance of the 41 species as columns of the main matrix and we used the Sorensen or Bray and Curtis method (McCune and Mefford 1999) to measure the distance/similarity. NMS allowed us to represent in a k -dimensional space (in this case $k = 2$) the species composition of the 72 plots, and distinguish differences between species composition of the plots, aggregating them into ecologically homogeneous groups of species (Legendre and Legendre 1998). PC-ORD software (McCune and Mefford 1999) was used.

The relationship between the 41 species considered and the 14 environmental covariates was then studied using a modeling approach similar to that previously described for Species Richness and Shannon index. A Poisson generalized linear mixed model was fitted with a logarithmic link function for all species including a random intercept for the tree, using again a variance–covariance matrix with a first order autoregressive structure. The multicollinearity of the regressors was also taken into account using

the Variance Inflation Factor (VIF). All statistical tests are considered significant throughout the text with $\alpha = 0.05$.

Results

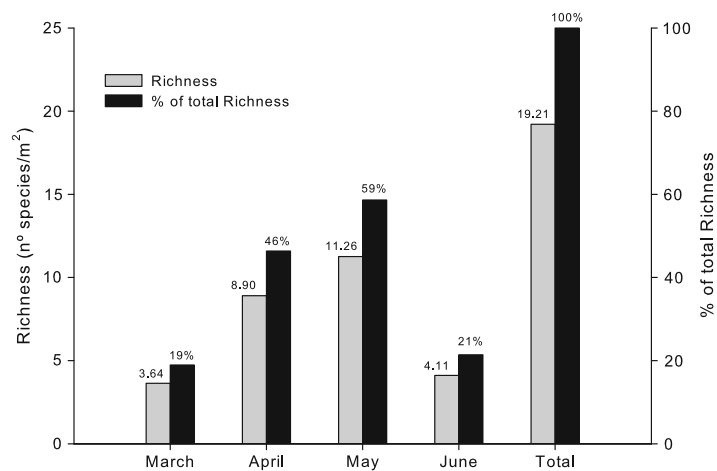
Intra-seasonal temporal variation in species richness

The number of species recorded during the spring months is shown in Fig. 2. These results confirm important differences in the Species Richness over the course of the study year. During May, the number of species observed was the highest, coinciding with the end of the vegetative season, hence the peak in accumulated biomass. However, it still represented only 58.64% of the total number of species identified over the whole annual life cycle.

Influence of soil fertility and light availability on floristic diversity

The Shannon index was directly related to soil texture (positive response to % of silt), as shown in Table 3. Both the Species Richness and the Shannon index decreased in the positions closest to the tree bole ($\chi_1 = 19.93$; $P < 0.0001$; $\chi_1 = 15.41$; $P < 0.0001$), as shown in Fig. 3. In addition, Richness was higher only on the South West side of the tree beyond the

Fig. 2 Differences between the Species Richness recorded at different times throughout the spring. Total richness accounts for all species found per m^2 over the whole year, whereas richness refers to the number of species/ m^2 found on each sampling date. % of Total Richness accounts for the Species Richness recorded monthly compared to Total Richness



canopy, at distances 1.5R ($\chi_1 = 6.56$; $P = 0.0104$), 2R ($\chi_1 = 4.31$; $P = 0.0379$), and 2.5R ($\chi_1 = 19.16$; $P < 0.0001$), as was the Shannon index (for 1.5R, $\chi_1 = 13.6$, $P = 0.0002$; for 2R, $\chi_1 = 4.9$, $P = 0.0266$; for 2.5R, $\chi_1 = 27.6$, $P < 0.0001$). No differences between orientations were found within the canopy projection.

Influence of soil fertility and light availability on species distribution

The results from the NMS of the 72 studied plots, based on the species composition and abundance, showed two clearly differentiated species groups

Table 3 Lognormal GLMMs between Shannon index and the environmental covariates

Parameter	Estimate	Std. error	Z statistic	P value	EF (%)
Intercept	0.971	0.0268	36.19	<0.0001	
Silt	0.0046	0.0023	1.98	0.0474	26.43

Only significant fixed effects are shown
EF (%), efficiency of the model

(Figs. 4, 5): within and beyond the canopy projection; as has been extensively described in the literature (see “Discussion” section). Figure 5 shows the influence of the different species on the axis construction during the NMS process. Two main groups were identified as having the main influence on plot definition within and beyond the canopy projection, as summarized on Table 4. These groups of species characterized the “within canopy” group and the “beyond canopy” group.

Distance and orientation indirectly determined soil parameters and light availability (see Gea-Izquierdo et al. 2010, for a more detailed discussion); according to these gradients, some of the characteristic species of the “within canopy and beyond canopy” groups showed significant statistical relationships with the environmental covariates (Table 5; Fig. 6). Among those species which characterize the “within canopy” group, the distribution of *Carduus tenuiflorus* was greatly influenced by the levels of clay and P (positively) in the soil and displayed a strong, negative relationship with light availability. Similarly, the distribution of *Lolium rigidum* showed a

Fig. 3 Mean Species Richness and Shannon index at different orientations and positions relative to the tree-bole. Error bars correspond to standard deviations

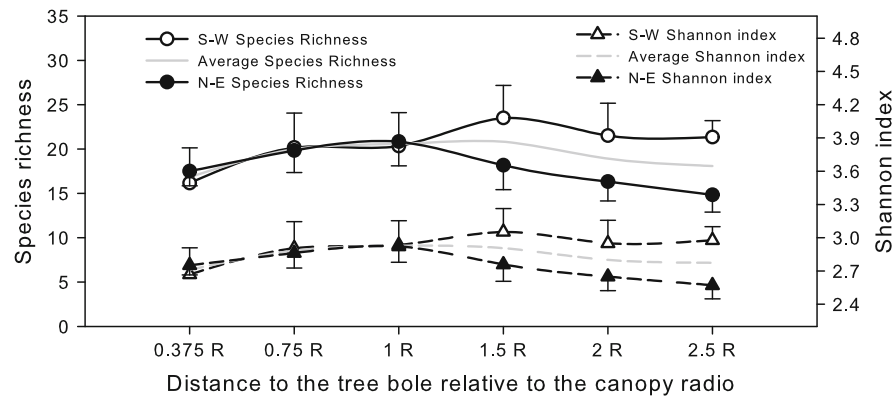


Fig. 4 Results of the NMS of the 72 plots. The plots marked as “within canopy” represent plots from 0.375R and 0.75R and the plots marked as “beyond canopy” from 1.5R, 2R, and 2.5R

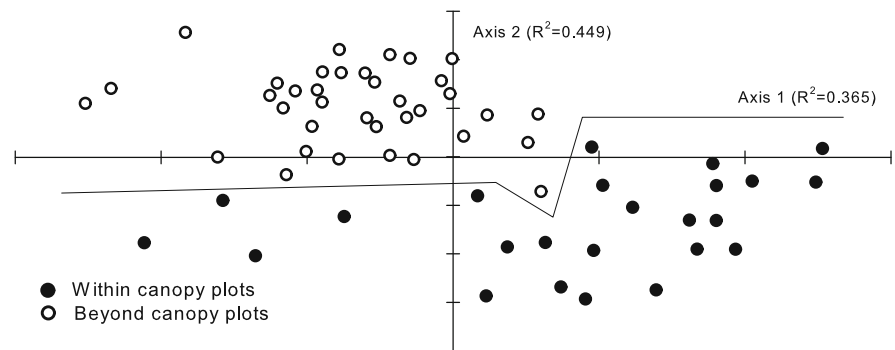


Fig. 5 Influence of the 41 most abundant species on the NMS of the 72 plots (Table 4)

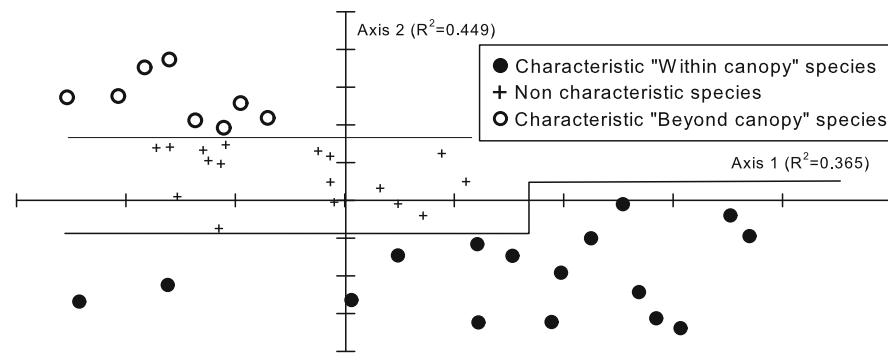


Table 4 Most abundant species recorded in each of the groups defined using NMS

Most abundant recorded species		
Characteristic “within canopy” species (●)	Characteristic “beyond canopy” species (○)	Non-characteristic species (+)
<i>Anagallis arvensis</i> L.	<i>Anthoxanthum aristatum</i> Boiss.	<i>Anthemis arvensis</i> L.
<i>Biserrula pelecimus</i> L.	<i>Linaria spartea</i> (L.) Chaz.	<i>Avena barbata</i> Pott ex. Link.
<i>Briza maxima</i> L.	<i>Mibora minima</i> (L.) Desv.	<i>Bellardia trixago</i> (L.) All.
<i>Bromus diandrus</i> Roth.	<i>Ornithopus sativus</i> Brot	<i>Bromus tectorum</i> L.
<i>Carduus tenuiflorus</i> Curtis	<i>Spergula arvensis</i> L.	<i>Campanula lusitanica</i> L.
<i>Cerastium gracile</i> (L.) Dufoura	<i>Teesdalia nudicaulis</i> (L.) R. Br.	<i>Cynodon dactylon</i> (L.) Pers.
<i>Echium plantagineum</i> L.	<i>Trifolium cherleri</i> L.	<i>Erodium moschatum</i> (L.) L’Hér.
<i>Geranium molle</i> L.	<i>Xolantha guttata</i> (L.) Raf.	<i>Holcus setiglumis</i> Boiss. & Reuter
<i>Hordeum murinum</i> L.		<i>Leontodon salzmannii</i> (Schultz Bip.) Ball.
<i>Lamium amplexicaule</i> L.		<i>Ornithopus compressus</i> L.
<i>Lolium rigidum</i> Gaudin		<i>Ornithopus pinnatus</i> (Miller) Druce
<i>Plantago lagopus</i> L.		<i>Raphanus raphanistrum</i> L.
<i>Plantago lanceolata</i> L.		<i>Rhynchosinapis hispida</i> (Cav.) Heywood
<i>Senecio sylvaticus</i> L.		<i>Rumex bucephalophorus</i> L.
<i>Viola kitaibeliana</i> Schultz in Roem. & Schultz		<i>Silene vulgaris</i> (Moench) Garcke
<i>Vulpia bromoides</i> (L.) Dumort.		<i>Tolpis barbata</i> (L.) Gaertner
		<i>Vulpia myuros</i> (L.) Gmel.

positive relationship with soil clay and Ca content, but a negative response to soil C/N as well as light irradiance between September and June. The models for these two species explained more than 50% of the variance (Table 5). In addition, the distribution of *Senecio sylvaticus* showed a very strong positive relationship with the soil organic matter content whilst the distribution of *Echium plantagineum* was negatively related to soil density and K content (Table 5).

In contrast, only two species characteristic of the “beyond canopy” group showed significant relationships with the environmental covariates studied. Both *Anthoxanthum aristatum* and *Trifolium cherleri* were

positively influenced by the soil silt content. In addition, the distribution of *Anthoxanthum aristatum* showed positive relationships with C/N and the light availability from September to June. This model had an efficiency of 47%.

A number of species were not included in either of the two groups: those which can grow within, beyond or just below the limit of the canopy projection. Among these latter species, the distribution of *Ornithopus compressus* showed a negative relationship with the soil organic matter content, although this model displayed a considerably poorer goodness-of-fit than most other models discussed. *Ornithopus pinnatus* seemed to be negatively influenced by the soil Ca level, which

Table 5 Most important Log–Poisson regressions between abundance of the main species identified and the environmental covariates (soil properties and light)

Group	Species	EF (%)	Parameter	Estimate	Std. Error	Z statistic	P value	EF (%)
Within canopy	<i>Carduus tenuiflorus</i>	54.82	Light (S-J)	−0.08	0.01	6.27	<0.0001	11.62
			Clay	1.94	0.17	11.18	<0.0001	29.77
			P	0.06	0.01	−14.85	<0.0001	54.82
	<i>Echium plantagineum</i>	13.18	Intercept	5.13	1.98	2.59	0.0095	
			Density	−3.24	1.33	−2.43	0.0151	5.96
			K	−0.01	0.01	−2.86	0.0042	13.78
	<i>Lolium rigidum</i>	53.32	Light (S-J)	−0.02	0.01	−2.41	0.0158	10.87
			C/N	−0.13	0.06	−2.31	0.0208	10.95
			Clay	1.36	0.24	5.66	<0.0001	41.37
	<i>Senecio sylvaticus</i>	43.15	Ca	0.45	0.09	5.25	<0.0001	53.32
			OM	0.54	0.04	13.16	<0.0001	43.15
Beyond canopy	<i>Anthoxanthum aristatum</i>	47.44	Intercept	−6.75	1.58	−4.27	<0.0001	
			Light (S-J)	0.06	0.02	5.07	<0.0001	28.54
			Silt	0.06	0.02	3.31	0.0009	45.99
	<i>Trifolium cherleri</i>	31.41	C/N	0.03	0.01	3.00	0.0027	47.44
			Intercept	−4.71	0.90	−5.25	<0.0001	
			Silt	0.22	0.04	5.45	<0.0001	31.41
Non-characteristic species	<i>Ornithopus compressus</i>	7.78	Intercept	1.39	0.17	8.12	<0.0001	
			OM	−0.31	0.15	−2.12	0.0339	7.78
	<i>Ornithopus pinnatus</i>	47.73	Ca	−0.83	0.19	−4.31	<0.0001	47.73
	<i>Rumex bucephalophorus</i>	14.21	Intercept	3.06	0.82	3.71	0.0002	
			pH	−0.37	0.16	−2.40	0.0162	14.21
<i>Tolpis barbata</i>	77.32	Light (M-J)	0.1	0.01	8.8000	<0.0001	74.63	
			P	0.1	0.01	3.5600	0.0004	77.32

Only significant fixed effects are shown

explained 48% of the variance of this species abundance. The distribution of *Rumex bucephalophorus* was positively influenced by soil pH. It is also interesting to note that the distribution of *Tolpis barbata* showed a significant relationship with soil P content and light between March and June, but did not figure among the characteristic species of the “beyond canopy” group. These two covariates explained together up to 77% of *Tolpis barbata* abundance (Table 5).

Discussion

Intra-seasonal temporal variation in species richness

Grassland Species Richness estimation in this study was based on four different floristic inventories

carried out during the spring of 2007 between March and June, which covers the period in which the flowering cycle of Mediterranean grassland species occurs (San Miguel-Ayanz 2001). By means of several inventories during the cycles of the different species it is possible to analyze the global community, taking into account the strong intra-annual variations of the annual communities. This use of several inventories is a key aspect of this study since other studies only performed one inventory per year to estimate Species Richness (González Bernáldez et al. 1969; Marañón 1985, 1986; Peco 1989; Montalvo et al. 1993). The estimation of species richness in this study would have been very different if it had been based on just one inventory at the end of the growing season. For example, if the estimation were based on the inventory performed in May (the month with the highest relative Species Richness) it

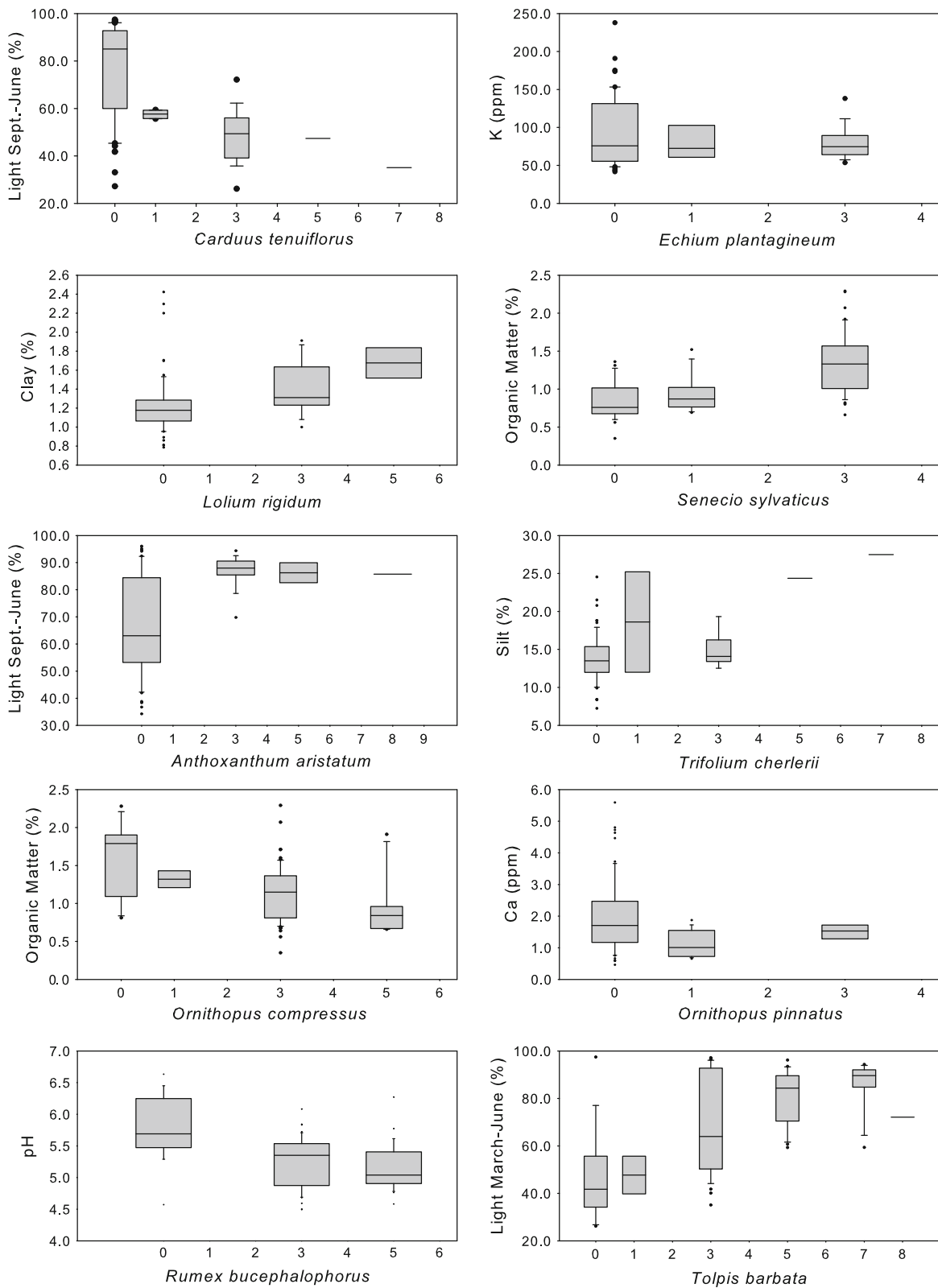


Fig. 6 Relationships between species abundance and environmental covariates

would have identified just 59% of the species richness. Although some species may be identified without flowers/fruits, it would still mean that much information would be missing as regards species diversity in the Mediterranean annual grassland studied. The yearly cycle in grasslands can be described as a dynamic replacement of species with different ecological requirements. Pioneer species show faster life cycles, being the first to flower and fruit (in March). These are replaced in the following months by other species from the same community.

In addition to this high intra-annual variability, Mediterranean grasslands are highly conditioned by strong inter-annual fluctuations (Peco 1989; Montalvo et al. 1993; San Miguel-Ayanz 2001). This study was only carried out in one year, so further differences in Species Richness might be expected if a similar study were to be undertaken using a multi-annual approach. Another factor that needs to be taken into account when comparing different species richness studies is the extent of the area analyzed and the size of the plots (Whittaker et al. 2001; de Miguel et al. 2005), especially in communities as dynamic as annual grasslands. In this regard, the total number of species in this study (110 in the 72 m²) falls slightly short of the 135 species in 0.1 ha measured by Marañón (1985), and the plot Species Richness (mean of 19.21 species/m²) is also lower than that observed by Marañón (1986): 16.5 spp./4 m² within the tree canopy; 27.4 spp./4 m² beyond the canopy projection; 31.9 spp./4 m² at the edge of the canopy projection. These differences may also be related to differences in soils and/or pasture management between sites.

Influence of soil fertility and light availability on floristic diversity

Plots with greater light availability and lower soil fertility, i.e., grasslands beyond the canopy projection and on the SW side of the tree (Gea-Izquierdo et al. 2009), showed higher floristic diversity. This supports the direct relationship between light availability and diversity, and also between low fertility sites and increasing diversity (González Bernáldez et al. 1969; Marañón 1986). However, no direct relationship was found between light availability (or any particular nutrient content) and species diversity within the

canopy projection. Therefore, the lower species diversity within the tree canopy projection is likely to be determined by a combination of factors and plant-plant interactions. An increase in fertility reduces the stress and intensifies competition for light, supporting the dominance of fewer, more competitive species, a phenomenon generally occurring in ecology, where highly productive sites tend to be less diverse (Marañón 1986; Mamolos et al. 1995; Hacker and Gaines 1997; Gross et al. 2005). The intense competition for nutrients instead of light in the micro-habitats beyond the canopy projection is likely to result in higher species diversity favored by the microspatial heterogeneity and the different requirements of the plants (Mamolos et al. 1995; Reynolds et al. 1997).

Soil texture (% silt) has been identified as a key parameter influencing species diversity at our sites. Soil texture is considered an important factor affecting the dynamics of semi-natural grasslands, influencing seed production, seed storage in the soil, and seedling establishment, since these are normally related to water availability and indirectly to the rate of organic matter accumulation (Martínez-Turanzas et al. 1997).

Influence of soil fertility and light availability on species distribution

There are generally held beliefs regarding the ecology of some of these species that were not reflected by our modeling approach. However, there could be scale-dependent relationships, i.e., species could show a certain ecological preferences in a macro-scale including a broader range within the covariates values that we did not appreciate in our small-scale environmental gradient design. Positive relationships were expected 'a priori' between the characteristic species of the within canopy group and OM, N, K, Ca, Mg, and CEC, as well as negative relationships with light and bulk density (e.g., Gallardo 2003; Gea-Izquierdo et al. 2010). Some species which are characteristic of the within canopy group such as *Carduus tenuiflorus*, *Echium plantagineum*, *Senecio sylvaticus*, and *Lolium rigidum* have generally been recognized on a global scale as being ruderal species, displaying a strong relationship to soil N content, which is consistent with the higher N content of the

soils within the tree canopy projection (Gallardo 2003; Gea-Izquierdo et al. 2010). However, no direct relationship was found between those species and soil N content. Some studies in Mediterranean Australia show a positive relationship between *Carduus tenuiflorus* and soil phosphate (Austin et al. 1985). This finding coincides with the positive relationship identified with soil P in our results. It has also been reported in previous studies that *Echium plantagineum* responds positively to soil clay content (Narwal et al. 2008), which seems to be consistent with the strong negative relationship identified between the distribution of the species and soil bulk density. According to other studies (González Ponce and Sentín Montoya 2007), the populations of *Lolium rigidum* are positively influenced by N fertilizers (González Ponce and Sentín Montoya 2007) which can be explained by the low N mineralization rate of the plots where it is most abundant. The distribution of *Senecio sylvaticus* was also related to soil mineral nutrients, specially N and P (van Andel and Vera 1997), however, our data only reveals a positive relationship with the soil organic matter content, which in turn is considered a general indicator of soil fertility (Gallardo et al. 2000). *Anthoxanthum aristatum*, one of the most characteristic species of the beyond canopy group, showed a significant relationship with light availability during the whole vegetative period, which is characteristic of areas not affected by the trees (e.g., Gallardo 2003; Gea-Izquierdo et al. 2010). Additionally, both *Anthoxanthum aristatum* and *Trifolium cherlerii* showed a clear positive relationship with soil silt content, contrasting with several positive relationships observed between soil clay content and a number of characteristic species belonging to the within canopy group (*Lolium rigidum* and *Carduus tenuiflorus*). The two differentiated groups suggested by the ordination and described in the literature for isolated trees in natural grasslands (e.g., González Bernáldez et al. 1969; Marañón 1986; Scholes and Archer 1997) are the result of the classic resource competition or niche separation theory, in which an unstable equilibrium is maintained between different species communities (competitors) which avoid competition by requiring slightly different resources in time and/or space (e.g., Callaway and Walker 1997; Reynolds et al. 1997; Gross et al. 2005). Herbaceous species compose the understory vegetation of the

dehesa system creating a complex matrix of several species groups which follows a set of small-scale ecological micro-gradients generated by the tree and other factors such as livestock and micro-topography. Specific relationships, when downscaling from qualitative covariates (distance and orientation) to quantitative soil and light covariates, were not always evident. Soils below the crown are more fertile (considering most nutrients) which coincides with low light availability. This, together with the high correlation among some of the nutrient variables (e.g., OM and N) is likely to shadow a number of specific effects, even though covariate colinearity was avoided in the models. In addition, we observed different responses to fertility from species belonging to the same group; this could be an example of differential ecological strategies of the different species, even among those belonging to the same group.

Finally, it should be mentioned that legume species such as *Trifolium cherleri*, *Ornithopus compressus*, and *O. pinnatus* showed no relationship with P. This finding was surprising since existing grassland management theories suggest otherwise, hence the common use of P fertilizers to improve the production of annual legumes (Olea and San Miguel-Ayanz 2006). Species like *O. compressus* and *O. pinnatus* showed a negative relationship with OM and Ca, respectively, which probably does not indicate a negative influence but rather a deficit resistance which allows them to be more competitive than other species in Ca and OM deficient soils. The model for *Ornithopus compressus*, which is the most abundant legume in the grassland, did not explain much of the variability, probably because at the study site this species is able to establish itself regardless of the microhabitat changes induced by trees, or because of the high number of outliers observed in Fig. 6.

The relationships between some of these herbaceous species and soil properties should be taken into account for pasture management and environmental restoration in areas of degraded soils. In addition, they could also be of interest in areas where some of the studied species are invasive.

The results of our analyses expose the difficulties involved in non-manipulative sampling designs to determine which specific factors or combinations of factors influence species abundance. The nature of the analysis make possible to show relations that should be validated in the future with manipulative

designs to obtain cause-effects relationships in more detailed species specific studies. They also point towards the existence of complex trade-offs between factors that determine the abundance and dominance of specific species.

Conclusions

Sampling only at the end of the growing season is likely to lead to an underestimation of plant diversity and composition of annual grasslands. The Mediterranean annual grassland studied had high intra-annual variability which, together with the strong inter-annual variability exhibited by this dynamic ecosystem, determines their ecology, diversity and composition. The strong intra-annual dynamics of Mediterranean grasslands greatly affected the degree of local floristic diversity. Thus, to obtain reliable information regarding the ecology of annual species we would recommend sampling twice each year, during the spring period. We identified some clear responses of specific species to soil and light factors, but these relationships did not always match general empirical beliefs concerning the ecology of some of these species. Some species considered ruderal, such as *Carduus tenuiflorus*, *Echium plantagineum*, *Lolium rigidum*, *Rumex bucephalophorus*, and *Tolpis barbata*, showed a clear response to nutrient and light availability but no relationship with soil nitrogen. The important group of legumes *Ornithopus* sp. and *Trifolium* sp. included in our grassland did not exhibit any relationship at all with P but they did display a negative relationship with OM. This could be a consequence of grass being less competitive in poor soils, with some legume species maybe having some advantage through nitrogen fixing. The response of these plants is not straightforward and depends on the interactions of different factors. Some of these relationships between the distribution of herbaceous species and soil properties may prove useful in pasture management, ecosystem restoration, and biodiversity conservation.

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References

- Austin MP, Williams OB, Belbin L (1985) Grassland dynamics under sheep grazing in an Australian Mediterranean type climate. *Vegetatio* 47:201–211
- Belsky AJ (1994) Influences of trees on savanna productivity: tests of shade nutrients and tree-grass competition. *Ecology* 75:922–932
- Brooker RW, Maestre FT, Callaway RM, Lortie CL, Cavieres LA, Kunstler G, Liancourt P, Tielbörger K, Travis MJM, Anthelme F, Armas C, Coll L, Corcket E, Delzon S, Forey E, Kikvidze Z, Olofsson J, Pugnaire F, Quiroz CL, Saccone P, Schifffers K, Seifan M, Touzard B, Michalet R (2008) Facilitation in plant communities: the past the present and the future. *J Ecol* 96:18–34
- Callaway RM, Walker LR (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology* 78(7):1958–1965
- de Miguel JM, Ramírez-Sanz L, Castro I, Costa-Tenorio M, Casado MA, Pineda FD (2005) Plant species richness and spatial organization at different small scales in western Mediterranean landscapes. *Plant Ecol* 176:185–194
- Diggle PJ, Heagerty P, Liang K-Y, Zeger SL (2002) *Analysis of longitudinal data*. Oxford University Press, Oxford
- Frazer GW, Canham CD, Lertzman KP (1999) Gap Light Analyzer (GLA): imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs users manual and program documentation. Simon Fraser University Burnaby BC and the Institute of Ecosystem Studies, Millbrook
- Gallardo A (2003) Effect of tree canopy on the spatial distribution of soil nutrients in a Mediterranean Dehesa. *Pedobiologia* 47:117–125
- Gallardo A, Rodríguez-Saucedo JJ, Covelo F, Fernández Alés R (2000) Soil nitrogen heterogeneity in a Dehesa ecosystem. *Plant Soil* 222:71–82
- Gea-Izquierdo G, Sasha G, Bartolome JW (2007) Assessing plant-nutrient relationships in highly invaded Californian grasslands using non-normal probability distributions. *Appl Veg Sci* 10:343–350
- Gea-Izquierdo G, Montero G, Cañellas I (2009) Changes in limiting resources determine spatio-temporal variability in tree-grass interactions. *Agrofor Syst* 76:375–387
- Gea-Izquierdo G, Allen-Diaz B, San Miguel-Ayanz A, Cañellas I (2010) How do trees affect spatio-temporal heterogeneity of nutrient cycling in Mediterranean annual grassland? *Ann For Sci* 67:112
- González Bernáldez F, Morey M, Velasco F (1969) Influences of *Quercus ilex rotundifolia* on the herb layer at the El Pardo forest (Madrid). *Boletín de la Real Soc Española de Historia Nat (Biol)* 67:265–284
- González Ponce R, Sentín Montoya I (2007) Effect of nitrogen fertilization schedule and herbicide on competition between barley and ryegrass. *J Plant Nutr* 30:783–794
- Gross KL, Mittelbach GG, Reynolds HL (2005) Grassland invisibility and diversity: responses to nutrients seed input and disturbance. *Ecology* 86(2):476–486
- Guisan A, Edwards TC Jr, Hastie T (2002) Generalized linear and generalized additive models in studies of species distributions: setting the scene. *Ecol Model* 157:89–100

- Hacker SD, Gaines SD (1997) Some implications of direct positive interactions for community species diversity. *Ecology* 78:1990–2003
- Huenneke LF, Mooney HA (1989) Grassland structure and function: California annual grassland. Kluwer, London
- Legendre P, Legendre L (1998) Numerical ecology. Elsevier Science BV, Amsterdam
- Mamolos AP, Veresoglou DS, Barbayiannis N (1995) Plant species abundance and tissue concentrations of limiting nutrients in low-nutrient grasslands: a test of competition theory. *J Ecol* 83(3):485–495
- Marañón T (1985) Diversidad florística y heterogeneidad ambiental en una dehesa de Sierra Morena. *Anal de Edafol y Agrobiol XLIV(7-8):1183–1197*
- Marañón T (1986) Plant species richness and canopy effect in the savanna-like “dehesa” of S.W. Spain. *Ecol Mediterr XII(1-2):131–141*
- Martínez-Turanzas GA, Coffin DP, Burke IC (1997) Development of microtopography in a semi-arid grassland: effects of disturbance size and soil texture. *Plant Soil* 191:163–171
- McCullagh P, Nelder JA (1989) Generalized linear models. Chapman & Hall, London
- McCune B, Mefford MJ (1999) PC-ORD. Multivariate analysis of ecological data. MjM Software Design, Gleneden Beach, OR
- Molenberghs G, Verbeke G (2005) Models for discrete longitudinal data, 1st edn. Springer Berlin Heidelberg, New York, NY
- Montalvo J, Casado MA, Levassor C, Pineda FD (1993) Species diversity patterns in Mediterranean grasslands. *J Veg Sci* 4:213–222
- Narwal S, Sindel BM, Jessop RS (2008) Dormancy and longevity of annual ryegrass (*Lolium rigidum*) as affected by soil type depth rainfall and duration of burial. *Plant Soil* 310:225–234
- Olea L, San Miguel-Ayanz A (2006) The Spanish dehesa: a traditional Mediterranean silvopastoral system linking production and nature conservation. *Grassl Sci Eur* 11:3–13
- Peco B (1989) Modelling Mediterranean pasture dynamics. *Vegetatio* 83:269–276
- Reynolds HL, Hungate BA, Chapin FS III, D’Antonio C (1997) Soil heterogeneity and plant competition in an annual grassland. *Ecology* 78(7):2076–2090
- Rhoades CC (1997) Single-tree influences on soil properties in agroforestry: lessons from natural forest and savanna ecosystems. *Agrofor Syst* 35:71–94
- Rivas-Martínez S, Rivas-Saenz S (2010) Worldwide bioclimatic classification system 1996–2009. Phytosociological Research Center, Spain. <http://www.globalbioclimatics.org>. Accessed 10 April 2010
- San Miguel-Ayanz A (2001) Pastos naturales españoles. Mundi-Prensa-Fundación Conde del Valle de Salazar, Madrid
- SAS Institute Inc. (2002) SAS/STAT® 9.0 User’s Guide. SAS Institute Inc., Cary, NC
- Scholes R, Archer SR (1997) Tree-grass interactions in savannas. *Annu Rev Ecol Syst* 28:517–544
- van Andel J, Vera F (1997) Reproductive allocation in *Senecio sylvaticus* and *Chamaenerion angustifolium* in relation to mineral nutrition. *J Ecol* 65:747–758
- Westhoff W, van der Maarel E (1978) The Braun-Blanquet approach. In: Whittaker RH (ed) Classification of plant communities. Junk, The Hague
- Whittaker RJ, Willis KJ, Field R (2001) Scale and species richness: towards a general hierarchical theory of species diversity. *J Biogeogr* 28:453–470