Do biotic interactions modulate ecosystem functioning along stress gradients? Insights from semi-arid plant and biological soil crust communities

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Climate change will exacerbate the degree of abiotic stress experienced by semi-arid ecosystems. While abiotic stress profoundly affects biotic interactions, their potential role as modulators of ecosystem responses to climate change is largely unknown. Using plants and biological soil crusts, we tested the relative importance of facilitative–competitive interactions and other community attributes (cover, species richness and species evenness) as drivers of ecosystem functioning along stress gradients in semi-arid Mediterranean ecosystems. Biotic interactions shifted from facilitation to competition along stress gradients driven by water availability and temperature. These changes were, however, dependent on the spatial scale and the community considered. We found little evidence to suggest that biotic interactions are a major direct influence upon indicators of ecosystem functioning (soil respiration, organic carbon, water-holding capacity, compaction and the activity of enzymes related to the carbon, nitrogen and phosphorus cycles) along stress gradients. However, attributes such as cover and species richness showed a direct effect on ecosystem functioning. Our results do not agree with predictions emphasizing that the importance of plant–plant interactions will be increased under climate change in dry environments, and indicate that reductions in the cover of plant and biological soil crust communities will negatively impact ecosystems under future climatic conditions.

Keywords: facilitation; competition; climate change; Mediterranean; biotic interactions

1. INTRODUCTION

The study of biotic interactions among plants, understood as ‘the effect of one individual plant on another individual of either the same or a different species’ (Brooker 2006), has been a core research theme since the early days of ecology (Oosting 1948; Keddy 2001; Callaway 2007). These plant–plant interactions are ubiquitous in most terrestrial ecosystems, and interact with habitat suitability and dispersal to determine the structure of plant populations and communities (Callaway et al. 2005; Valiente-Banuet & Verdú 2007; Chu et al. 2008; but see Mitchell et al. 2009). Because the important roles they play in determining the array of functional traits within plant communities, which interact with the environment to affect processes such as nutrient-cycling, positive (facilitative) and negative (competitive) interactions have been frequently invoked as major controls of ecosystem functioning (Hooper et al. 2005; Michalet et al. 2006; Yachi & Loreau 2007). However, relatively few studies have empirically examined how these interactions affect the functioning of whole ecosystems (Mulder et al. 2001; Kikvidze et al. 2005). Therefore, the importance (sensu Welden & Slauson 1986) of facilitation and competition for maintaining ecosystem functioning is largely unknown (Callaway 2007).

Assessing the direct effects of biotic interactions on ecosystem functioning using the large body of literature available is not an easy task because the vast majority of studies have simplified the complexity of natural communities by evaluating the interaction between a single or a few pairs of species. Such studies constituted over 81 per cent of the ca 400 studies reviewed in recent syntheses on facilitation in plant communities (Flores & Jurado 2003; Brooker et al. 2008). In response, the study of facilitative–competitive interactions among all the members of a given community is now gaining increased attention
(e.g. Cavieres et al. 2006; Dullinger et al. 2007; Valiente-Banuet & Verdú 2007; Maestre et al. 2008), and there is a clear need to devote more research efforts to explore such interactions at this level if we aim to substantially advance our understanding of their role as drivers of ecosystem functioning (Brooker et al. 2008; Maestre et al. 2009a). However, conducting field experiments to assess the outcome of biotic interactions at the community level is logistically difficult, if not impossible, in most ecosystems. Although the attribution of patterns to processes cannot be made without uncertainty using solely observational approaches, they are being increasingly used and recommended for this aim (Brooker et al. 2008; Maestre et al. 2009a). In this direction, recent experimental studies provide evidence that spatial aggregation promotes coexistence in plant communities (Stoll & Prati 2001; Monzeglio & Stoll 2005).

Other studies have shown that processes such as competition and facilitation may be inferred through the observation of segregation and aggregation patterns, respectively (Purves & Law 2002; Tirado & Pugnaire 2005), and that changes in the net outcome of interactions promoted by abiotic stress may be tracked by parallel shifts in the fine-scale spatial arrangement and aggregation of plant communities (Kikvidze et al. 2005).

Semi-arid ecosystems, which cover 41 per cent of Earth's land surface and support over 38 per cent of the total global population of 6.5 billion (Reynolds et al. 2007), are among the most sensitive ecosystems to climate change (Körner 2000). In the semi-arid areas of the Mediterranean basin, predicted modifications in climate—a sharp decrease in water availability, an increase of temperature by up to 7°C in summer by the end of the twenty-first century and a higher overall climate variability (de Castro et al. 2005; Rowell & Jones 2006)—are going to substantially exacerbate the degree of abiotic stress these communities experience (Schröter et al. 2005). Substantial research efforts have been devoted over the last decade to predict how the interplay of facilitative and competitive interactions varies along abiotic stress gradients driven by precipitation and temperature (e.g. Callaway et al. 2002; Maestre & Cortina 2004; Holzapfel et al. 2006). This body of research has shown that modifications in the degree of abiotic stress have major impacts on plant–plant interactions, although the specific effects of such modifications on the magnitude and direction of these interactions are still being debated (Maestre et al. 2005a, 2006, 2009a; Lortie & Callaway 2006; Smit et al. 2009).

Because co-occurring species differ in their tolerance to abiotic stress, which in turn affects the outcome of plant–plant interactions (Liancourt et al. 2005; Wang et al. 2008), the study of one or a few particular pairs of species—followed by most facilitation–competition research carried out to date (Keddy 2001; Callaway 2007)—may not be sufficient to accurately predict how biotic interactions within a given community will change along abiotic stress gradients. Despite its importance, very few empirical studies have evaluated how the outcome of facilitative–competitive interactions at the community level vary along wide-ranging abiotic stress gradients (i.e. involving more than two levels; Kikvidze et al. 2005; Dullinger et al. 2007; Maestre et al. 2009b), and how joint changes in these interactions and in climate affect ecosystem functioning (Kikvidze et al. 2005). Therefore, and despite it having been hypothesized that climate change may also exert indirect effects on ecosystem functioning by influencing competitive and facilitative interactions (see Brooker 2006; Tylianakis et al. 2008 for reviews), it is difficult to know whether these interactions can control ecosystem responses to climate change, particularly when compared with community attributes with important functional roles, such as diversity (Reiss et al. 2009).

In this article we focus on the following question: do biotic interactions and other community attributes modulate ecosystem functioning along climatic gradients in semi-arid Mediterranean ecosystems? We hypothesize that both biotic interactions and attributes such as cover and diversity (species richness and evenness) will drive variations in ecosystem functioning along abiotic stress gradients, and thus have the potential to drive ecosystem responses to climate change. To test this hypothesis, we used multiple biotic communities (vascular plants and biological soil crusts (BSCs) formed by mosses and lichens) and experimental approaches (natural and manipulated climatic gradients at different spatial scales). We also sought to include a comprehensive set of ecosystem processes and variables related to ecosystem functioning, something critical when assessing the functional role of biotic communities (Reiss et al. 2009). Our combined use of multiple experimental approaches, biotic communities and spatial scales to test the role of stress and biotic interactions on ecosystem functioning has not, to our knowledge, been attempted before. Such an integrated approach can provide broader insights on the functional role of biotic interactions and other attributes of biotic communities, and on their respective potential as drivers of ecosystem responses to climate change.

2. MATERIAL AND METHODS

To achieve our objective, four independent studies have been carried out in various semi-arid environments of Spain. Three of these studies (hereafter named as Studies 1, 2 and 3) use BSCs as a model system. The last study (Study 4) targets vascular perennial vegetation. In Studies 1, 2 and 4, variations in abiotic stress were induced by natural changes in climatic and/or topographic conditions, while in Study 3 such variations were promoted through experimental manipulations. We decided to focus on BSC in most of the studies because they are a key biotic component of semi-arid environments (Belnap & Lange 2003), a good model system to explore the question posed here (Bowker et al. 2010) and are clearly under-represented in the facilitation–competition literature (e.g. Callaway 2007). The results presented from Studies 1 and 4 are new re-analyses of previously published data (Maestre et al. 2008, 2009b; Maestre & Escudero 2009).
Species richness was estimated as the number of lichen species present in each plot. For the estimation of cover, species evenness and biotic interactions (described below), each plot was divided into 100 sampling quadrats of 5 × 5 cm, and the cover of every lichen species was estimated. The average of the cover of all lichens in the 100 quadrats was used as our estimate of total plot cover. Species evenness was calculated using the Pielou’s J index (Pielou 1975). Abiotic stress promoted by changes in resource availability was indirectly measured in every plot using two variables: slope angle and soil surface roughness (for details see the electronic supplementary material, appendix A). Slope angle is related to the radiation dose and heat load, and is an important abiotic factor controlling the distribution of soil lichens (Hauck et al. 2007). Soil surface roughness is related to the runoff and infiltration dynamics, and thus to water availability. An independent calibration proved that soil surface roughness measured with this index was negatively related to soil moisture at the 0–2 cm depth after spring rainfalls at the study site (electronic supplementary material, figure S2).

(b) Study 1: variations in biotic attributes, interactions and ecosystem functioning in BSC communities along a small-scale natural environmental gradient

This study was conducted in gypsum outcrops located next to Belmonte del Tajo, in Central Spain (40°7′3″N, 3°18′30″W, 686 m a.s.l.). The climate is Mediterranean semi-arid, with a mean annual temperature and rainfall of 14 °C and 452 mm, respectively. The studied gypsum outcrops are surrounded by a well-preserved forest of Quercus ilex L. and Pinus halepensis Miller, but perennial plant cover within them remains below 20 per cent (electronic supplementary material, figure S1).

A total of 63 plots (50 × 50 cm), spread over a homogeneous area of 1.3 ha, were placed non-randomly on bare ground areas with well-developed BSC-forming lichen communities. This non-random placement of plots is commonly followed with BSCs because of the small size and high within-site spatial variability of the organisms constituting them (Maestre et al. 2005b; Bowker et al. 2006; Martinez et al. 2006). However, a minimum separation distance of 0.7 m between sampling units was established to minimize the risk of sampling non-independent areas owing to the spatial structure of BSC. Much of the spatial variation in the cover of BSC organisms in semi-arid Mediterranean areas occurs at spatial scales smaller than the 50 × 50 cm quadrats used (Maestre 2003), and with this separation distance we sought to remove potential sources of non-independence between sampling quadrats. With this survey, we aimed to capture the greatest possible contrast in lichen and moss community composition and structure, avoiding changes in the proportion of suitable habitat among the plots that could confound the interpretation of the co-occurrence patterns observed (Dullinger et al. 2007).

Species richness was estimated as the number of lichen species present in each plot. For the estimation of cover, species evenness and biotic interactions (described below), each plot was divided into 100 sampling quadrats of 5 × 5 cm, and the cover of every lichen species was estimated. The average of the cover of all lichens in the 100 quadrats was used as our estimate of total plot cover. Species evenness was calculated using the Pielou’s J index (Pielou 1975). Abiotic stress promoted by changes in resource availability was indirectly measured in every plot using two variables: slope angle and soil surface roughness (for details see the electronic supplementary material, appendix A). Slope angle is related to the radiation dose and heat load, and is an important abiotic factor controlling the distribution of soil lichens (Hauck et al. 2007). Soil surface roughness is related to the runoff and infiltration dynamics, and thus to water availability. An independent calibration proved that soil surface roughness measured with this index was negatively related to soil moisture at the 0–2 cm depth after spring rainfalls at the study site (electronic supplementary material, figure S2).
Richness was estimated as the total number of moss and lichen species found in each transect. Cover was estimated as the proportion of the total length of the transect covered by these organisms. Species evenness was calculated using the Pielou’s J index. For the estimation of biotic interactions (detailed below), we divided each transect into 30 segments of 5 cm and for each, we recorded the presence of every bryophyte and lichen species intercepted along the segment. Climatic attributes (annual radiation, temperature and rainfall) were collected for each site using the available climatic interpolations for the Iberian Peninsula (Ninyerola et al. 2005).

(d) Study 3: simulated climate change impacts on ecosystem functioning in BSC communities

This study is part of an ongoing research programme, started in July 2008, devoted to evaluate climate change impacts on BSC-dominated ecosystems. It is being conducted in gypsum outcrops located in the vicinity of Aranjuez, in the centre of the Iberian Peninsula (40°02′ N – 3°37′ W; 590 m a.s.l.). The climate is Mediterranean semi-arid, with an average annual rainfall and temperature of 456 mm and 13.8°C, respectively. Perennial plant cover is below 40 per cent, and is dominated by S. tenacissima; isolated individuals of the evergreen shrub Retama sphaerocarpa (L.) Boiss. are also present. The open areas between perennial plants are colonized by a well-developed BSC, dominated by lichens such as D. diacapsis, Squamarina lentigera, F. subbracteata and Psora decipiens.

We set-up a factorial experiment at this site with three treatments: BSC (poorly developed BSC communities with cover less than 5% versus well-developed BSC communities with cover greater than 50%); temperature increase (control versus increased temperature); and rainfall reduction (control versus a 20% reduction in annual rainfall). The working plots (1 m² size) were randomly selected among suitable bare ground areas, and the eight combinations of treatments were randomly assigned to the plots. Ten replicates per combination of treatments were established, resulting in a total of 80 plots. As the rainfall reduction treatment was installed in November 2008, we report here preliminary results of the temperature increase treatment (July 2008–September 2009). In this experiment, we aim to achieve an annual increase in air temperature of 2–4°C, according to model predictions for Central Spain by the late twenty-first century (de Castro et al. 2005). For doing this, we used open top chambers (OTC) as described in the electronic supplementary material, appendix A (see also in the electronic supplementary material, figures S4 and S5).

Within each plot, we installed a PVC collar (20 cm diameter, 8 cm height) for soil CO2 measurements. The cover, species richness and evenness (Pielou’s J index) of the BSC community (bryophytes and lichens) were estimated within each collar at the beginning of the experiment using the point-sampling method (1 × 1 cm grid; 120 sampling points per collar). For the estimation of biotic interactions (detailed below), we divided each collar into 19 circles with a diameter of 4 cm, spaced by about 2 cm which was not sampled, and for each we recorded the presence of every bryophyte or lichen species.

(e) Study 4: variations in biotic attributes, interactions and ecosystem functioning in S. tenacissima grasslands along a regional natural environmental gradient

This study was conducted in 29 S. tenacissima steppes located along the same central to southeastern Spain gradient described for Study 2. Most sites (22) were located on soils derived from limestone, while seven sites were located on gypsum-rich soils. All sites were placed on south-facing gentle slopes. Vegetation was in all cases open grasslands dominated by S. tenacissima (electronic supplementary material, figure S2), and contained shrub species like Quercus coccifera L. and Rosmarinus officinalis L. in calcareous soils, and Lepidium subulatum L. and Gypsophila struthium L. in gypsum soils. Perennial plant cover ranged between 15 and 68 per cent (see Maestre & Escudero (2009) for further information on the study sites).

In each site, we established a 30 × 30 m plot for assessing the attributes of the perennial plant community. In the upper left corner of each plot, we located one 30 m long transect downslope. Three parallel transects of the same length, each 8 m apart across the slope, were added. In each transect, we collected a continuous record of all perennial vegetation patches intercepting the transect for estimating total cover. In each transect, we also placed 20 consecutive quadrats (1.5 × 1.5 m size), and the cover of every perennial species was visually recorded. These data were used to assess biotic interactions (described below) and species evenness (Pielou’s J index). The number of perennial species per 900 m² plot was used as an estimate of total species richness. Climatic attributes (annual radiation, temperature and rainfall) were also estimated for each site using the available climatic interpolations for the Iberian Peninsula (Ninyerola et al. 2005).

(f) Assessment of biotic interactions through co-occurrence patterns

To estimate the outcome of biotic interactions at the community level, we carried out null model analyses of co-occurrence patterns (Gotelli 2000). This approach has often been employed to evaluate the importance of competitive interactions as a force structuring biotic communities (see Gotelli & Graves 1996 for a review), and in recent years it is being used to explore both competitive and facilitative interactions in vascular plant and lichen communities (Dullinger et al. 2007; Maestre et al. 2008; Rooney 2008). We acknowledge that species co-occurrence can be affected by processes such as limited dispersal, habitat selection and clonal growth (Gotelli & Graves 1996; Abrahamson et al. 2005). However, we believe that these aspects can only marginally affect co-occurrence in the studied communities because of the characteristics of the surveys employed (which minimized the sampling of non-suitable habitat), the dispersal characteristics of the species studied (which

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make them quite unlikely to be dispersal-limited in the studied sites) and the prevalence of clonal species such as *S. tenacissima* in all the grasslands evaluated.

For each plot/transect, the data were organized as a presence–absence matrix, where each row and column represents a different species and quadrat/transect segment, respectively. We estimated co-occurrence in each of the sampled plots/transects (matrices) with the C-score index. It is calculated for each pair of species as $(R_i - S)(R_j - S)$, where $R_i$ and $R_j$ are the matrix row totals for species $i$ and $j$, and $S$ is the number of squares in which both species occur; this score is then averaged over all possible pairs of species in the matrix (Gotelli 2000). If a community is structured by competitive or facilitative interactions, the C-score should be significantly larger or smaller than expected by chance, respectively. We selected the C-score among different available indices because it is robust to the presence of noise in the data and has good statistical properties (see Gotelli 2000 for a review).

Because the values of the C-score are dependent on the number of species and co-occurrences 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 10 000 null communities (Gotelli & Entsminger 2006). Values of SES higher and lower than 0, respectively, indicate prevailing spatial segregation and aggregation among the species within a community. Null model analyses were conducted with EcoSim 7.22 (Gotelli & Entsminger 2006).

(g) Statistical analyses

In Studies 1 and 2, we evaluated the relationships between biotic interactions, other attributes of biotic communities (species richness and cover), and the different surrogates of ecosystem functioning using structural equation modelling (SEM; Grace 2006). Generally, an investigator will propose an *a priori* model that features variables and hypothesized relationships among variables in a path diagram (figure 1). The second step is to estimate path coefficients using the maximum likelihood estimation technique. These are obtained for each pathway of the model by optimally adjusting the observed variance–covariance matrix to the path diagram. Standardized path coefficients range from 0 to 1, are analogous to regression weights or partial correlation.
coefficients, and describe the effect size of relationships in the model. At this time, researchers typically test the overall goodness-of-fit of the model against the dataset, poor goodness-of-fit indicating that a model is not a plausible causal scenario, which could have resulted in the covariance matrix of the dataset (Grace 2006). Our a priori model was saturated, meaning that there was a direct uni- or bi-directional relationship between every possible pairing of variables, because all were plausible hypotheses. We fitted the saturated model to estimate the path coefficients, and worked backward removing weak pathways which did not strongly impact the model fit (generally path with coefficients <0.05). The exception was that we retained paths from the C-score to ecosystem function variables in all models because these were a primary focus of the study. In this way we simplified to more parsimonious models which could be subjected to goodness-of-fit criteria, and various models may differ somewhat in their final structure. We used the traditional $\chi^2$ goodness-of-fit test, but since it is prone to some type I error, the RMSEA index and the Bollen-Stine bootstrap test were also considered as alternative indices of model fit (Grace 2006). These generally agree closely, and yield the probability of the implied covariance structure of the model fitting that of the data. Thus, and unlike many statistical tests, low probability values are not desired.

In our SEM models we employ composite variables, which allow an additive combination of the effects of multiple conceptually related variables upon a response variable (Grace 2006). Composite variables are primarily a graphical and numerical interpretation tool, and do not change the underlying model. In both datasets, we used a composite entitled ‘community properties’ that represents the summed effects of total cover, species richness and species evenness. Likewise, we used composites (‘environmental effects’) to pool the effects of variables related to climate or microclimate: annual precipitation, mean annual temperature and mean annual radiation in the dataset from Study 2, and slope angle and surface roughness in that from Study 1. However, and to determine which individual variables were explicitly active within each composite, we also examined the underlying observed variable models with the composite variables removed. SEM analyses were performed using AMOS (SPSS Inc., Chicago, IL, USA).

In the case of the dataset gathered from Study 4, there was insufficient sample size for carrying out SEM analyses ($n = 29$), and instead we used path analysis (Shipley 2001) based upon partial Mantel statistics (Smouse et al. 1986) to construct a model with similar structure. The interpretation is similar, except that a ‘variable’ is actually a distance matrix based upon multiple conceptually related variables using squared Euclidean distance. The environment matrix contained annual precipitation, mean annual temperature and mean annual radiation. The community properties matrix contained total cover, species richness and species evenness. The ecosystem function matrix contained soil compaction, soil C, respiration, water-holding capacity and enzyme (phosphatase, $\beta$-glucosidase and urease) activities. The C-score matrix was simply the C-score data expressed in distance matrix format. Our approach was similar to that of Leduc et al. (1992), except that we did not remove paths based upon probability values, and emphasize the path coefficient (equivalent to the partial Mantel statistic) as our measure of effect size. Partial and bivariate Mantel statistics were obtained in R 2.6.2 (www.r-project.org), using the Ecodist package (Goslee & Urban 2007). $r^2$ of endogenous variables was calculated using the formula in McCune & Grace (2002). As a post hoc test to determine the individual effects of particular community attributes on ecosystem functions, we repeated the analyses three times, substituting the community attributes matrix with matrices representing cover, richness and evenness alone. We did the same with the environment matrix to see the effect of each abiotic factor measured separately on the C-score.

Soil respiration data from Study 3 were analysed separately for plots with low and high BSC cover. Data from the former were analysed using repeated-measures analysis of variance (ANOVA), with OTC as a fixed factor. Data from the high cover plots were analysed using repeated-measures analysis of covariance (ANCOVA), with OTC as a fixed factor and biotic interactions, cover, species evenness and species richness as covariates. Separate analyses were carried out for each of these covariates. Prior to these analyses, respiration data were log-transformed to achieve the homogeneity of variances in their distribution. ANOVA analyses were performed using SPSS for WINDOWS 17.0 (SPSS Inc.).

3. RESULTS (a) Variations in biotic attributes, interactions and ecosystem functioning in BSC communities

In Study 1 (figure 2a), our SEM model was able to explain greater than one-third of the variance in both $\beta$-glucosidase and urease ($r^2 > 0.35$ in both cases), respectively, but had little explanatory power for phosphatase ($r^2 = 0.08$). This model was satisfactorily fitted to our data, as indicated by the different goodness-of-fit statistics (figure 2a). Most of the variance was accounted for by community properties other than species interactions, particularly cover, whereas direct effects of environmental variables and species interactions were relatively small (absolute value of $r \leq 0.26$). The abiotic variables measured exerted important indirect effects on the soil enzymes via their direct influence upon species interactions and other community properties (absolute value of $r \geq 0.47$).

In Study 2 (figure 2b), our SEM was able to explain 50 and 38 per cent of the variance in $\beta$-glucosidase and phosphatase, respectively. Our data satisfactorily fitted this model, as shown by the different goodness-of-fit statistics (figure 2b). In contrast to experiment 1, the largest contribution to the variance explained was the direct effect regional climatic variables ($\beta$-glucosidase $r = 0.73$, phosphatase $r = 0.63$). Of secondary importance were the effects of community properties such as total cover, which in the case

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of phosphatase were moderately strong \((r = 0.40)\). These effects were in turn moderately affected by the environmental gradients (absolute value of \(r\) between 0.20 and 0.30). Both the effects of species interactions and evenness upon the soil enzymes evaluated and the effects of environmental gradients upon species interactions were weak. However, species interactions indirectly affected ecosystem functioning via their moderate effects on community properties.

In Study 3, plots with well-developed BSCs showed higher soil respiration than plots without BSCs (figure 3). The OTCs promoted an average increase in temperature of 2.6°C compared with the control treatment over the study period.

![Figure 2](http://rstb.royalsocietypublishing.org/)
b) Variations in biotic attributes, interactions and ecosystem functioning in *S. tenacissima* grasslands

In Study 4, the overall variation explained by the model was relatively low ($r^2 = 0.026$; figure 4). Variations in both the C-score and the attributes of biotic communities were poorly related to changes in abiotic conditions ($r^2 < 0.01$ in both cases). The greatest direct effect upon the ecosystem function matrix was the environmental matrix ($r = 0.49$). The effects of both the C-score and the community properties matrix on the ecosystem function matrix were non-significant. However, when the effects of the different biotic attributes were considered in isolation from each other, the cover was significantly related to ecosystem functioning ($r = 0.17$).

4. DISCUSSION

Overall, we found little evidence to suggest that biotic interactions, as measured by the standardized effect of the C-scores, are a major direct influence upon indicators of ecosystem function along abiotic stress gradients driven by changes in water availability and temperature. This result was consistent in all the studies conducted, regardless of the spatial scale considered, the experimental approach followed and the organisms being targeted. Despite the lack of direct effects of species interactions, in most cases over one-quarter of the variation was explained in most ecosystem function indicators by direct effects of environmental gradients or other community properties. Semi-arid ecosystems are primarily abiotically driven (Whitford 2002), and the ecosystems studied are not an exception. However, attributes such as cover, and to a lesser degree, species richness directly and significantly influenced many of the functional surrogates measured. Therefore, our results provide evidence for a strong biotic control on ecosystem functioning in Mediterranean semi-arid ecosystems dominated by both plants and BSCs.

(a) Effects of biotic interactions and community attributes on ecosystem functioning

Although currently under revision (Maestre et al. 2009a; Smit et al. 2009), conceptual models widely employed when studying the outcome of plant–plant interactions along abiotic stress gradients (e.g. the ‘stress-gradient hypothesis’ of Bertness & Callaway (1994)) have promoted the idea that facilitative interactions should be more frequent, and thus more important for ecosystem structure and functioning, under high abiotic stress conditions (see Callaway 2007 for a review). Indeed, it has been suggested that the importance of facilitation in dry Mediterranean environments should increase with the ongoing climatic change (Brooker 2006). On the other hand, and across latitudinal gradients, there is a large body of literature on trophic interactions suggesting that biotic interactions are more important at low latitudes (see Schemske et al. 2009 for a recent review). Co-occurrence patterns, our surrogate for biotic interactions, were found to vary substantially with abiotic stress only at small spatial scales (figure 1b). Regardless of these variation patterns, our results clearly indicate that the relative importance of the outcome of facilitative–competitive interactions at the community level as a driver of variations in ecosystem functioning along abiotic stress gradients is lower than that of other attributes of biotic communities.
and semi-arid steppes, which have found strong and the same surrogates. The patterns found also always lower than that between cover/species richness and the surrogates of ecosystem functioning was magnitude of the relationship between spatial pattern in the same ecosystems studied here, found that the Maestre & Escudero 2009). These studies, conducted in ecosystem processes (Brooker 2005; Lamb & Cahill 2008; Mitchell et al. 2008). Do our results indicate that biotic interactions are not important for ecosystem functioning? While this may be the first impression drawn from our analyses, we cannot extrapolate beyond our data and the ecosystems studied, and more empirical examples are clearly needed to evaluate the generality of our results. It is worth noting that finding ample evidence of competition and facilitation does not imply that such processes are necessarily playing a predominant role in ecosystem processes (Brooker et al. 2008). While the intensity of these interactions can be very high, their impact relative to other processes (i.e. their importance) to determine ecosystem structure and functioning may vary from high to low (see Brooker et al. 2005; Lamb & Cahill 2008; Mitchell et al. 2009 for recent examples).

Our findings resemble those from previous studies evaluating the relative importance of spatial pattern, which is commonly determined by biotic interactions (e.g. Eccles et al. 1999), and cover/richness as drivers of ecosystem functioning (Maestre et al. 2003b; Maestre & Escudero 2009). These studies, conducted in the same ecosystems studied here, found that the magnitude of the relationship between spatial pattern and the surrogates of ecosystem functioning was always lower than that between cover/species richness and the same surrogates. The patterns found also agree with many studies conducted in a wide variety of environments, including Mediterranean shrublands and semi-arid steppes, which have found strong and positive relationships between biotic attributes, such as cover and species richness on different surrogates of ecosystem functioning (e.g. Troumbis & Memtsas 2000; Martinez-Mena et al. 2002; Bastida et al. 2008; Montés et al. 2008).

Direct effects of biotic interactions on ecosystem functioning might be related to an increase in the efficiency of the whole community for using resources and recycling nutrients, the so-called ‘complementarity effect’—that implies a reduction in the competitive effects of some species on others (Callaway 2007)—or to direct or indirect facilitative effects leading to an increase in community productivity and ecosystem functioning (Knops et al. 1999; Mulder et al. 2001). In semi-arid environments, it is well known that facilitative–competitive interactions among plant species are important determinants of the heterogeneous spatial distribution of vegetation commonly found in these areas and of the formation of resource islands under the canopy of isolated shrub and grass species (Aguirre & Sala 1999). Why are we not observing significant effects of biotic interactions upon the surrogates of ecosystem functioning measured, particularly when compared with attributes such as total cover? While the mechanisms highlighted above can be important drivers of changes in vascular plant productivity, the surrogate of ecosystem functioning used in virtually all the relevant studies (Callaway 2007), they may not be so important when using soil variables acting as surrogates of processes related to nutrient-cycling. Direct effects of biotic interactions on these variables are likely to work primarily at the scale of plant patches (Cortina & Maestre 2005), and may not be translated to the inter-patch areas, which are the dominant land cover in arid and semi-arid ecosystems. However, soil attributes in these areas may be

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**Figure 4.** Final partial Mantel path analysis for the dataset gathered in Study 4. Boxes represent matrices of conceptually related variables. Numbers adjacent to arrows are partial Mantel coefficients, analogous to regression weights among matrices rather than univariate variables, and indicative of the effect size of the relationship. Width of arrows is proportional to path coefficients. Inset tables reflect the results from separate post hoc tests, wherein univariate matrices of the various community attributes and abiotic variables substituted the ‘community properties’ and ‘environmental effects’ matrices, respectively. The changes in the rest of the paths observed during these tests are negligible, and are not shown for clarity. Significance levels are as follows: *p < 0.05, ***p < 0.01 and **p < 0.001.
largely affected by plant patches (Maestre et al. 2009c),
these can modify the microclimate in their
surroundings through shading, and can provide
carbon, water and nutrients through processes such
as hydraulic lift, lateral root growth and litter inputs
(Breshears et al. 1998; Caldwell et al. 1998; Boeken &
Orenstein 2001). The influence of plant patches on
the functioning of the inter-patch areas is going to be
increasingly important with changes in cover, irrespec-
tive of whether such changes are driven by facilitation
or not.

Regarding BSC communities, positive interactions
among soil lichens can occur through mechanisms
such as increased nutrient availability close to
N-fixing species like Collema spp. (Belnap 2002),
and increased water availability in the surroundings
of those species capturing dew (Kidron et al. 2002). On
the other hand, negative interactions can arise through
mechanisms such as allelopathy (Souza-Egipsy et al.
2002), genuine competitive displacement (Armstrong &
Welch 2007) and reduced moisture availability by
those species sealing the soil surface (Cantón et al.
2004). While the outcome of these processes will
have functional consequences at the scale of the inter-
acting organisms, the multiplicity of competitive and
facilitative interactions happening within whole
communities, and their opposite effects on processes
such as nutrient-cycling (e.g. N fixation versus
reduced soil moisture) can dilute the functional impor-
tance of biotic interactions at this scale. Our results
provide, to our knowledge, the first empirical test of
the relative importance of biotic interactions, cover
and richness as drivers of ecosystem functioning in
BSC-dominated ecosystems. They also agree with
recent studies and syntheses conducted in BSC-
dominated areas describing an important control
of total cover and richness on soil nutrient-cycling
(Maestre et al. 2005b; Bowker et al. 2010).

(b) Indirect and direct effects of changes in
abiotic stress on ecosystem functioning

While the direct effects of increased temperature and
modifications in rainfall amount and pattern are caus-
ing the most evident impacts of climate change on
ecosystem functioning (e.g. Dormann & Woodin
2002; Fay et al. 2003; Emmett et al. 2004), indirect
effects mediated through impacts upon biota can also
play an important role in the way ecosystem
function responds to such changes (Canadell et al.
2007). Recent studies conducted with model grass-
land communities have shown that biotic attributes
such as biodiversity interact in complex ways with
global change drivers such as elevated CO2 and
increases in nutrient availability to determine ecosys-
tem productivity and community nutrient use (e.g.
Reich et al. 2004; Maestre & Reynolds 2006). Despite
the recognized importance of these interactions and
indirect effects, no studies like these have been con-
ducted with biotic communities other than vascular
plants.

Although widely employed to explore the impacts
of climate change on organisms and ecosystem processes,
results from observational approaches such as those
employed here must be interpreted with caution
because response to climate change might involve
adaptation/plasticity that is neglected when working
on natural environmental gradients, as populations
are possibly already adapted to the local conditions
(Giménez-Benavides et al. 2007; but see Gimeno
et al. 2008). These limitations can be overcome by
using experimental approaches such as those
employed in Study 3. The preliminary results obtained
from this study provide evidence of strong short-term
effects of climate change on soil respiration, a response
that was controlled by the total cover of BSCs (i.e.
areas of low BSC cover did not show any response to
the warming treatment), but not by species richness,
evenness or biotic interactions. If warming increases
soil respiration, particularly in areas of high BSC
cover, BSC-forming organisms such as lichens and
mosses may be at risk of C deficits, particularly when
air temperatures are high and moisture is limited
(Wilske et al. 2008). This situation may be exacerbated
by other ongoing climatic alterations, such as increases
in UV radiation, which has been shown to negatively
affect the physiological functioning of BSC-forming
organisms (Belnap et al. 2008). Our results indicate
that BSCs will probably experience added stress under
the forecasted future climatic conditions, which may in
turn have negative feedbacks on many ecosystem functions
modulated by these organisms (see Belnap & Lange
2003 for a review). The increase in soil respiration observed in the OTC treatment also
agree with results from warming experiments con-
ducted in shrublands and grasslands (Emmett et al.
2004; Zhou et al. 2006; but see Luo et al. (2001)
and Lellei-Kovács et al. (2008)). Our measurement
period is not long enough to meaningfully extrap-
olate the observed soil respiration responses, and to discuss
the mechanisms driving them, but we would like
to highlight the role played by BSC cover as a driver
of these responses to experimental warming. These
experimental results further add to the observations
obtained from Studies 1, 2 and 4 to emphasize the
role of total cover as another potentially key biotic
attribute modulating the responses of semi-arid
ecosystems to the ongoing environmental change.

5. CONCLUDING REMARKS

Important research efforts are being devoted to incor-
porating the multiplicity of factors affecting ecosystem
functioning when evaluating its responses to environ-
mental change, when using both experimental (e.g.
Reich et al. 2004; Maestre & Reynolds 2006) and
modelling (e.g. Savage et al. 2007; Shen et al. 2008)
approaches. At the same time, the study of the
facilitation–competition continuum along environ-
mental gradients has been a major topic of study
during the last two decades (Callaway 2007; Brooker
et al. 2008). However, most of this research has tar-
geted single or a few pairs of species, have not
directly evaluated the potential roles of climate,
biotic interactions and other community attributes as
drivers of ecosystem functioning and have been con-
ducted using environmental gradients involving just
two levels. Our results contribute to filling this gap,
and indicate that attributes of biotic communities such as total cover, but not the outcome of biotic interactions, exert important controls on ecosystem functioning along environmental gradients in semiarid ecosystems dominated by BSCs and vascular plants. They also indicate that factors negatively affecting biotic attributes such as cover, and to a lesser degree richness, will probably have a major negative impact on ecosystem functioning under future climatic conditions, exacerbating the impacts of climate on this functioning. Although challenging (Freckleton et al. 2009), future facilitation–competition research should explicitly consider the importance of biotic interactions, as this is crucial for a full understanding of the role of these interactions as drivers of ecosystem functioning and of its responses to climate change.

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REFERENCES


Cortina, J. & Maestre, F. T. 2005 Plant effects on soils in drylands: implications on community dynamics and


Dormann, C. F. & Woodin, S. J. 2002 Climate change in the


Phil. Trans. R. Soc. B (2010)


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