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Microhabitat amelioration and reduced competition among understorey plants as drivers of facilitation across environmental gradients: towards a unifying framework

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Abstract

Studies of facilitative interactions as drivers of plant richness along environmental gradients often assume the existence of an overarching stress gradient equally affecting the performance of all the species in a given community. However, co-existing species differ in their ecophysiological adaptations, and do not experience the same stress level under particular environmental conditions. Moreover, these studies assume a unimodal richness-biomass curve, which is not as general as previously thought. We ignored these assumptions to assess changes in plant-plant interactions, and their effect on local species richness, across environmental gradients in semi-arid areas of Spain and Australia. We aimed to understand the relative importance of direct (microhabitat amelioration) and indirect (changes in the competitive relationships among the understorey species: niche segregation, competitive exclusion or intransitivity) mechanisms that might underlie the effects of nurse plants on local species richness. By jointly studying these direct and indirect mechanisms using a unifying framework, we were able to see how our nurse plants (trees, shrubs and tussock grasses) not only increased local richness by expanding the niche of neighbouring species, but also by increasing niche segregation among them, though the latter was not important in all cases. The outcome of the competition-facilitation continuum changed

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Supplementary Material

Supplementary data associated can be found in the online version of this article.

Appendix A. Characteristics of vegetation types and sites selected.

Appendix B. Measuring community composition changes across environmental gradients.

Appendix C. An alternative approach to measuring changes in competitive outcomes depending on microsite

depending on the study area, likely because the different types of stress gradient considered. When driven by both rainfall and temperature, or rainfall alone, the community-wide importance of nurse plants remained constant (Spanish sites), or showed a unimodal relationship along the gradient (Australian sites). This study expands our understanding of the relative roles of plant-plant interactions and environmental conditions as drivers of local species richness in semi-arid environments. These results can also be used to refine predictions about the response of plant communities to environmental change, and to clarify the relative importance of biotic interactions as a driver of such responses.

Keywords

competitive exclusion model; niche expansion; niche segregation; plant-plant interactions; richness-biomass relationship; stress-gradient hypothesis

Introduction

The study of the mechanisms controlling the composition of plant communities has been a major topic in ecology since the early days of this science (see Gotelli and Graves, 1996 for a review). Recent research suggests that local-scale, non-random processes such as abiotic constraints and biotic interactions, determine the species that are able to successfully colonize a given environment (Holzapfel and Mahall, 1999; Huston, 1999; Lortie et al., 2004a; Rajaniemi et al., 2006; Gotelli et al., 2010). Pioneering studies have suggested that, among local-scale processes, competition regulates species richness at high levels of productivity, while limited physiological tolerances to abiotic stress or disturbances reduce species richness at low productivity levels (Grime, 1973; Huston, 1979). Overall, the combined effects of abiotic constraints and competition should generate a hump-shaped relationship between species richness and local productivity, which is reputed to be ubiquitous in nature (Grime, 1973; Huston, 1979). However, several studies question the generality of the hump-shaped richness-biomass relationship, particularly at both local and regional scales (Grace, 1999; Waide et al., 1999; Gillman and Wright, 2006; Whitakker, 2010). These discrepancies may relate to the fact that plant-plant interactions are key drivers of community structure not only in high biomass/productivity environments, as assumed by the hump-shaped model, but also in low biomass/productivity environments (Tilman, 1988; Callaway, 2007). In spite of this controversy, ecologists have only considered the hump-shaped richness-biomass relationship to explore the potential effects of positive, non-trophic interactions (hereafter ‘facilitation’; Hacker and Gaines, 1997; Michalet et al., 2006). It remains uncertain, therefore, how the relative effects of plant-plant interactions on species richness change along environmental gradients, and how such interactions influence the richness-biomass relationship (Rajaniemi et al., 2006).

Previous studies addressing the importance of facilitation in maintaining community richness (e.g., Hacker and Gaines, 1997; Michalet et al., 2006) have focussed mainly on the environmental buffering promoted by nurse plants at low to moderate biomass levels as the key mechanism underlying the increase in species richness promoted by facilitation. In addition to microclimatic amelioration, protection from herbivory by nurse species can also

increase the habitat available for the less adapted species, and therefore increase local richness (microhabitat amelioration). Although the relationship between the importance and frequency of positive plant-plant interactions and abiotic/biotic stress was originally thought to be monotonic and positive (Bertness and Callaway, 1994), some studies have suggested a waning of positive interactions under either extreme abiotic stress levels or intense herbivory (e.g. Kitzberger et al., 2000; Ibañez and Schupp, 2001; Smit et al., 2007), thus obviating microhabitat amelioration or “*niche expansion*” (Bruno et al., 2003; Michalet et al., 2006). Explanations invoked to explain this lack of microhabitat amelioration under such conditions are: 1) the competitive effects of nurse plants may outweigh the positive effects of environmental amelioration, particularly when abiotic stress is promoted by scarcity of resources such as water (Tielbörger and Kadmon, 2000a; Maestre and Cortina, 2004; Maestre et al., 2009), and 2) nurse plants may not be able to ameliorate harsh abiotic conditions sufficiently to promote plant recruitment in extremely stressful environments (Michalet et al., 2006; Forey et al., 2009; Soliveres et al., *in press*). We henceforth refer to both of these potential mechanisms as *facilitation waning models*.

Although previous research describing facilitatory mechanisms in relation to the diversity-biomass relationship have focused on low to moderately-low biomass environments, other studies have revealed that ecological processes such as niche segregation (Silvertown, 2004), competition intransitivity (lack of hierarchy in competition networks: Gilpin, 1975), and indirect facilitation (Levine, 1999; Brooker et al., 2008) may be key mechanisms enhancing species richness in more productive conditions. Changes from a marked competitive hierarchy in plant interaction networks to a less marked hierarchy (intransitivity), or higher niche segregation scenarios, can have important implications for the maintenance of plant species richness due to the reduction of competitive exclusion (Silvertown, 2004; Laird and Schamp, 2006). It is known that both mechanisms (niche segregation and competition intransitivity) might be enhanced due to an increase in the spatio-temporal heterogeneity of resources or to a higher species pool (Tilman, 1994; Grace et al., 1993; Bowker et al., 2010). The more benign conditions often found under nurse plants (Franco and Nobel, 1989) may not only increase the local species pool because of microclimatic amelioration, but also augment the variability of available niches by providing a higher degree of heterogeneity in the availability of light, nutrients or mycorrhizas in comparison with open areas (Pugnaire et al., 1996; Zabinsky et al., 2002; Maestre and Cortina, 2005). Although largely ignored in facilitation research (Callaway, 2007; Brooker et al., 2008; but see Tielbörger and Kadmon, 2000b), these localized effects may potentially increase niche segregation and competition intransitivity beneath nurse plant canopies, thereby reducing competitive exclusion among understory plants and enhancing overall local richness and productivity.

Despite the interest generated in the combined effects of nurse plants on both microhabitat amelioration and changes in competitive outcomes in order to understand the role of plant-plant interactions as a driver of plant community diversity, these mechanisms have largely been explored separately in the literature. There is a clear need, therefore, to develop a unifying framework that integrates our understanding of the roles of microhabitat amelioration and changes in interaction networks when explaining the effects of plant-plant

interactions on local species richness (Callaway, 2007; Brooker et al., 2008). Furthermore, previous research on facilitation considers environmental gradients as a surrogate of the stress level for all the species within a community (Lortie et al., 2004a; Travis et al., 2005; Michalet et al., 2006). This may however be a problematic assumption, as the degree of stress under particular environmental conditions depends on the particular adaptations of each species. Stress is a concept that is applicable to individual species not whole communities (see Körner, 2003, 2004; Lortie et al., 2004b; for a discussion). In this study, we aimed to jointly study two key mechanisms underlying plant facilitation (microclimate amelioration and changes in competitive networks) without the assumptions of the existence of an overarching stress gradient or a unimodal richness-biomass relationship. To do so, we examined the effects of nurse plants on local richness and surrogates of biomass across environmental gradients in semi-arid environments in Australia and Spain. We selected these two regions because they have contrasting vegetation communities and management histories, and thus can help us to test the generality of our hypotheses and results. Our approach quantified empirically the two main processes by which plant-plant interactions may affect local diversity: 1) habitat increase for less adapted species due to microclimate amelioration, and 2) changes in the outcome of competitive interactions among species beneath nurse canopies. We also included in this framework an assessment of changes in the intensity, importance and frequency of positive interactions on community-level plant richness and biomass along wide environmental gradients. We tested the following hypotheses: 1) nurse plants will enhance local species richness via microhabitat amelioration and changes in the competitive networks (niche segregation and/or competition intransitivity) of their understorey vegetation; and 2) There is no overarching stress-gradient affecting equally all the species in a given community, as is often assumed. Rather, different species co-occurring in a community will differ in their relative tolerances to given environmental conditions. Hence, the intensity, importance and frequency of facilitation at the community level, and therefore microhabitat amelioration, will remain constant across stress gradients as the identity, but not the amount, of facilitated species changes along such gradients (Greiner La Peyre et al., 2001; Gross et al., 2010).

Methods

Study area

Two semi-arid regions were selected for this study: one located in the steppes of central and south-eastern Spain, and the other in open eucalypt woodlands of south-eastern Australia. We surveyed 11 sites in Spain along a climatic gradient ranging from 13°–17° C mean annual temperature and 273–488 mm average annual rainfall, and 10 sites ranging from 16°–19° C and 280–630 mm in Australia. Plot selection included the full range of environmental conditions existing within the distribution of both vegetation types in the countries studied, as recommended to test the relationships between plant-plant interactions, community processes and environmental gradients (Brooker et al., 2008; Lortie, 2010). Within each country, the selected plots shared the same soil type and current management, and were located on landscapes of similar aspect and slope to minimize any experimental noise that could potentially influence the effect of climatic conditions on the stress experienced by the different plant species forming the community (see Appendix A for a

detailed description of the study sites). The Spanish sites were located on soils derived from limestone. Vegetation was in all cases an open steppe (mean cover ranged from 35 to 68%) dominated by the perennial tussock grass *Stipa tenacissima* L., with sparse resprouting shrubs such as *Quercus coccifera* L., *Pistacia lentiscus* L. and *Rhamnus lycioides* L. Sites in south-eastern Australia were open woodlands located on clay loam soils. Canopy cover, which ranged from 18-70%, was dominated by *Eucalyptus populnea* F. Muell., *E. intertexta* R. T. Baker, *E. microtheca* F. Muell., *Geijera parviflora* Lindley and several shrub species (*Eremophila mitchellii* Benth, *E. sturtii* R. Br., *Dodonaea viscosa* (L.) Jacq. Chapulixtle, *Acacia* spp. and *Senna* spp.).

Vegetation survey

At each site, we established a 30 m × 30 m plot containing the representative vegetation of the surrounding area. This plot size permitted the inclusion of several grass, shrub and tree patches within this area, and was large enough to conduct the facilitation surveys described below. Within each plot we established four 30 m long transects, located 8 m apart, down the slope for the vegetation survey. Along each transect we placed 20 contiguous 1.5 m × 1.5 m quadrats, and recorded the cover and abundance of all perennial plant species within the quadrat. Clonal ramets were considered independent individuals when growing more than 3 cm apart. These data provided us with a presence/absence matrix of 80 cells (four transects by 20 quadrats) for each plot. The total cover of each plot, which has been shown to be a good surrogate of biomass in semi-arid environments (Flombaum and Sala, 2009), was derived from the average cover of perennial plants across the 80 quadrats. This survey was used to examine differences in community composition by using the frequency of each species in the 80 quadrats (see Appendix B for a detailed description of this analysis).

Facilitation survey

Because of differences in vegetation physiognomy, we conducted facilitation surveys using slightly different protocols in Spain and Australia. In each plot located in Spain, ten *Stipa* tussocks (hereafter *Stipa* microsite) were randomly selected, and the total area under their canopies sampled with 0.5 m × 0.5 m quadrats. The abundance and cover of all perennial plant species were recorded within each quadrat. Since most of the species in the studied areas have canopies smaller than the 0.5 m × 0.5 m area, we believe that this is an appropriate size to evaluate species co-occurrence on a distance close enough to ensure that the species found were interacting somehow. Ten paired open areas, located at least 1 m from any *Stipa* tussock or resprouting shrub (hereafter Open microsite), were randomly selected adjacent to these tussocks. We balanced our sampling effort among microsites by sampling the same area (i.e. the same number of 0.25 m² quadrats) of Open and *Stipa* microsites. Finally, we sampled the same area under the canopies of five *Quercus coccifera* (or another resprouting shrub when *Q. coccifera* was absent from the plot; hereafter Shrub microsite). In each one of these shrubs, we sampled an average of six quadrats, equivalent to the sum of the quadrats sampled in the two closest *Stipa* tussocks. This number ranged from 4 to 12, depending on the size of the tussock sampled. These quadrats were randomly placed underneath each shrub.

For the Australian plots we sampled three different microsites: Open, Shrub and Tree. Shrub microsites were represented as inverse cone-shaped shrubs (*sensu* Whitford, 2002) such as *Eremophila mitchellii*, *Dodonaea viscosa*, *Senna artemisioides* (Gaudic. ex DC.) Randell, or juvenile *Callitris glaucophylla* J. Thomps. & L.P. Johnson, depending on the species present in each plot. Our sampling protocol changed slightly depending on the canopy size of shrubs. Where the canopy was sufficiently large, we sampled six 0.5 m × 0.5 m quadrats under each of five shrubs. Where shrubs were smaller, a larger number of shrubs was sampled in order to sample a total of 30 quadrats. Paired Open microsites (> 1 m from any shrub or tree) were also sampled adjacent to these shrubs to yield the same sampling area. Finally, we sampled the same number of quadrats under the Tree microsites, which were represented by different species of eucalypts (*E. populnea*, *E. intertexta*, *E. microtheca*), *Casuarina pauper* F.Muell. ex L.A.S. Johnson or *Geijera parviflora*. Because of the large area occupied by these tree species (up to 200 m² for some *Eucalyptus* trees), we sampled only three trees in each plot, with 10 randomly placed quadrats sampled underneath each one. Although the tree or shrub species sampled changed among different plots across the gradient studied, we sampled the same species of shrubs and trees within each plot, to avoid the confounding effect of sampling different species using such a low number of sampled individuals (five or three, depending on the microsite). The canopy area of all shrubs and trees was calculated based on the area of a circle. To have an estimation of the area of each nurse plant, we averaged the canopy area of all the individuals of each microsite within a plot, this gave us an estimation of how the size of each nurse was affected by local environmental conditions. For both Spain and Australia, plot-level richness was calculated by summing up the total number of different species found in the 0.5 m × 0.5 m ($n = 30$), and 1.5 m × 1.5 m ($n = 80$) quadrats sampled. We acknowledge that the different number of individuals sampled for each microsite could affect somehow our results. However, we feel that this is unlikely because we averaged all the metrics at the plot level to do our calculations and analyses (see below). Since the focus of this study was the community level, all of these calculations were based on the ~30 quadrats sampled for each microsite and plot as a whole, considering them as a representative sampling for each microsite-type, rather than being separated by each individual sampled within each microsite.

Statistical analyses

REDUCTION OF CLIMATIC DATA—Six climatic variables (annual radiation, minimum, maximum and mean temperature, and rainfall, and temperature range [maximum–minimum temperature]) were collected for each site using available climatic models in Spain (Ninyerola et al., 2005), and data from the Bureau of Meteorology in Australia (www.bom.gov.au). Since the focus of the paper was to understand how plant-plant interactions behave along climatic (abiotic) gradients, the effect that the canopy of the plants (a biotic component) may have had on either incident radiation or any other climatic variable was not included in these environmental measures. We reduced these climatic variables to a single synthetic variable for each country using PCA. Summarizing environmental variables in a PCA allowed us to obtain a more general assessment of the influence of all of our environmental variables at both community and species-specific levels. This approach is highly recommended for testing relationships between plant-plant interactions and abiotic stress along environmental gradients (Lortie, 2010). We used the

first PCA axis as our surrogate for the climatic gradient present at our sites in both countries (hereafter referred to as *climate*). This axis explained 88.6% (Eigenvalue = $8.08 \cdot 10^3$) and 86.2% (Eigenvalue = $1.07 \cdot 10^4$) of the variance in the climatic data for Spain and Australia, respectively. This axis was highly correlated with rainfall and radiation in Spain (Eigenvectors = -0.864 and 0.502 for rainfall and radiation, respectively; the remainder of the eigenvectors were < 0.03 in all cases) but only with rainfall in Australia (Eigenvector = 0.996 ; the rest of eigenvectors were < 0.1 in all cases). Principal Component Analyses were carried out in Primer v. 6 statistical package for Windows (PRIMER-E Ltd., Plymouth Marine Laboratory, UK).

We evaluated the relationships between *climate* and both cover and species richness at the plot level, and the relationship between cover and richness, using both linear and quadratic regressions. Regression analyses were carried out using SPSS 13.0 for Windows (Chicago, Illinois, USA).

ASSESSING BIOTIC INTERACTIONS—Because plant-plant interactions cannot be measured in a simple way (Maestre et al., 2005; Brooker et al., 2005), we applied three complementary indices to quantify their intensity, importance and frequency. The intensity and importance of plant-plant interactions were assessed using both plant richness and cover. To measure the intensity of the interactions, i.e., the effect that neighbours have on species richness and cover irrespective of other environmental factors (Brooker et al., 2005), we used the Relative Interaction Index (RII; Armas et al., 2004). This index is calculated for each microsite pair as $(P_{\text{Nurse}} - P_{\text{Open}})/(P_{\text{Nurse}} + P_{\text{Open}})$, where P_{Nurse} is either mean cover or mean species richness under the canopy of a nurse plant (*Stipa*, Shrub or Tree microsites) and P_{Open} is either mean cover or mean species richness in the Open microsite. This index has statistical properties which make it suitable for comparing the intensity of plant-plant interactions across environmental gradients; it has defined limits ($-1, +1$), is symmetrical around zero, and has identical absolute values for competition and facilitation. It is also linear, unbiased at low interaction intensity, and has no discontinuities in its range (Armas et al., 2004). For each plot we calculated the mean index obtained from all the Nurse-Open microsite pairs sampled.

To assess the importance of plant-plant interactions, i.e., the relative effect that *Stipa*, shrubs and trees had on richness and cover compared to that of other environmental factors (Brooker et al., 2005), we used the Interaction Importance Index (I_{imp} ; Seifan et al., 2010). It has similar statistical properties to RII, and is therefore comparable among sites located across the environmental gradients sampled. This index is calculated as $I_{\text{imp}} = N_{\text{imp}} / (|N_{\text{imp}}| + |E_{\text{imp}}|)$, where N_{imp} and E_{imp} are the nurse plant and environmental contributions to species richness or total cover, respectively. N_{imp} is calculated as $P_{\text{Nurse}} - P_{\text{Open}}$, and E_{imp} as $P_{\text{Open}} - MP_{\text{Open/Nurse}}$, where $MP_{\text{Open/Nurse}}$ is the maximum value of species richness or mean cover found in the entire gradient, irrespective of the microsite sampled.

Finally, the frequency of positive interactions was measured as the percentage of either *facilitation obligates* and *facilitation beneficiaries* (*sensu* Butterfield, 2009), as a percentage of the total species pool of each plot. We considered as facilitation obligates those species

found only under the canopy of a given nurse plant but not in the Open microsites (regardless of the identity of the nurse plant), while facilitation beneficiaries were species with more individuals growing under the canopy of a nurse than in the Open microsites. We used the number of recruited individuals because seedling germination and establishment, particularly during the first year after germination, are known to be the principal bottlenecks in plant recruitment in semi-arid environments such as those sampled (Eldridge et al., 1991; Escudero et al., 1999; Maestre et al., 2001). Thus, we believe that a higher number of individuals recruiting in a given microsite is indicative of superior environmental conditions experienced in this microsite for a given species, an approach followed by previous studies on the topic (e.g., Valiente-Banuet et al., 2006).

Previous facilitation research predicts that the frequency (Bertness and Callaway, 1994) or importance (Brooker et al., 2005) of positive interactions is monotonically and positively related to abiotic conditions. Other studies suggest that these plant-plant interaction indicators follow a unimodal relationship with abiotic stress, indicating a collapse of facilitation at both extremes of the stress gradient, which may be or not mediated by the size of nurse plant canopies (the facilitation waning models; Maestre and Cortina, 2004; Michalet et al., 2006; Maestre et al., 2009). In order to test the validity of these models, the intensity, importance and frequency of plant-plant indicators were plotted against *climate*, and their relationships were tested with both linear and quadratic regressions. In the case of frequency, we calculated the percentage of obligate and beneficiary species for each microsite and country separately, and then for the whole community (including the two different microsites sampled in each plot). Hereafter, we will refer to “community” throughout this paper as the plant species assemblage found in each 30 × 30 m plot. This battery of approaches provided us with a complete assessment of the relationships between plant-plant interactions and abiotic conditions.

MEASURING CHANGES IN PLANT-PLANT INTERACTION OUTCOMES DEPENDING ON MICROSITE

—We aimed to assess the effect of nurse plants on the competitive outcomes of their understorey species by increasing or decreasing competitive intransitivity or niche segregation among them. Conducting empirical tests of the degree of intransitivity or niche segregation in a community is exceptionally difficult because it would require measuring the competitive ability or the different niches of resource uptake for every species in a community, which can also change along environmental gradients (Grace et al., 1993; Huston, 1999). Henceforth, we attempted to measure changes in competition intransitivity or the degree of niche segregation in the community by using null models of guild structure based on patterns of species co-occurrence (Gotelli and Graves, 1996; Gotelli et al., 2010) and their relationship with the plot-scale richness of species found in each microsite. These null models are organized *a priori* by groups of ecological significance, and allow testing of the role of competition in structuring the community within each of these groups separately (Gotelli and Graves, 1996). In our case, we were interested in see how the C-score index changed among the different microsites studied, so these microsites were our *a priori* guilds. These null models are useful to measure the degree of niche segregation in the community (more niche segregation, higher level of co-occurrence), but are unable to disentangle the degree of competition intransitivity. Both competition intransitivity and

transitivity render the same small-scale co-occurrence pattern: less co-occurrence due to small-scale competitive exclusion. However, is easy to differentiate between both types of competitive networks when analyzing jointly this increase in small scale competitive outcomes and local richness. While networks with a marked hierarchy in the competition have a few dominant species and, therefore, a reduced local richness, intransitive ones generate a high species turnover and therefore, a high local richness (Laird and Schamp 2006).

For co-occurrence analyses, we organized our presence/absence data (obtained from the 0.5 m × 0.5 m quadrats) by microsite guilds. Specifically, we calculated species co-occurrence (C-score index, explained below) independently for the Stipa, Shrub and Tree microsities by pooling all the sampled quadrats of each microsite within each plot ($n = 30$), obtaining one unique value per microsite and plot. Most of the species sampled in Spain and Australia are small shrubs or grasses, and therefore the quadrat size used is particularly suitable for including interactions among these species without including random co-occurrence or exclusions not related to competition among them. We estimated species co-occurrence with the C-score index, a metric commonly used in this kind of analyses (e.g. Dullinger et al., 2007; Rooney et al., 2008; Bowker et al., 2010). This index is calculated for each pair of species as $(R_i - S)(R_j - S)$, where R_i and R_j are the number of total occurrences for species i and j , and S is the number of quadrats in which both species occur. This score is then averaged over all possible pairs of species in the matrix (Gotelli, 2000). The C-score is related to the competitive exclusion concept of “*checkerboardness*” i.e., how many of the possible species pairs in a given community never appear in the same quadrat together. Thus, positive and large values of this index indicate that competition may be the prevalent mechanism determining the co-occurrence patterns observed (Gotelli, 2000).

To determine the strength of co-occurrence in a sample, the observed C-score value is compared against a set of null models which serve as a baseline for what a community unstructured by species interactions would look like (Connor and Simberloff, 1979). Furthermore, since the values of the C-score are based upon presence/absence matrices, this value is only sensitive to changes in interspecific co-occurrence patterns (our surrogate of biotic interactions), and completely independent of the existence of intraspecific interactions. As the values of the C-score are dependent on the number of species and co-occurrences observed within each plot, we obtained a standardized effect size (SES) as $(I_{obs} - I_{sim})/S_{sim}$, where I_{obs} is the observed value of the C-score, and I_{sim} and S_{sim} are the mean and standard deviation, respectively, of this index obtained from the n simulations performed (Gotelli and Entsminger, 2006). We used ‘fixed rows–equiprobable columns’ null models and 5000 simulations. With this approach, we conserve the abundance of each species (rare species remained rare and common species remained common), and each quadrat was assumed to have the same probability of being colonized, regardless of the number of species found in each quadrat, during the simulations. This null model has been recommended for standardized samples (i.e. data collected in quadrats of the same size) collected in homogenous habitats (i.e. each separated microsite; Gotelli, 2000), such as the ones gathered in this study. We also used the “fixed rows–fixed columns” algorithm (both species and quadrats conserved their relative abundance and richness, respectively) to add

confidence to our conclusions. The results obtained with this analysis were very similar to those obtained with the ‘fixed rows–equiprobable columns’ null model, and thus are not shown.

Standardized Effect Size (SES) values of the C-score less than or greater than zero indicate prevailing higher co-occurrence (spatial aggregation) or lower co-occurrence (spatial segregation) among the species within a community, respectively. To assess the extent to which changes in competitive outcomes affect local diversity, we compared the SES obtained with the plot-level richness found in each microsite (hereafter ‘plot richness’). If we analyze the effects of nurse plants on SES and plot richness separately, there are four possible responses: 1) Nurse plants have a joint effect reducing SES and increasing plot richness compared to Open microsites: these plants promote the development of understorey/ neighbour species *via* niche segregation (reduced SES indicates reduced competition), and this has positive effects on the overall plot-scale richness; 2) Nurse plants increase both SES and plot richness: these plants increase quadrat-scale competition, but species with competitive advantage vary among quadrats, generating a high species turnover, and therefore increasing plot-scale richness (intransitivity); 3) Nurse plants increase SES and reduce plot richness: competitive exclusion is the dominant interaction between understorey species, and a smaller set of competition winners dominate all quadrats, which reduce plot richness; and 4) Nurse plants do not affect SES, regardless of their effects on plot richness: changes in the competitive outcomes are not an important factor modulating the effect of nurses on plot-scale richness.

Differences in SES and plot richness values obtained among microsites were compared using separate one-way ANCOVAs for each variable. In these models, microsite (Open, Stipa and Shrub – Spain; Open, Shrub and Tree –Australia) was introduced as a fixed factor, and mean plot cover (our surrogate of overall site biomass) was used as a covariate. Standardized Effect Size data were $(x+1)$ transformed to meet the assumptions of ANCOVA analysis. Tukey’s HSD *post-hoc* tests were used to assess differences among the three microsites of each country. We tested for relationships among the residuals of the ANCOVA and nurse plant canopy area using Spearman correlations. This was necessary in order to assess the importance of nurse size, as we could not use nurse size as a covariate in our model because Open microsites do not have a size, and the relationships between nurse area and their effect on SES and plot richness might not necessarily be linear. Null model analyses were conducted with Ecosim 7.22 (Gotelli and Entsminger, 2006). ANCOVA analyses were carried out using SPSS 13.0 for Windows (Chicago, Illinois, USA). See Appendix C for methodological details and results of an alternative approach.

ASSESSMENT OF MICROHABITAT AMELIORATION—We calculated the Chao-Jaccard abundance-based similarity index (hereafter *Chao index*; Chao et al., 2005) to assess the relative role of microhabitat amelioration by nurse plants on community species richness. This index is based on the probability that two randomly chosen individuals, one from each of two samples (referred to as a “pair”), belong to species shared by both samples. It takes into account not only the number of shared species among different microsites, but also differences in their relative abundances (Chao et al., 2005). We assume that, as the influence of a given nurse plant increases, more species should be present by means of

microhabitat amelioration, or because these species are more abundant due to the microenvironmental amelioration in comparison with Open microsites. We calculated the *Chao index* at the plot level by summing over the number of individuals and species recorded in all quadrats for a given microsite within each plot. Thus, the higher the dissimilarity among nurse microsites and open areas in a given plot, the higher the effect of niche on the plot species richness. Differences in the *Chao index* between microsite *pairs* (Stipa/Shrub vs. Open, Shrub/Tree vs. Open, Stipa/Shrub vs. Shrub/Tree) within each country were compared with one-way ANOVA, with microsite *pair* as fixed factor. Tukey's *post-hoc* HSD tests were used to assess significant differences among pairs. After conducting this analysis, and to assess the influence of *climate* in the effect promoted by nurse plants on their understorey populations, we evaluated the relationship between *climate* and *Chao index* using both linear and quadratic regressions. With both approaches we can adequately evaluate the differences in the understorey populations between each microsite (each nurse-type may have different effects on a given target plant, and this may translate into a high dissimilarity, not only between Nurse/Open microsites, but also between different nurses), and to account for the possible non-linear relationships between *climate* and the effects of the different nurse plants tested on their understorey vegetation. The *Chao index* was calculated using EstimateS 8.2.0 for Windows (Colwell, 2000; <http://viceroy.eeb.uconn.edu/estimates>). ANOVA and correlation analyses were carried out using SPSS 13.0 for Windows (Chicago, Illinois, USA).

Results

Diversity-biomass relationships and the effect of climate

A total of 96 and 131 perennial species were found in Spain and Australia, respectively, with a plot-level richness ranging from 9 to 47 in Spain, and from 16 to 51 in Australia. The number of species found in each plot was linearly and negatively related to mean plot cover in Spain (Fig. 1A). Maximum richness was found near the centre of the climatic gradient, under conditions of both moderate drought and medium radiation levels (Fig. 1C). In contrast, richness was largely independent of either cover or *climate* (the first axis of the PCA ordination of the climatic variables) in Australia (Figs. 1B and 1D). Mean cover at the plot level showed a monotonic and positive increase with *climate* in both Spain and Australia, reaching its maximum value in the more mesic (maximum rainfall and/or lowest radiation levels) plots.

Plant-plant interactions and abiotic stress

While neither intensity nor importance of plant-plant interactions were related to abiotic conditions in Spain, we detected a hump-shaped relationship between most RII and Iimp values and rainfall for the Australian sites (Fig. 2). Both the percentage of facilitation beneficiaries and obligates tended to decrease with rainfall. This negative trend was found to be significant for facilitation beneficiaries in Spain and for obligates in Australia (Fig. 3). The percentage of plants with more individuals under the canopy of any nurse plant than in Open microsites (facilitation beneficiaries) decreased from about 50% in the drier and warmer sites to about 30% in the wetter sites for Spain. This relationship was different, however, for Australia, where facilitation obligates followed a unimodal hump-shaped

relationship with *climate*. At the community level, only 10% of species required a nurse plant to occur at the wettest sites, but this percentage increased up to 40% in the driest sites, and showed a maximum (*ca.* 60%) at the middle of the environmental gradient (Fig. 3). When we tested the relationship between frequency of positive interactions of each nurse plant and *climate* separately, we found different results depending on the nurse plant analyzed in both regions. We detected a similar relationship with *climate* at the community level for Spanish Shrub microsites and Australian Tree microsites, but there were no significant relationships for *Stipa* (Spain) or Shrub (Australia) microsites. There was a marginally significant linear relationship between nurse area and the percentage of the species growing under *Stipa* ($R^2 = 0.34$; $P = 0.061$); the same relationship for Shrub microsites was not significant. Relationships among nurse size and interaction indices were not significant for any microsites in Australia.

Competitive exclusion, as indicated by SES values, was lower under the canopy of both *Stipa* and Shrub microsites than in Open microsites along the entire Spanish gradient (Table 1). Tukey's HSD post-hoc tests revealed differences between *Stipa* and Open ($P = 0.028$), and between Shrub and Open ($P = 0.015$) microsites, but not between *Stipa* and Shrub microsites ($P = 0.961$). Mean plot cover did not affect SES results, but when analyzing the effects of cover and microsite on plot-level richness, we found a significant effect of cover and a marginal positive effect of microsite (Table 1). However, this positive effect decreased substantially with mean plot cover (Spearman correlation between residuals of the ANOVA and mean plot cover = -0.78 ; $P < 0.0001$). Nurse size showed no relationship with the residuals of the ANCOVA models fitted with both SES and plot richness in Spain or in Australia. Nurse plants significantly increased plot richness in Australia. While the highest richness was found in Shrub microsites (Tukey HSD for Shrub and Open microsites: $P = 0.029$), this effect was less marked in Tree microsites (Tukey HSD: $P = 0.093$ for Tree vs. Open microsites). However, differences in competitive exclusion among microsites, as measured with SES, were not found in this country (Table 1). In contrast to the results found in Spain, mean plot cover did not modify the effect of microsite on SES or plot richness in Australia (Table 1).

Microhabitat amelioration and the effect of nurse plants on their understorey populations

Although the similarity index was slightly lower for Shrub vs. Open microsites (0.64 ± 0.08 , mean \pm SE, $n = 11$) than for *Stipa* vs. Open microsites (0.74 ± 0.08), we did not find significant differences in the similarity index among nurse microsites in Spain (*Stipa* vs. Shrub, 0.68 ± 0.06 , $n = 11$; ANOVA: $F_{2,29} = 0.622$; $P = 0.54$). The understorey community composition of both Shrub and *Stipa* was more similar (70%) to each other than those found in Open sites. Significant differences in similarity among microsite pairs were found, however, in Australia ($F_{2,26} = 4.57$; $P = 0.02$), suggesting some degree of microhabitat amelioration. The community composition between Shrub-Open or Shrub-Tree pairs were also very similar (60% of similarity), though the similarity index for Tree-Open microsites was markedly lower (40%; Tukey HSD *post-hoc* test; $P = 0.034$, 0.710 and 0.171 for Open-Shrub vs. Open-Tree, Open-Shrub vs Shrub-Tree, Open-Tree vs Shrub-Tree pairs, respectively). Similarity among the microsites tested did not show any relationship with *climate* in Spain, but two of the three indices calculated showed a quadratic relationship with

this variable in Australia (Fig. 4), suggesting a trend of higher increasing similarity at both ends of the climatic gradient.

Discussion

We present a unifying framework that considers all key mechanisms driving the effects of facilitative interactions on local species richness i.e. microhabitat amelioration and changes in the interaction network of the understorey vegetation. Moreover, to our knowledge, our analyses provide one of the first rigorous tests of the Stress Gradient Hypothesis (SGH) within the context where it was originally formulated (i.e. the *frequency* of positive interactions at community level increases with abiotic harshness; Bertness and Callaway, 1994). Our results indicate that nurse plants increase local richness by expanding the niche of less-adapted species (Fig. 3), and by promoting the coexistence of understorey species through niche segregation (Table 1). Our study suggests that the effects of plant-plant interactions on diversity extend across entire biomass or environmental gradients (discussed below) and are not restricted to locations at mid to low levels of biomass (*sensu* Hacker and Gaines, 1997; Michalet et al., 2006). Although this hypothesis deserve further experimentation, this could explain why facilitation operates even in very productive environments (Vieria et al., 1994; Rigg et al. 2002) and is readily apparent once we question the notion of an overarching stress gradient that affects all species in a given community. The importance of facilitation along environmental gradients seems therefore to be largely independent of the degree of ‘stress’ represented by the gradient. Rather, it depends on the number of, and interrelationships among, different stressors driving such a gradient and the ability of those species within the community to deal with these stressors.

Plant-plant interactions along environmental gradients

The negative trend found in the frequency of positive interactions with increasing rainfall in Spain (related significantly to cover and therefore biomass) provides support for the original predictions of the SGH (Bertness and Callaway, 1994; Fig. 3). However, at the Australian sites, which experienced higher levels of environmental harshness (plot cover declined to 17% in some cases; see Appendix A), positive effects of nurses on community richness waned at the highest water stress levels, supporting facilitation waning models (Michalet et al., 2006; Maestre et al., 2009). The latter result is consistent with studies from other water-limited environments (Kitzberger et al., 2000; Maestre and Cortina, 2004; Anthelme et al., 2007), and suggests that the frequency of facilitative interactions will decrease at sites with conditions harsh enough to prevent recruitment of most species, even under the canopy of nurse plants.

Our results also demonstrated inconsistent relationships among environmental stress and both interaction intensity and importance, contrary to prevailing facilitation theory (Brooker et al., 2005; Callaway, 2007). In Spain, neither interaction intensity nor importance were related to the environmental gradient we evaluated, but a hump-shaped relationship for both facilitation/competition indicators was found in Australia (Fig. 2). How can we account for this difference? Environmental stress along the Spanish gradient was driven by two negatively correlated and distinct stressors; water and radiation. Thus, it is likely that in the

different extremes of this gradient, plants less well adapted to local conditions would benefit from nurse canopies, respectively (Choler et al., 2001; Liancourt et al., 2005; Gross et al., 2010). This may explain why the net positive effects of nurse plants were equally intense or important at the community level along the entire gradient (Maestre et al., 2005). Indeed, this is suggested by the fact that the proportion of facilitation obligate species was not significantly related to *climate* in this region. The higher number of facilitation beneficiary species under drier conditions could be related to the fact that germination and recruitment of most semi-arid species are limited by periods of adequate soil moisture, irrespective of physiological adaptations or environmental optima (Westoby, 1978/79). These periods are more easily achieved under the more shaded conditions beneath nurse plants than in Open microsites (Franco and Nobel, 1989). Higher levels of soil moisture and shading are typical of the environment under the canopy of the nurse plants we studied, in comparison with bare ground areas (Moro et al., 1997; Maestre et al. 2003; Warnock et al., 2007; Howard et al., *unpublished data*). Therefore, under drier environmental conditions we would likely record more individuals of most species on the less water-stressed situation found under the canopy of nurse plants (Kitzberger et al., 2000).

Conversely, the environmental stress in the Australian gradient was predominantly driven by a single stressor (rainfall) through its influence on soil moisture availability. Consequently, nurse plants could conceivably have an important role in allowing recruitment and persistence of taxa less well adapted to low soil moisture (Kitzberger et al., 2000; Soliveres et al., 2010). However, facilitation collapsed when, unlike Spain, extremely drought levels were reached. The most parsimonious explanation for this result is that species with low tolerances to drought and/or herbivory were unable to overcome the environmental filters controlling their recruitment, regardless of the presence of nurse plants (Kitzberger et al., 2000; Ibañez and Schupp, 2001; Soliveres et al., *in press*). Thus, the positive effects on richness and biomass at the plot level collapsed under these extremely harsh conditions, as discussed by the '*facilitation waning models*' (Tielbörger and Kadmon, 2000a; Maestre and Cortina, 2004; Michalet et al., 2006). This is consistent with observations of a higher percentage of facilitation obligate species and lower similarity at moderate levels of water stress, and with the breakdown of these effects under extremely high levels of drought (Figs. 3 and 4).

We found some evidence that increasing nurse size could explain increasing facilitative effects of *Stipa* in the Spanish sites. Percentage of facilitation obligate species and the intensity of the positive effects on community diversity increased with increasing average canopy area of *Stipa*, suggesting an increased capacity to ameliorate harsh environmental conditions by larger tussocks. However, and in contrast to previous research (Michalet et al., 2006), nurse area was not related to the importance, intensity or frequency of positive interactions at the community level in the Shrub and Tree microsites tested. The model developed by Michalet et al. (2006) was based on empirical data using mostly tussock-like nurses (species similar to *Stipa*), or cushion-like plants (e.g. Choler et al., 2001; Callaway et al., 2002; Liancourt et al., 2005; Anthelme et al., 2007). Small increases in the size of these nurses are likely to have relatively large facilitatory effects on understorey species (Michalet et al., 2006; Anthelme et al., 2007). However, relatively small changes in large nurse plants,

such as the Australian eucalypt trees (with canopy areas up to 200 m²) or the shrubs we studied, would be less influential. Although our conclusions could be affected somehow by the fact that we averaged individual nurse sizes within each plot, instead of analyzing each individual separately, these results suggest caution in generalizing the effects of nurse plant size in semi-arid environments without considering the non-linear responses of microclimatic amelioration to changing canopy area. When the smallest nurse plants almost always exceed an area of 3 m², such as the microsites tested in this study (excepting *Stipa*), we would not expect a strong relationship between nurse size and their tendency to alleviate environmental stress.

The effects of plant-plant interactions on diversity: microhabitat amelioration and niche segregation

The results from both the percentage of facilitation obligates and the *Chao index*, and the lack of a relationship between these indices and *climate* in Spain (Figs. 3 and 4), suggest that facilitation from nurse plants increases the habitat for the species that are less well adapted to the given environmental conditions present in a particular site across the gradient, rather than providing microhabitat amelioration only for competitive species under harsh conditions (Travis et al., 2005; Michalet et al., 2006). We argue that this is not caused by the stress-tolerator or competitor strategy of the species involved, but rather by the distance of each target species from its ecophysiological optimum (Greiner la Peyre et al. 2001; Gross et al., 2010). It should be noted that about 20-30% of the sampled species recruited only under the canopies of nurse plants (Fig. 3), regardless of the environmental conditions present in each plot. The total percentage of facilitation obligate species (when considering both microsites together) was greater than those for separate microsites in both Spain and Australia. These results suggest that the identity of these species changed according to the particular nurse plant examined, and highlight the species-specific nature of plant interactions (Callaway, 2007). These changes in the identity of facilitated species depending on the nurse plants were, perhaps, a result of their different phylogenetic relationships (Valiente-Banuet and Verdú, 2007; 2008) or idiosyncratic ecological requirements (Prider and Facelli, 2004; Maestre et al., 2009).

In contrast with previous studies (Tielbörger and Kadmon, 2000b), our results showed that nurse plants affected the competitive outcomes of their understorey vegetation in comparison with Open areas through increases in niche segregation, but not by increasing competition intransitivity (Table 1). While this increase in niche segregation beneath the canopies of nurse plants was apparent in Spain, it was not evident in Australia. Over the Spanish gradient, competitive exclusion was significantly lower under nurse canopies than in Open microsites. Although we did not measure microclimatic heterogeneity in our study, previous research suggests that nurse plants may not only allow more species to recruit, increasing the species pool due to microclimatic amelioration, but also increase such environmental heterogeneity (e.g. Zabinsky et al., 2002; Maestre and Cortina, 2005). The joint effect of both processes (increase in the pool of species and the heterogeneity in the availability of resources that these species compete for) might increase niche segregation, and therefore local diversity (Huston, 1979; 1999; Silvertown, 2004). We found, however, that despite the relatively constant effect of nurse plants upon the interaction network of

their understorey vegetation, the effect of nurses on local diversity decreased with biomass. It is conceivable that the relative differences in the microenvironmental conditions between nurse and Open microsites that allowed more species to recruit under nurses than in the unvegetated interspaces, declined under wetter conditions. However, changes in interaction networks among neighbours did not seem to be an important driver of facilitative interactions in Australia. The combination of the lack of nurse effects on competitive outcomes with a positive effect of nurses on local richness highlights the major importance of direct facilitation in this region. In spite of its theoretically important implications for diversity (Gilpin, 1975; Laird and Schamp, 2006; Bowker et al., 2010), we did not find an important contribution of competition intransitivity to local species richness in any of the studied regions, even in the more productive and heterogeneous environments beneath nurse plants.

Concluding remarks

By using a variety of conceptual and analytical approaches, and an appropriate dataset collected in contrasting semi-arid regions, our study provides a more complete mechanistic understanding of the relative role of biotic, non-trophic interactions shaping local richness along natural environmental gradients. Although our conclusions should be taken with caution, since they are based solely on our results, the results obtained and the conceptual framework that we propose should improve current theory in relation to the behavior of plant-plant interactions along environmental gradients. The joint consideration of the species-specific nature of 'stress' (Körner, 2003) together with the existence of different types of environmental gradients (Figs. 2-4), offer a potential explanation for the contrasting results reported in the literature, not only in the context of plant-plant interactions (Maestre et al., 2005, 2006; Lortie and Callaway, 2006), but also in relation to the putative universality of the hump-shaped relationship between richness and biomass (Grace, 1999; Waide et al., 1999; Gillman and Wright, 2006; Fig. 1). Plant-plant interactions will be unrelated (e.g. Maestre et al., 2005) with environmental gradients if these gradients are driven by different stressors in opposite directions. Conversely, such interactions will present a unimodal relationship with an environmental gradient (e.g. Michalet et al., 2006) when such gradient is driven by one single stressor. The lack of a unique shape in the relationship between richness and biomass at local scales would be explained, at least in part, by 1) the differential effect of plant-plant interactions on local richness in response to the types and interrelationships among different stressors along productivity gradients, and 2) by changes in the relative importance of both facilitative mechanisms (microhabitat amelioration and changes in competitive networks of neighbour plants) along such gradients. This understanding helps to refine our predictions of the response of plant communities to environmental changes, and clarifies the relative importance of biotic interactions as a driver of such responses. We believe that the findings of our study will have important implications for interpreting previous studies evaluating the interplay between facilitation and competition along stress gradients, and should be considered by future research on this important community ecology topic.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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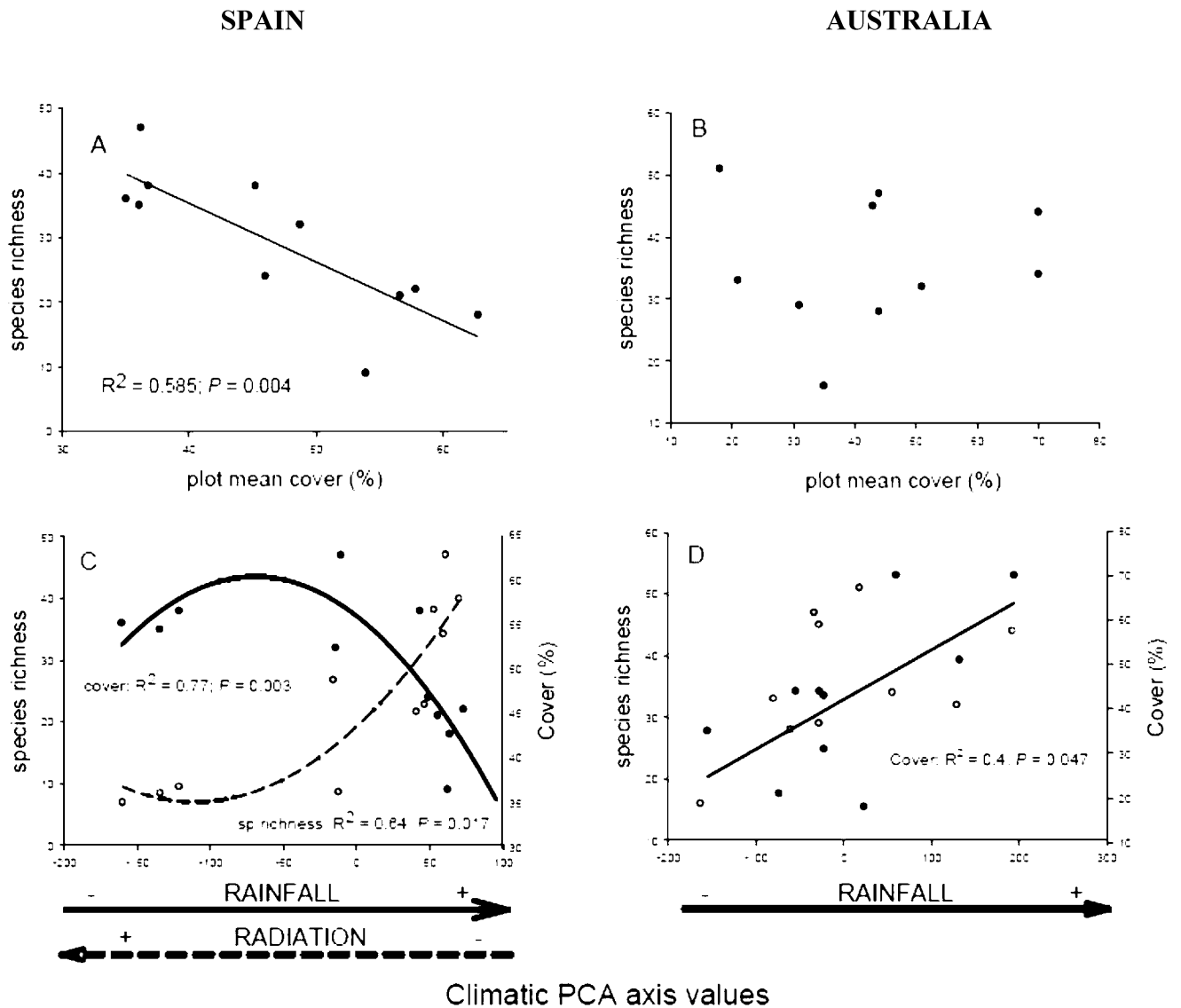


Figure 1. Relationships between cover and species richness at the community level in Spain (A) and Australia (B), respectively. The relationship between both cover (open circles) and richness (closed circles) and the first axis of a PCA derived from climatic variables is shown for both Spain (C) and Australia (D), respectively. Significant relationships ($P < 0.05$) for cover and richness are shown as continuous and dashed lines, respectively.

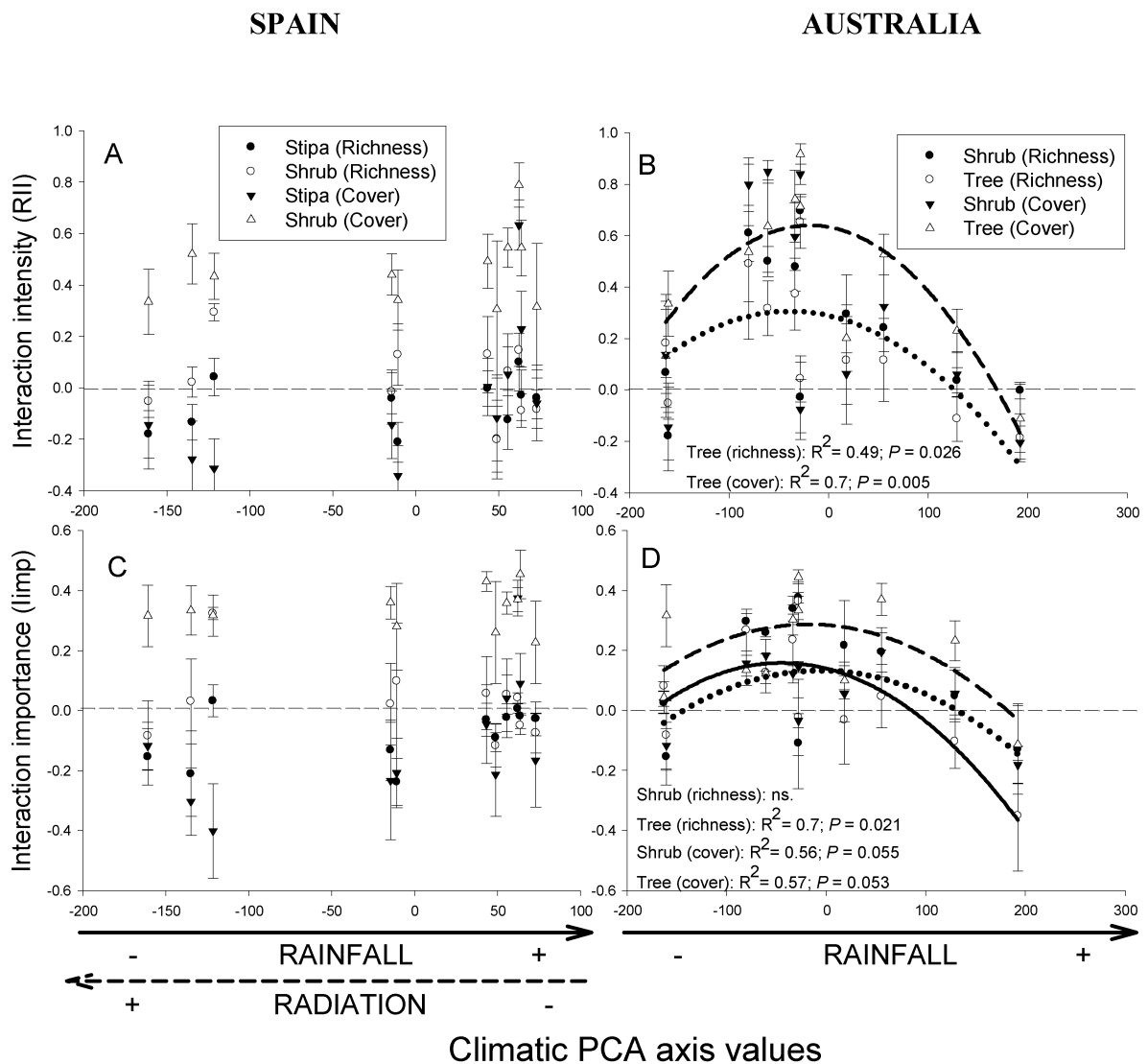


Figure 2. Relationships between the indicators of interaction intensity (Relative Interaction Index, RII) and importance (Interaction importance index, Iimp), calculated for both community richness and cover, and the first axis of a PCA derived from climatic variables. Values higher or lower than zero indicate positive (facilitation) or negative (competition) effects of the nurse plants, respectively. Values are means \pm SE per plot and microsite. Significant ($P < 0.05$), and marginally significant ($0.05 < P < 0.10$) relationships are shown in the figure as bold (Shrub [richness]), dashed (Tree [cover]) and dotted (Tree [richness]) lines.

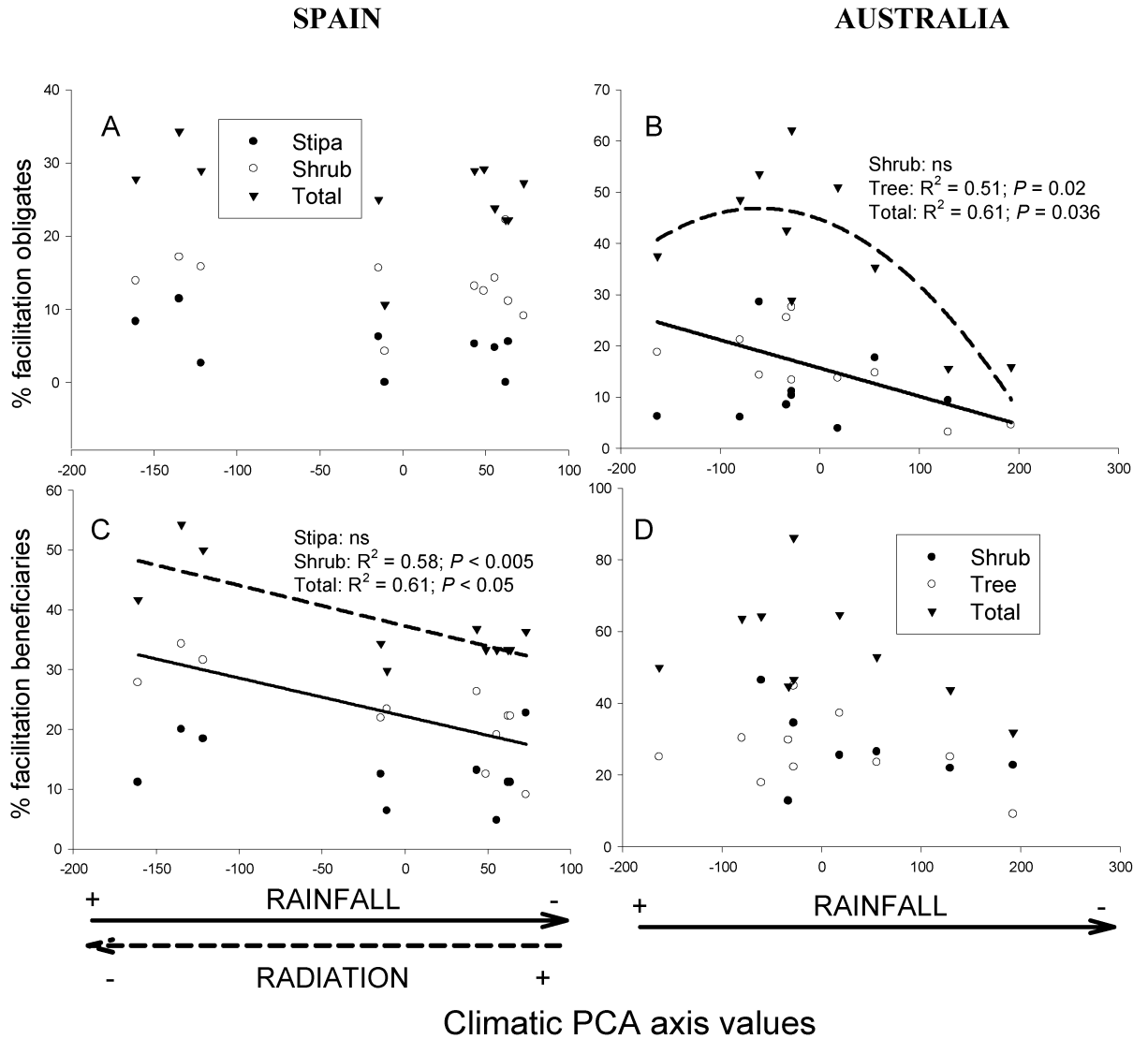


Figure 3.

Relationships between the number facilitation beneficiaries (species with more individuals recruiting under nurse plants than in Open microsites) and facilitation obligates (species that only recruit under the canopy of nurse plants), as a percentage of total species richness in each plot, and the first axis of a PCA derived from climatic variables. Stipa (Spain) or Shrub (Australia) microsites are shown with closed circles. Shrub (Spain) and Tree (Australia) microsites are shown with open circles and by using continuous lines to point significant ($P < 0.05$) relationships. Total (summing both microsites) facilitated species are shown with triangles and by using dashed lines to point significant relationships.

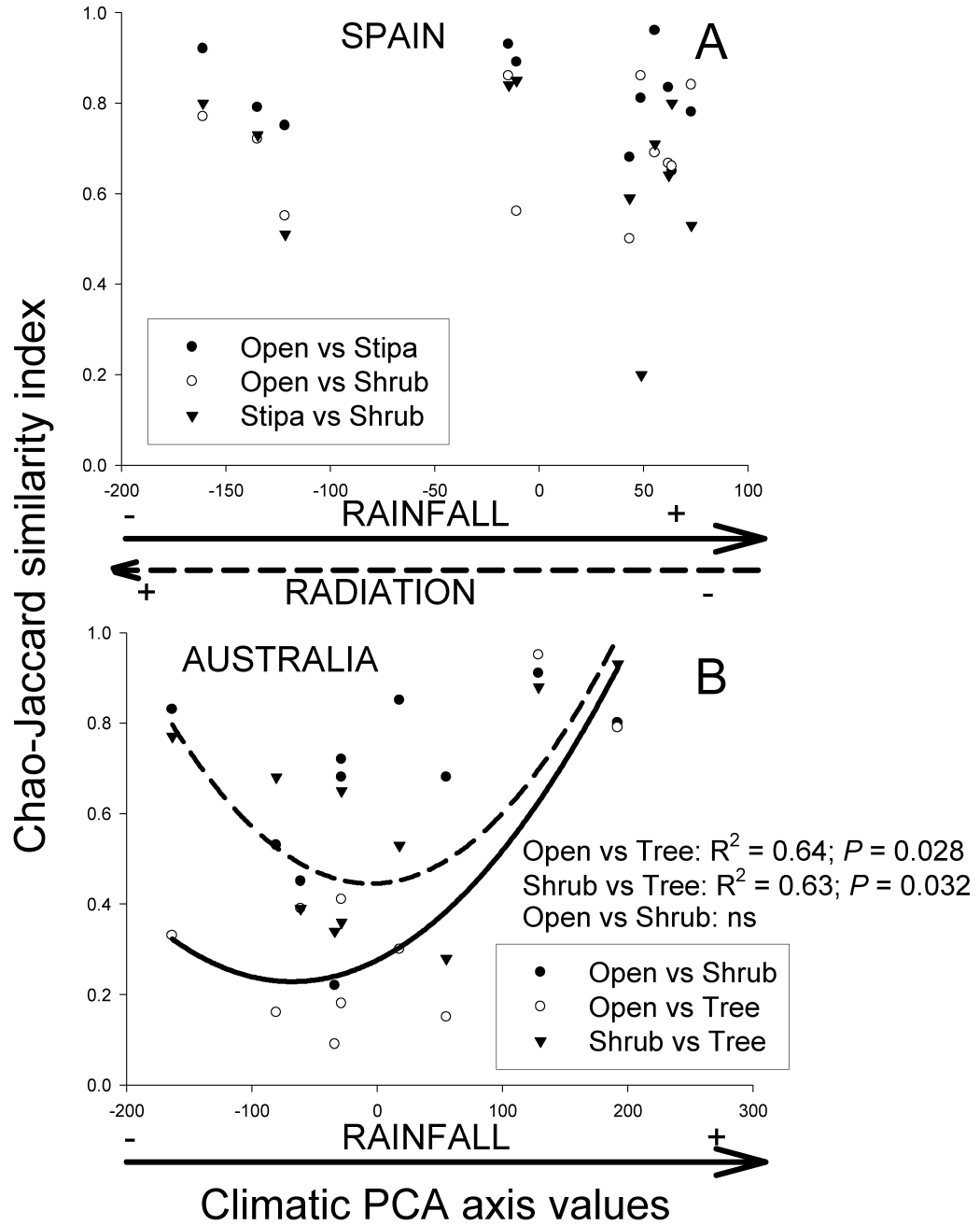


Figure 4. Relationships between Chao-Jaccard similarity index, our surrogate of microhabitat amelioration, and *climate* in Spain and Australia. Data from the three different microsite pairs for each country (Open vs Stipa, Open vs Shrub, and Stipa vs Shrub for Spain; Open vs Shrub, Open vs Tree, and Tree vs Shrub for Australia) are shown in closed circles, open circles and triangles, respectively. Significant relationships ($P < 0.05$) are shown as bold (Open vs Tree) and dashed (Tree vs Shrub) lines.

Table 1

C-score standardized effect size (SES) and plot-scale richness (Richness) values for each microsite tested (mean \pm SE for the total plots sampled) at both studied regions. ANCOVA results for both variables and their theoretical implications (explained in the main text) are also given in the table.

Spain	Variable values			Factor	Df	F	P-value	Underlying mechanism
	Open	Stipa	Shrub					
SES	1.0 \pm 0.4	0.1 \pm 0.1	0.0 \pm 0.2	Cover	1,29	2.05	0.162	Higher niche segregation and richness under nurse plants: this difference declines with increasing biomass
				Microsite	2,29	5.70	0.008	
Richness	11.4 \pm 1.5	12.3 \pm 1.7	14.8 \pm 1.9	Cover	1,29	53.9	<0.001	
				Microsite	2,29	3.00	0.065	
Australia	Open	Shrub	Tree	Factor	Df	F	P-value	Underlying mechanism
SES	-0.1 \pm 0.5	2.0 \pm 0.8	0.8 \pm 0.7					
				Microsite	2,26	2.40	0.101	
Richness	11 \pm 1.5	17 \pm 1.7	15.8 \pm 1.5	Cover	1,26	0.24	0.628	
				Microsite	2,26	4.050	0.030	