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Original article

Early environments drive diversity and floristic composition in Mediterranean old fields: Insights from a long-term experiment

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ABSTRACT

While many studies have explored how previous and current environmental conditions affect the performance of individual organisms, their relative importance as drivers of current diversity and composition of communities is virtually unknown. We evaluated the response of herbaceous communities to previous (experienced during early establishment) and current environmental conditions by comparing their composition and diversity in an abandoned Mediterranean cropland planted with *Quercus ilex* subsp. *ballota* L. seedlings. These seedlings received different experimental treatments (summer irrigation and artificial shading) during the first three years after planting, and were interrupted from then on. We tested two complementary hypotheses: (i) the previous environments experienced by the herbaceous communities during their establishment have a long-term carry-over effect on diversity and composition of species assemblages and (ii) these communities are influenced by their current environment, particularly by the woody layer and the soil conditions. Overall, we observed an important contribution of initial environmental conditions in determining the current diversity and composition of herbaceous communities. Amelioration of environmental conditions, particularly water stress, during community establishment resulted in a decrease in alpha and beta diversity, possibly as a consequence of decreasing environmental heterogeneity. Previous environments accounted for 26.3% of the explained variance in current community composition. Annuals, legumes and forbs also responded significantly to previous environments, which explained 27.9%, 36.2% and 30.1%, respectively, of the variance in their composition. Our results suggest that those species present at a particular site early in succession pre-empt the site and influence vegetation dynamics on that site for a long time. This study provides important insights for understanding the mechanisms underlying the ecological effects of issues like cropland reforestation and woody vegetation encroachment.

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1. Introduction

It is well known that early plant establishment is basic for determining the spatial and temporal variation in the composition and structure of plant communities (Grubb, 1977; Silvertown, 2004). Establishment is a complex process driven by a series of factors interacting to determine emergence and net recruitment. These include mechanisms such as stochastic processes (Sale, 1977; Chesson and Warner, 1981), neighbour plant interactions (Goldberg, 1987; Callaway and Walker, 1997), dispersal (Gill and Marks, 1991), and abiotic constraints determined by resources such as light, water and nutrients (Escudero et al., 2000; Maurer and Zedler, 2002; Maestre and Reynolds, 2007). Consequently, environmental conditions prevailing during the establishment of communities (i.e. previous environments) are important determinants of the structure and composition of species assemblages in the long term. Furthermore, it can be argued that short term environmental conditions, such as severe drought, experienced during the establishment of a given community may shape its diversity and composition long after such conditions had ceased. Although many studies have emphasised the role of both previous and current environmental conditions on the performance of individual organisms (Bazzaz, 1996; Reekie et al., 1997; Metcalfe and Monaghan, 2001; Relyea, 2002; Weing and Delph, 2001; Rey Benayas and Camacho-Cruz, 2004; Grether, 2005), little research has been conducted at the community level (Lortie et al., 2004). Therefore, the relative contribution of previous and current environmental conditions as drivers of diversity and composition of plant communities is virtually unknown.

In the face of the existing vacuum of knowledge, we propose a conceptual three-pathway model to illustrate the potential effects of previous (during early establishment) and current environments on the diversity and composition plant communities (Fig. 1), and tested it experimentally with

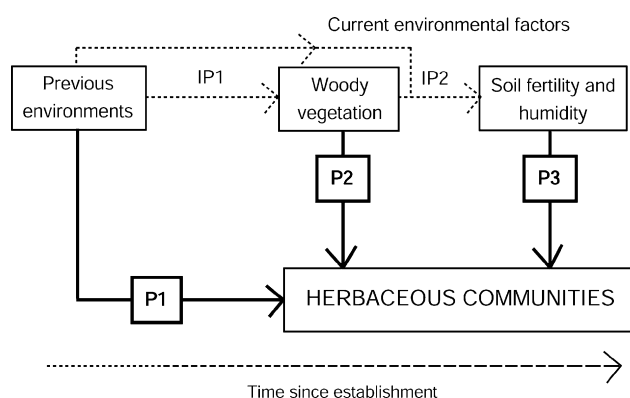


Fig. 1 – Schematic pathways through which previous and current environmental factors influence herbaceous communities in reforested old fields. Because previous environments (P1) can exert some effect on current environmental conditions, the direct effects of current environmental variables (P2, P3) are separated from the indirect effects (IP1, IP2) that previous environments might have through their influence on current environmental conditions.

herbaceous communities in a Mediterranean old field afforested with woody species. To our knowledge, no previous study has used an experimental approach to explore how responses to previous environments affect the current arrangement of plant communities. Such studies are essential to advance our understanding on the interplay between previous and current environmental conditions as drivers of the composition and diversity of plant communities. The first pathway (P1) of our conceptual model (Fig. 1) hypothesizes that previous environments influence the diversity and species composition of herbaceous communities by favouring a particular assemblage of species during early establishment. The second pathway (P2) establishes the hypothesis that, with time, woody vegetation will exert an influence on the herbaceous community by modifying resource levels, particularly water and nutrient level amelioration and light deprivation (McMurtrie and Wolf, 1983; Wu et al., 1985). Therefore, different levels of woody cover may favour different herbaceous species. Since tree performance has been shown to be partly determined by previous environments (Rey Benayas and Camacho-Cruz, 2004), it is essential to separate the direct effect exerted by the woody vegetation (P2) from indirect effects of previous environments on herbaceous communities through an intermediary effect on the woody vegetation (IP1). Finally, the third proposed pathway (P3) refers to soil conditions, which can influence species diversity and composition, particularly under stressful Mediterranean conditions (Bonet, 2004). At the same time, soils can be affected by both previous environments and woody vegetation. Previous environments might affect soil fertility, for instance, through mitigation of water stress for soil organisms. Likewise, different studies have shown that soils developing under tree canopies are richer in nutrients and organic matter, have a greater water-holding capacity, and their macroporosity promotes infiltration and the redistribution of soil water (Boettcher and Kalisz, 1990; Joffre and Rambal, 1988, 1993). As a result of all these potential interactions, it is important to separate the direct effects of soil conditions (P3) from the indirect effects that other factors might have on the herbaceous flora through an intermediate effect on different soil properties (IP2).

Our study is part of a long-term study aiming to investigate the effects of experimentally manipulated environments (summer irrigation and artificial shading) on the performance of different life history traits of *Quercus ilex* subsp. *ballota* L. seedlings (Rey Benayas, 1998; Rey Benayas and Camacho-Cruz, 2004). In the present study, we evaluated how these environmental manipulations affected the diversity and composition of herbaceous communities long after they had ceased. We hypothesized that the assemblage of plants into communities should reflect both a carry-over effect of previous environments, and responses to current environmental conditions. Our main objective was to test our conceptual model and to determine the relevance of previous environments in opposition to current environments. More specifically, we wanted to understand how stable herbaceous communities change once the initial environmental conditions that triggered a particular arrangement of species had been interrupted, and how long does the effect of certain management decisions last. Additionally, we wanted to investigate if there were different responses in species with different life

cycles (annuals and perennials) and functional attributes (graminoids, legumes and forbs). Our experimental approach provides not only an excellent opportunity to test how responses to previous environments affect the current arrangement of herbaceous communities, but may also provide useful insights to forecast potential effects of processes such as shrub encroachment (Van Auken, 2000) and woody plant invasion (Richardson and Rejmánek, 2004) in the future development of herbaceous communities.

2. Material and methods

2.1. Study area and field experiment

The study site is an abandoned cropland located in central Spain (40°3'N, 4°24'W, altitude 450 m). It has a typically Mediterranean climate, characterized by a long summer drought that imposes severe water stress on the vegetation, and cold winters. Total annual precipitation is 480 mm and mean annual temperature is 15 °C. The soil is a luvisol type derived from arkoses.

Quercus ilex subsp. *ballota* seedlings were planted in 1993 in a 1 ha site which had been cultivated for grain for several years until the plantation was established (Rey Benayas, 1998). The experiment included four treatments, resulting from the factorial combination of summer irrigation and artificial shading (control, irrigation, shading, irrigation and shading), with four replicated plots (10 m × 10 m) per combination of treatments (16 plots in total). Plots were separated by a distance of 20 m from each other. Each plot was planted with 50 one-year-old seedlings, regularly distributed and separated 2 m from each other. The irrigation treatment consisted of water applied by sprinklers at the peak of the dry season (60 mm in July and August; 120 mm in total per year); water was added uniformly to the entire plot area. The artificial shading treatment consisted of a reduction in incident radiation by 68% by placing a black polyethylene net that stood 2 m above ground. Herbivores were excluded from all plots with a wire mesh because hares and rabbits caused severe damage to seedlings. The artificial shading and summer irrigation treatments were interrupted in the winter of 1996. All plots have been under natural water and radiation inputs since then, but protection from herbivores has been maintained to the present date.

2.2. Sampling design

Sampling of herbaceous communities took place between April and May 2005. Nine 0.5 m × 0.5 m quadrats were set in each of the 16 plots with a regular distribution. We recorded the presence of all vascular plant species in each quadrat. Relative abundance (from 0 to 9) was then calculated at the plot level by adding up all presences across quadrats. We used Fisher's alpha as a measure of plot alpha diversity (Rosenzweig, 1995). Beta diversity was estimated as the mean Bray–Curtis dissimilarity distance (Magurran, 1988) between quadrats within a plot. All species were classified, according to their life cycles, into annuals (including biannuals) and perennials. Likewise, all species were classified into guilds,

according to their functional attributes, as graminoids, legumes and forbs.

The four different management treatments applied to reforested plots between 1993 and 1995 were considered in this study as surrogates of the environmental conditions during the three first years of succession in the former cropland (i.e. conditions during early establishment). To account for the effect of current environmental conditions, we measured tree canopy volume, soil fertility and soil moisture at the time of sampling the herbaceous communities (spring of 2005).

Tree canopy volume was estimated using the height and crown projected area per plot in 2005. Mean tree canopy volume was highest in plots subjected to irrigation (mean = 209.8 m³ ± SD = 90.70), followed by plots subjected to shading (mean = 171.7 m³ ± SD = 42.05) and both irrigation and shading (mean = 137.8 m³ ± SD = 42.60). The plots that were not subjected to any treatment displayed the lowest mean tree canopy volume (mean = 103.17 m³ ± SD = 41.11).

To analyze soil fertility, we collected three 20 cm deep soil cores per plot, which were combined into a single composite sample per plot. In each composite sample the following variables were measured (we report mean, standard deviation, and range across plots): carbon content (0.505 ± 0.123 [0.349–0.755]%), total nitrogen (0.049 ± 0.012 [0.036–0.074]%), carbon/nitrogen ratio (10.309 ± 0.652 [9.499–11.743]), NH₄⁺ concentration (2.246 ± 1.420 [0.000–4.058] µg/g), NO₃⁻ concentration (0.608 ± 1.551 [0.000–3.093] µg/g), potential nitrification rate (0.258 ± 0.156 [0.032–0.582] µg/g day), potential mineralization rate (0.212 ± 0.147 [0.000–0.523] µg/g day), potential ammonification rate (-0.046 ± 0.057 [-0.096–0.163] µg/g day), P₂O₅ (69.0 ± 62.4 [30.0–295.0] mg/kg), phosphorus content (28.144 ± 25.461 [12.237–120.329] mg/kg), potassium content (186.0 ± 78.8 [90.0–380.0] mg/kg), and pH (5.977 ± 0.538 [5.120–7.000]). Carbon content was analyzed using K₂Cr₂O₇ in a H₂SO₄ environment. Total N was analyzed using the Kjeldahl method (H₂SO₄ with Se and K₂SO₄). Potential net mineralization, nitrification and ammonification rates were determined by incubating 5 g of dry soil with 15 g of pure sand and 6 mL of water during 14 days in the dark at 30 °C. Soil samples were extracted with 100 mL of KCl 2 M, shaken for 1 h and the suspension filtered through 0.45 µm millipore filters. NH₄⁺-N and NO₃⁻-N in the extract were measured by colorimetry (indophenol blue method), using a microplate reader. Potential net mineralization rate was calculated as the difference between the NH₄⁺-N + NO₃⁻-N concentration before and after the incubation period. Net nitrification was the difference between the NO₃⁻-N concentration over the same period. Potential ammonification rate was the difference between potential net mineralization rate and potential nitrification rate. For P₂O₅ we used the method reported by Burriel and Hernando (1950). Phosphorus content was extracted with acetic acid 2.5% and determined by colorimetry (molybdenum blue method, Allen et al., 1986). Potassium was analyzed according to MAPA (1986) using an Optic PLASMA ICP (Perkin-Elmer, model 4300 DV). In addition, soil moisture was measured with a time domain reflectometer (TDR, Topp et al., 1980) at five sampling points per plot. Measurements were taken on three dates (13 May, 23 May, and 7 June 2005), and the mean value per plot was calculated (5.644 ± 1.710 [3.380–9.493]). A principal component analysis (PCA) was

then used to reduce the dimensionality of the soil dataset. The first PCA axis explained 37.1% of the variance and was positively related to the mineralization and nitrification rates, and negatively related to organic matter content, carbon content, potassium content, total nitrogen, P_2O_5 , pH and humidity. This axis was therefore interpreted as a gradient of soil fertility and humidity, with negative values indicating more humid and fertile soil conditions, and used henceforth as a surrogate of soil fertility and humidity. To avoid autocorrelation between the two environmental variables used in this study (soil fertility/humidity and tree canopy volume, $r = 0.40$, $p = 0.124$, $n = 16$), we extracted the variability in the first PCA axis not explained by tree canopy volume by means of a linear regression. The residuals of this model were then used in the subsequent analyses to account for the effects of soil fertility and humidity which were not explained by tree canopy volume.

2.3. Data analysis

We used analyses of covariance (ANCOVA) to account for the relative effects of previous environments (a factor including the four management treatments) and current environmental conditions (two covariates: tree canopy cover, and soil fertility and humidity) on alpha (Fisher's alpha) and beta diversity (mean Bray–Curtis dissimilarity distance between-quadrats).

Non-metric multidimensional scaling (NMDS) was used to: (i) explore the patterns of species composition in reforested plots subjected to different treatments and (ii) relate the variability in species composition to environmental variables representing previous (summer irrigation, artificial shading) and current environmental conditions (tree canopy volume, soil fertility and humidity). We used the Bray–Curtis dissimilarity distance to compute the resemblance matrix between plots. Values of relative species abundance and environmental variables were then fitted onto the first two axes of the NMDS ordination. Squared correlation coefficients (R^2) and empirical p -values (p) were calculated for these linear fittings.

We used the semi-parametric permutational multivariate analyses of variance (hereafter PERMANOVA) developed by Anderson (2001) to test the simultaneous responses of floristic composition to previous and current environments. The model included two factors related to previous environments (artificial shading and summer irrigation) and two covariates representing current environmental conditions (tree canopy volume and soil fertility and humidity). To separate the effects of these two former variables from those of previous environments, we partitioned the variance of species abundance data into three different components, following our conceptual model: (1) direct effects of previous environments (P1 in Fig. 1); (2) direct effects of current environmental variables (P2 and P3 in Fig. 1); and (3) indirect effects of previous environments through their effects on current environmental variables (IP1 and IP2 in Fig. 1). We followed a two-step procedure to partition the variance. In the first step, we conducted a PERMANOVA with all the experimental treatments and covariates (tree canopy volume, soil fertility and humidity). This allowed us to calculate the direct effects of previous environments and the full effect of the covariates on species

composition. In a second step, we extracted the variability of the covariates not explained by previous environments by means of univariate linear models. We then ran a PERMANOVA with the factors representing previous environments and the residuals of the corresponding linear models in order to account for the direct effects of the covariates on species composition. By doing this, we inflated the amount of variance explained by the previous environments, but could accurately estimate the variance explained by the direct effects of the covariates. Finally, to account for indirect effects, we simply calculated the differences in the explained variance of the covariates between the first and the second model. Note that this did not allow the statistical testing of significant indirect effects. This set of analyses was also performed for annuals and perennials and for different functional types (graminoids, legumes, forbs). PERMANOVA analyses were performed with the program PERMANOVA 1.6 (Anderson, 2005; downloaded from <http://www.stat.auckland.ac.nz/~mja/Programs.htm>). We used the Bray–Curtis distance and 10,000 permutations for these analyses (permutations of residuals under the reduced model; Anderson and Ter Braak, 2003).

3. Results

We did not detect significant effects of current environmental conditions on the diversity of herbaceous communities (Table 1). Linear models revealed a statistically significant negative effect of summer irrigation on beta diversity (Table 1b). Likewise, there was a marginally significant negative effect of the interaction between irrigation and shading on both alpha and beta diversity ($p < 0.1$, Table 1, Fig. 2).

Overall, we recorded 51 herbaceous species in 16 plots: 39 annuals and 12 perennials; 16 graminoids, five legumes and 30 forbs (Table 2). Non-metric multidimensional scaling (NMDS) showed within and between-group dissimilarities for reforested plots under each of the four combinations of summer irrigation and artificial shading (Fig. 3). A total of 17 species showed a significant relationship with the NMDS ordination axes (Table 2, Fig. 3), of which there were 13 annuals (76%), and four perennials (24%); and seven were graminoids (41%), two were legumes (12%), and eight were forbs (47%).

There were significant linear effects of summer irrigation (a factor representing previous environmental conditions), and soil fertility and humidity (a variable representing current environmental conditions) on the two axes of the species ordination (Table 3). This information, however, does not include the shared effects among all the independent variables and factors. Table 4 summarizes the results of the two-step procedure used to separate the direct and indirect effects of previous and current environmental conditions on species composition. In the first step, we estimated the direct effects of previous environments, which explained 26.3% of the variance (Table 4, Panel A). There were significant effects of both shading and irrigation on species composition, but not of their interaction. In the second step, we extracted the amount of variance explained by the direct effects of tree canopy volume (8.3%) and soil fertility and humidity (7.1%), although the latter was not significant (Table 4,

Table 1 – Summary of analyses of covariance used to test the effects of previous (summer irrigation and artificial shading during the first three years of establishment) and current environmental conditions (13 years after establishment) on: (Panel A) alpha diversity (Fisher's alpha) and (Panel B) beta diversity (between-quadrats mean Bray–Curtis dissimilarity distances)

Source	df	SS	EV (%)	MS	F	p(permutation)
Panel A						
Previous environments						
Artificial shading	1	3.355	6.2	3.355	1.229	0.293
Summer irrigation	1	3.710	6.9	3.710	1.359	0.271
Shading × irrigation	1	9.559	17.8	9.559	3.502	0.090
Current environments						
Tree canopy volume	1	0.861	1.6	0.861	0.315	0.587
Soil fertility and humidity	1	8.814	16.4	8.814	3.229	0.103
Residual	10	27.297	50.9	2.729		
Total	15	53.596				
Panel B						
Previous environments						
Artificial shading	1	0.001	2.4	0.001	1.029	0.334
Summer irrigation	1	0.011	26.2	0.011	6.951	0.025
Shading × irrigation	1	0.007	16.7	0.007	4.466	0.060
Current environments						
Tree canopy volume	1	0.005	11.9	0.005	3.173	0.105
Soil fertility and humidity	1	0.002	4.7	0.002	1.277	0.285
Residual	10	0.016	38.0	0.016		
Total	15					

df = Degrees of freedom; SS = sum of squares; EV = explained variance (%); MS = mean squares; F = F-statistic; p(permutation) = p value based on permutations.

Panel B). The indirect effects of previous environments on floristic composition were then calculated as the difference between the explained variance of the covariates in both steps (Fig. 4a).

We observed similar trends when annual species were considered (Fig. 4b), yet the response of perennials to both previous and current environments was not significant (Fig. 4c). Different guilds also showed different responses. While graminoids (Fig. 4d) showed a weak, non-significant response to both previous and current environments, legumes (Fig. 4e) and forbs (Fig. 4f) showed a marked response to previous environments, the latter being also significantly affected by tree canopy volume. The response to soil fertility and humidity was not significant in all cases.

Indirect effects of previous environmental conditions, mediated by their effects on tree canopy volume, had a weak influence on the composition of herbaceous communities. In contrast, indirect effects through soil humidity and fertility were, in most cases, relatively large (Fig. 4). Nonetheless, the statistical significance of these indirect effects could not be tested. In all cases where previous environments had significant effects, they were due to the effect of shading and/or irrigation individually (see Web version, Appendix 1), but not to their interaction. Legumes were an exception to this trend, for only irrigation contributed significantly to most of the explained variance in the model.

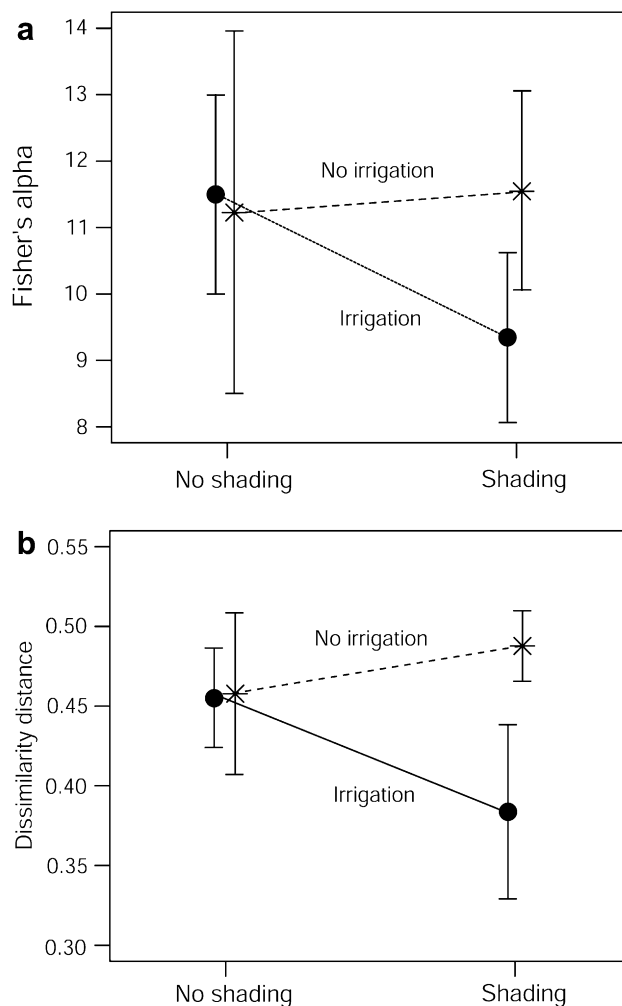


Fig. 2 – Profile plots showing the individual and interacting effects of previous environments (summer irrigation, artificial shading) on: (a) alpha diversity (Fisher's alpha) and (b) beta diversity (mean Bray–Curtis dissimilarity distances between-quadrats). Data represent mean values and ±1 standard deviations (n = 4).

4. Discussion and conclusions

Our study provides a tool to link empirical data and theory in order to evaluate the imprint of specific events on the inertia of ecological communities during the early stages of their establishment. Overall, we observed an important contribution of initial environmental conditions in determining the diversity and composition of herbaceous communities almost 12 years after community establishment. Summer irrigation was the most important factor in explaining patterns of species composition in herbaceous communities (Table 4). It is likely that past irrigation stimulated soil fertility through mitigation of water stress for soil organisms. We believe that this increase in fertility persisted in time, a fact supported by the presence of species that indicate relatively high nitrogen concentrations, such as *Bromus hordeaceus* or *Daucus carota*, in plots subjected to irrigation or both irrigation and shading,

Table 2 – List of the 51 species sampled in this study and their assignment to different groups according to their life form (A = annual, P = perennial) and guild (G = graminoid, L = legume, F = forb)

Species	Functional traits	Guild structure	Treatments				R ²	p
			C	I	S	I × S		
<i>Agrostis castellana</i> Boiss. et Reuter	P	G	2	0	0	0	0.48	0.009
<i>Aira caryophylla</i> L.	A	G	4	3	4	1	0.39	0.035
<i>Anagallis arvensis</i> L.	A	F	1	0	0	1	0.12	0.449
<i>Andryala integrifolia</i> L.	A	F	4	4	4	4	0.36	0.065
<i>Anthemis arvensis</i> L.	A	F	1	2	0	0	0.45	0.013
<i>Avena barbata</i> Pott ex Link	A	G	4	4	4	4	0.06	0.697
<i>Bellardia trixago</i> (L.) All.	A	F	2	4	3	2	0.13	0.425
<i>Bromus hordeaceus</i> L.	A	G	3	4	3	4	0.72	< 0.001
<i>Bromus madritensis</i> L.	A	G	1	1	0	0	0.06	0.640
<i>Bromus rubens</i> L.	A	G	4	3	3	4	0.19	0.221
<i>Bromus tectorum</i> L.	A	G	3	4	2	1	0.71	< 0.001
<i>Campanula rapunculus</i> L.	P	F	0	1	0	0	0.04	0.677
<i>Centranthus calcitrapa</i> (L.) Dufresne	A	F	4	3	4	4	0.50	0.016
<i>Cerastium pumilum</i> Curtis	A	F	1	1	4	3	0.12	0.448
<i>Chondrilla juncea</i> L.	P	F	4	4	4	4	0.27	0.153
<i>Convolvulus arvensis</i> L.	P	F	3	3	1	4	0.17	0.282
<i>Crepis capillaris</i> (L.) Wallr.	A	F	4	4	4	4	0.64	0.004
<i>Crepis vesicaria</i> L.	A	F	4	4	2	4	0.03	0.818
<i>Cynodon dactylon</i> (L.) Pers.	P	G	2	0	1	0	0.48	0.002
<i>Dactylis glomerata</i> L.	P	G	2	1	2	1	0.03	0.841
<i>Daucus carota</i> L.	P	F	3	3	2	4	0.58	0.004
<i>Eruca sativa</i> Millar	A	F	0	1	0	0	0.04	0.638
<i>Eryngium campestre</i> L.	P	F	1	0	2	2	0.34	0.060
<i>Foeniculum vulgare</i> Millar	P	F	0	0	0	1	0.19	0.259
<i>Galium parisiense</i> L.	A	F	3	4	4	2	0.18	0.271
<i>Geranium molle</i> L.	A	F	4	4	4	4	0.06	0.676
<i>Hordeum marinum</i> Hudson	A	G	0	1	0	0	0.04	0.677
<i>Hordeum vulgare</i> L.	A	G	1	0	0	0	0.29	0.129
<i>Jassione montana</i> L.	A	F	0	3	3	1	0.00	0.998
<i>Lathyrus cicera</i> L.	A	L	0	1	0	0	0.04	0.638
<i>Leontodon hispidus</i> L.	A	F	3	4	4	4	0.74	< 0.001
<i>Lolium perenne</i> L.	P	G	0	1	0	1	0.27	0.124
<i>Papaver dubium</i> L.	A	F	0	2	0	0	0.05	0.728
<i>Picnoman acarna</i> (L.) Cass.	A	F	1	1	0	0	0.44	0.016
<i>Plantago lagopus</i> L.	A	F	0	0	0	1	0.01	0.942
<i>Rumex acetosa</i> L.	A	F	1	1	2	1	0.18	0.294
<i>Scolymus hispanicus</i> L.	P	F	3	2	3	1	0.22	0.169
<i>Senecio jacobaea</i> L.	P	F	2	1	2	1	0.19	0.242
<i>Senecio vulgaris</i> L.	A	F	1	0	0	0	0.29	0.129
<i>Silene colorata</i> Poirer	A	F	0	0	0	1	0.19	0.259
<i>Sonchus asper</i> (L.) Hill	A	F	0	1	1	2	0.40	0.035
<i>Taeniatherum caput-medusae</i> (L.) Nevski	A	G	3	3	3	4	0.53	0.005
<i>Torilis nodosa</i> (L.) Gaertner	A	F	0	0	0	1	0.27	0.193
<i>Trifolium arvense</i> L.	A	L	2	4	4	4	0.63	0.002
<i>Trifolium campestre</i> Schreber	A	L	4	4	2	3	0.45	0.015
<i>Trifolium tomentosum</i> L.	A	L	0	0	1	0	0.03	0.839
<i>Veronica arvensis</i> L.	A	F	1	3	2	0	0.21	0.263
<i>Vicia sativa</i> L.	A	L	1	0	0	0	0.15	0.299
<i>Vulpia ciliata</i> Dumort	A	G	4	4	4	3	0.62	< 0.001
<i>Vulpia geniculata</i> (L.) Link	A	G	4	4	4	4	0.19	0.232
<i>Vulpia muralis</i> (Kunth) Nees	A	G	1	0	1	0	0.35	0.052

The number of plots in which species occur in each of the four experimental treatments (C = control, I = summer irrigation, S = artificial shading, I × S = summer irrigation and artificial shading), the squared correlation coefficient (R²) and the statistical significance (p) of fitted values of relative species abundances onto the first two axes of the NMDS ordination are shown. p-values < 0.05 are in bold.

in association with more fertile conditions along the first NMDS axis (Fig. 3). In addition, species indicating low nitrogen concentrations (e.g. *Aira caryophylla*, *Vulpia ciliata*) were more abundant in control plots and plots subjected to shading, in

association with low soil fertility (Richard Michalet, pers. comm.).

Plots that had been subjected to summer irrigation were less heterogeneous in terms of species composition than

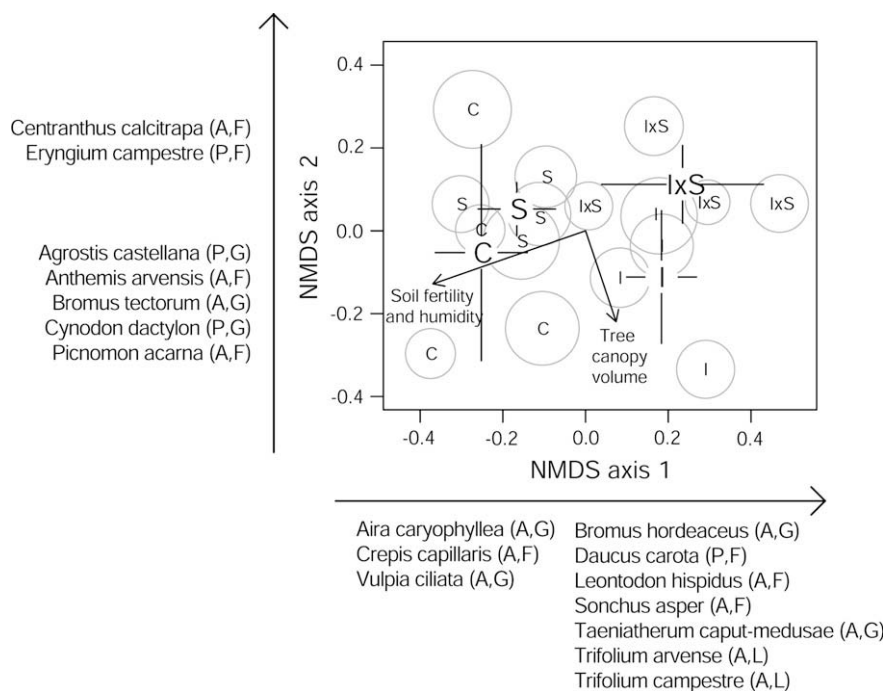


Fig. 3 – Non-metric multidimensional scaling (NMDS) ordination of herbaceous species in 16 reforested plots subjected to four combinations of summer irrigation and artificial shading (C = control, I = summer irrigation, S = artificial shading, I × S = summer irrigation and artificial shading), with four replicates per combination. Mean values and standard deviations of both NMDS axes are shown for each group as an indicator of between-plot variability. The size of the circles, representing each sampled plot, is proportional to the plot alpha diversity, as estimated by Fisher’s alpha. Next to each axis there is a list of species showing positive and negative correlation coefficients ($p < 0.05$) with the ordination axes (see Table 2 for details, A = annual, P = perennial, G = graminoid, L = legume, F = forb). Environmental vectors (tree canopy volume and soil fertility and humidity) have been fitted onto the ordination diagram (see Table 3 for details).

those that had not been subjected to irrigation, as indicated by a decrease in beta diversity. Likewise, plots subjected to both irrigation and shading displayed lower values of alpha and

beta diversity (Fig. 2), albeit the statistical significance of these relationships was weak ($p < 0.1$). Such responses were possibly related to the amelioration of harsh environmental conditions, particularly summer drought, during the establishment of plant communities. As the environmental conditions in plots subjected to irrigation were more predictable, so were the species assemblages thriving on them. This would reduce within-plot heterogeneity (beta diversity) and overall plot diversity (alpha diversity). In contrast, we did not detect a significant relationship of alpha and beta diversity with current environmental conditions. Chirino et al. (2006) showed that overall diversity of herbaceous communities was reduced in semi-arid Mediterranean grasslands and shrublands planted with *Pinus halepensis* Miller 30 years ago. We could not detect similar patterns in our study, which reveals that there are other factors operating, at least at this stage of succession, that have a larger influence on the diversity of herbaceous communities.

Initial environmental conditions also contributed significantly to explain the composition of herbaceous communities. All species that were involved in our succession study had potentially the same opportunities of dispersal into the field during community establishment. At the same time, three of the treatments applied to the planted *Quercus* seedlings contributed to ameliorate water stress and strong irradiance, or both, and therefore constituted differing prevailing environmental conditions at the time of community establishment.

Table 3 – Values of previous environmental categorical factors (summer irrigation and artificial shading) and current environmental vectors (tree canopy volume and soil fertility and humidity) fitted onto the first two axes of the NMDS ordination

Environmental variable	NMDS 1	NMDS 2	R^2	p
Factors				
Summer irrigation			0.51	<0.001
Absence	-0.21	-0.00		
Presence	0.21	0.00		
Artificial shading			0.09	0.250
Absence	-0.03	-0.08		
Presence	0.03	0.08		
Vectors				
Tree canopy volume	0.32	-0.95	0.13	0.413
Soil fertility and humidity	-0.94	-0.33	0.37	0.056
Squared correlation coefficients (R^2) and empirical p -values (p) are shown for each environmental variable. p -values < 0.05 are in bold.				

Table 4 – Summary of two-step permutation analyses of variance (PERMANOVA) used to test the effects of previous and current environments on the species composition of herbaceous communities: (Panel A) PERMANOVA with two factors (artificial shading, summer irrigation) and two covariates (tree canopy volume, soil fertility and humidity). This model allows the calculation of direct effects of previous environments on species composition and (Panel B) PERMANOVA with the same two factors and the residuals of the covariates

Source	df	SS	EV (%)	MS	F	p (perm)
Panel A						
Previous environments (direct effects)			26.3			
Artificial shading	1	1027.0	9.6	1027.0	2.246	0.023
Summer irrigation	1	1180.6	11.1	1180.6	2.582	0.009
Shading × irrigation	1	597.6	5.6	597.6	1.307	0.224
Current environments (all effects)			30.8			
Tree canopy volume	1	969.4	9.1	969.4	2.001	0.048
Soil fertility and humidity	1	2314.0	21.7	2314.0	4.898	0.000
Residual	10	4571.8	42.9	457.2		
Total	15	10,660.6				
Panel B						
Previous environments (all effects)			41.7			
Artificial shading	1	837.7	7.8	837.7	1.832	0.065
Summer irrigation	1	2829.9	26.5	2829.9	6.190	0.001
Shading × irrigation	1	775.1	7.3	775.1	1.695	0.076
Current environments (direct effects)			15.4			
Tree canopy volume	1	887.6	8.3	887.6	1.832	0.070
Soil fertility and humidity	1	758.9	7.1	758.9	1.529	0.136
Residual	10	4571.8	42.9	457.2		
Total	15	10,660.6				

This model accounts for the direct effects of the covariates on species composition. Indirect effects can be calculated as the difference between the explained variance (EV) of the covariates in Panel A and Panel B. df = Degrees of freedom; SS = sum of squares; EV = explained variance (%); MS = mean squares; F = F-statistic; p(perm) = p value based on permutations.

Environmental conditions that can favour certain species (Chesson and Huntly, 1989; Lavorel and Lebreton, 1992), coupled with some community mechanisms such as some type of competitive hierarchies (Fraser and Keddy, 2005) may have resulted in different filtered initial assemblages. Although the amelioration of adverse conditions ceased three years after community establishment, and all plots were subjected to the same environmental conditions thereafter, initial differences in species composition persisted in time. Such results are consistent with the hypothesis of initial floristic composition (Egler, 1954), which states that those species present at a particular site early in succession pre-empt the site and influence the course of succession on it for a long time.

Observed composition differences can be simply due to the effect that previous environments had upon the tree canopy and soil conditions. For this reason it was important to separate correlated effects of previous environments and current environmental conditions (tree canopy volume and soil

fertility and humidity in this study) from the direct effects of these variables on the structure of herbaceous communities. Our proposed two-step approach is suited for a thorough partitioning of these direct and indirect effects. There was a weak indirect effect of previous environments through tree canopy volume on species composition. This can be due to the fact that trees in managed plots grew faster than those in unmanaged plots during the treatment period, but their growth slowed after ceasing management (Rey Benayas and Camacho-Cruz, 2004). Therefore, although there is a direct effect of tree canopy volume on species composition (Table 4, Fig. 4), this was not mediated by the effect that previous environments had on this variable. The indirect effect of previous environments through soil fertility and humidity was always greater than the direct effect of this variable on species composition, except for perennial species, for which the indirect effect was negligible. We did not detect a clear relationship between tree canopy volume and previous environments. However, this finding must be regarded with caution because previous environments also affect other life history traits, such as root development, which could ultimately influence different soil properties. Overall, our results indicated that the influence of the tree canopy on soil fertility and humidity was more important than the direct effect of soils on the composition of herbaceous communities.

The effects of previous environments on herbaceous communities have important implications for population and community development. These effects were most noticeable in annuals, for which seed bank and species composition have been shown to be highly correlated (Levassor et al., 1990; Lavorel and Lebreton, 1992; Olano et al., 2005). We did not detect any effect of previous and current environments in perennials. For different guilds, the response to previous environments was also quite distinct. Graminaceous species did not show a significant response to previous environments, but legumes and forbs did. This might be explained by the fact that forbs and legumes, under mesic conditions, are better competitors for light than graminoids (Pérez Camacho, 2004). In addition, the legumes in our study were all annual species, and therefore the patterns observed for this guild might be mimicking the patterns observed for annuals.

The unexplained variation found in this study may be due to a combination of stochastic processes and background noise (sampling and microscale variation) and mechanisms not accounted for in this study, such as biotic interactions between species (Connell and Slatyer, 1977; Tilman, 1987; Ne'eman and Izhaki, 1996). It must also be noted that our study focussed on the fraction of species that germinated during the sampling period and, consequently, we may have missed some effects on the species germinating in autumn. Despite these limitations, our study allowed us to evaluate the imprint of specific events (e.g. summer irrigation mimics a reduction in summer drought) on the inertia of ecological communities during the first stages of their establishment. Overall, our results indicated that floristic composition in herbaceous communities is primarily explained by the effects of previous environments, and, to a lesser extent, by current environmental conditions. These results agree with studies showing the importance of environmental conditions during early establishment for long-term dynamics of plant

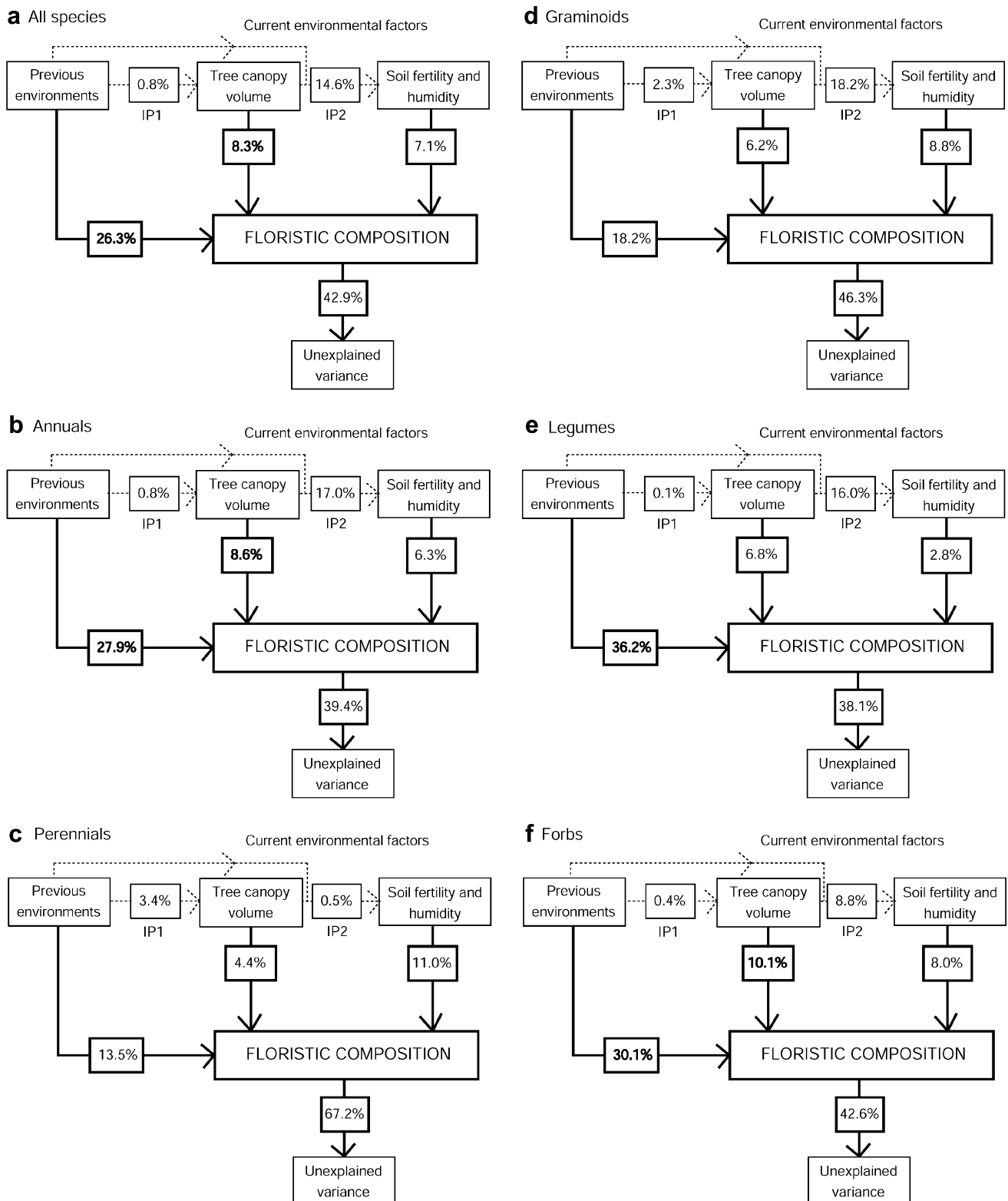


Fig. 4 – Schematic representations of the contribution of direct and indirect effects of previous and current environments to the variance of floristic composition of herbaceous communities based on PERMANOVA analyses for: (a) all species (see also Table 4 for details); (b) annuals; (c) perennials; (d) graminoids; (e) legumes; and (f) forbs (see also Web version Appendix 1 for details). Boxes with p-values lower than 0.05 are in bold.

populations in Mediterranean semi-arid regions (e.g. Escudero et al., 1999; Maestre et al., 2003), and suggest that monitoring of such conditions is crucial to predict long-term impacts of woody species on the composition and diversity of understorey species.

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Appendix I. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.actao.2008.06.006.

REFERENCES

- Allen, S.E., Grimshaw, H.M., Rowland, P., 1986. Chemical analysis. In: Moore, P.D., Chapman, S.B. (Eds.), *Methods in Plant Ecology*. Blackwell Scientific, Oxford, pp. 303–316.
- Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Aust. Ecol.* 26, 32–46.
- Anderson, M.J., 2005. PERMANOVA: a FORTRAN computer program for permutational multivariate analysis of variance. Department of Statistics, University of Auckland, New Zealand.
- Anderson, M.J., Ter Braak, C.J.F., 2003. Permutation tests for multi-factorial analysis of variance. *J. Stat. Comput. Simul.* 73, 85–113.
- Bazzaz, F.A., 1996. *Plants in changing environments: linking physiological, population, and community ecology*. Cambridge Univ. Press, Cambridge.
- Boettcher, S.E., Kalisz, P.J., 1990. Single-tree influence on soil properties in the mountains of eastern Kentucky. *Ecology* 71, 1365–1372.
- Bonet, A., 2004. Secondary succession of semi-arid Mediterranean old-fields in south-eastern Spain: insights for conservation and restoration of degraded lands. *J. Arid Environ.* 56, 213–233.
- Burriel, F., Hernando, V., 1950. El fósforo en los suelos españoles. V. Nuevo método para la determinar el fósforo asimilable en los suelos. *An. Edaf. Agrob.* 9, 611–622.
- Callaway, R.M., Walker, L.R., 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology* 78, 1958–1965.
- Chesson, P., Huntly, N., 1989. Short-term instabilities and long-term community dynamics. *Trends Ecol. Evol.* 4, 293–298.
- Chesson, P.L., Warner, R.R., 1981. Environmental variability promotes coexistence in lottery competitive systems. *Am. Nat.* 117, 923–943.
- Chirino, E., Bonet, A., Bellot, J., Sánchez, J.R., 2006. Effects of 30-year-old Aleppo pine plantations on runoff, soil erosion, and plant diversity in a semi-arid landscape in south eastern Spain. *Catena* 65, 19–29.
- Connell, J.H., Slatyer, R.O., 1977. Mechanisms of succession in natural community stability and organization. *Am. Nat.* 111, 1119–1144.
- Egler, F.E., 1954. Vegetation science concepts I. Initial floristic composition: a factor in old field vegetation development. *Vegetatio* 4, 412–417.
- Escudero, A., Iriondo, J.M., Olano, J.M., Rubio, A., Somolinos, R.C., 2000. Factors affecting establishment of a gypsophyte: the case of *Lepidium subulatum* (Brassicaceae). *Am. J. Bot.* 87, 861–871.
- Escudero, A., Somolinos, R.C., Olano, J.M., Rubio, A., 1999. Factors controlling the establishment of *Helianthemum squamatum* (L.) Dum., an endemic gypsophile of semi-arid Spain. *J. Ecol.* 87, 290–302.
- Fraser, L.H., Keddy, P.A., 2005. Can competitive ability predict structure in experimental plant communities? *J. Veg. Sci.* 16, 571–578.
- Gill, D.S., Marks, P.L., 1991. Tree and shrub seedling colonization of old fields in central New York. *Ecol. Monogr.* 61, 183–205.
- Goldberg, D.E., 1987. Neighborhood competition in an old-field plant community. *Ecology* 68, 1211–1223.
- Grether, G.F., 2005. Environmental change, phenotypic plasticity, and genetic compensation. *Am. Nat.* 166, 115–123.
- Grubb, P.J., 1977. The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biol. Rev.* 52, 107–145.
- Joffre, R., Rambal, S., 1988. Soil water improvement by trees in the rangelands of southern Spain. *Oecol. Plant.* 9, 405–422.
- Joffre, R., Rambal, S., 1993. How tree cover influences the water balance of Mediterranean rangelands. *Ecology* 74 (2), 570–582.
- Lavorel, S., Lebreton, J.D., 1992. Field evidence for lottery recruitment in Mediterranean old fields. *J. Veg. Sci.* 3, 91–100.
- Levassor, C., Ortega, M., Peco, B., 1990. Seed bank dynamics of Mediterranean pastures subjected to mechanical disturbance. *J. Veg. Sci.* 1, 339–344.
- Lortie, C.J., Brooker, R.W., Choler, P., Kikvidze, Z., Michalet, R., Pugnaire, F.I., Callaway, R.M., 2004. Rethinking plant community theory. *Oikos* 107 (2), 433–438.
- Maestre, F.T., Reynolds, J.F., 2007. Amount or pattern? Grassland responses to the heterogeneity and availability of two key resources. *Ecology* 88, 501–511.
- Maestre, F.T., Cortina, J., Bautista, S., Bellot, J., Vallejo, R., 2003. Small-scale environmental heterogeneity and spatio-temporal dynamics of seedling establishment in a semiarid degraded ecosystem. *Ecosystems* 6, 630–643.
- Magurran, A.E., 1988. *Ecological Diversity and its Measurement*. Princeton University Press.
- MAPA, 1986. *Métodos oficiales de análisis*, tomo III. Ministerio de Agricultura, Madrid.
- Maurer, D.A., Zedler, J.A., 2002. Differential invasion of a wetland grass explained by tests of nutrients and light availability on establishment and clonal growth. *Oecologia* 131, 279–288.
- McMurtrie, R., Wolf, L., 1983. A model of competition between trees and grass for radiation, water and nutrients. *Ann. Bot.* 52, 449–458.
- Metcalfe, N., Monaghan, P., 2001. Compensation for a bad start: grow now, pay later? *Trends Ecol. Evol.* 16, 254–260.
- Ne'eman, G., Izhaki, I., 1996. Colonization in an abandoned East-Mediterranean vineyard. *J. Veg. Sci.* 7, 465–472.

- Olano, J.M., Caballero, I., Loidi, J., Escudero, A., 2005. Prediction of plant cover from seed bank analysis in a semi-arid plant community on gypsum. *J. Veg. Sci.* 16, 215–222.
- Pérez Camacho, L., 2004. Dinámica de comunidades herbáceas mediterráneas: Influencia del régimen hídrico y el pastoreo. Ph.D. thesis, Universidad de Alcalá, Spain.
- Reekie, E., Parmiter, D., Zebian, K., Reekie, J., 1997. Trade-offs between reproduction and growth influence time of reproduction in *Oenothera biennis*. *Can. J. Bot.* 75, 1897–1902.
- Relyea, R.A., 2002. Costs of phenotypic plasticity. *Am. Nat.* 159, 272–282.
- Rey Benayas, J.M., 1998. Growth and mortality in *Quercus ilex* L. seedlings after irrigation and artificial shading in Mediterranean set-aside agricultural lands. *Ann. Sci. For.* 55, 801–807.
- Rey Benayas, J.M., Camacho-Cruz, A., 2004. Performance of *Quercus ilex* saplings planted in abandoned Mediterranean cropland alters long-term interruption of their management. *For. Ecol. Manag.* 194, 223–233.
- Richardson, D.M., Rejmánek, M., 2004. Invasive conifers: a global survey and predictive framework. *Div. Distribut.* 10, 321–331.
- Rosenzweig, M.L., 1995. *Species Diversity in Space and Time*. Cambridge Univ. Press, Cambridge, UK.
- Sale, P.F., 1977. Maintenance of high diversity in coral reef fish communities. *Am. Nat.* 111, 337–359.
- Silvertown, J., 2004. Plant coexistence and the niche. *Trends Ecol. Evol.* 19, 605–611.
- Tilman, D., 1987. Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. *Ecol. Monogr.* 57, 189–214.
- Topp, G.C., Davis, J.L., Annan, A.P., 1980. Electromagnetic detection of soil moisture: measurement in coaxial transmission lines. *Water Resour. Res.* 16, 574–582.
- Van Auken, O.W., 2000. Shrub invasions of North American semiarid grasslands. *Annu. Rev. Ecol. Syst.* 31, 197–215.
- Weinig, C., Delph, L.F., 2001. Phenotypic plasticity early in life constrains developmental responses later. *Evolution* 55, 930–936.
- Wu, H., Sharpe, P.J.H., Walker, J., Penridge, L.K., 1985. Ecological field theory: a spatial analysis of resource interference among plants. *Ecol. Model.* 29, 215–243.