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Original article

Lichen species co-occurrence patterns along an edge-interior Mediterranean forest gradient

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ABSTRACT

The co-occurrence pattern of epiphytic lichen communities was evaluated relative to micro-environmental heterogeneity along an edge-interior forest gradient. We collected data on the occurrence of 57 epiphytic lichen species from 452 plots in a Mediterranean forest remnant. We used two realistic null models to test for non-randomness in the structure of epiphytic lichen communities and generalized linear models to test the influence of micro-environmental variables on co-occurrence indices along the edge-interior gradient. We found that epiphytic lichen communities co-occurred less often than expected by chance, although the segregation pattern was not highly structured or recurrent. Less species co-occurrence was detected in the assemblage structure in the forest interior and with a southerly exposure. However, there were no significant relationships between co-occurrence and the number of coexisting species, the dominant species coverage or bryophyte coverage. In summary, our co-occurrence analyses suggested that epiphytic lichens were competitively structured, while we found no evidence of facilitation in more stressful conditions, as predicted by the stress-gradient hypothesis. Nevertheless, these results should be treated with caution because co-occurrence patterns may involve specific unknown modes of facilitation and competition, which relate to the life history of these organisms.

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1. Introduction

Understanding the factors that determine species coexistence has been a core research topic of community ecology for decades (Gotelli and McCabe, 2002; Callaway, 2007). The study of species co-occurrence patterns has been prevalent among the wide range of questions addressed in this research area (Brooker et al., 2008). One of the most significant research milestones in this area was the seminal work of Diamond and his assembly rules model (Diamond, 1975), which suggested that species should co-occur less often than expected by chance, thereby reflecting a major hypothesis at the time that competition was the driving force structuring plant communities. Subsequent community ecology studies have evaluated the underlying processes that may have

generated co-occurrence patterns (Gotelli and Graves, 1996; Sanders et al., 2007). Initially, a theoretical explanation for these patterns came from Grime (1979), who suggested the relative importance of competition as plant communities increased in higher primary productivity scenarios. Later, Bertness and Callaway (1994) developed the so-called “stress-gradient hypothesis” which explicitly considered positive plant–plant interactions in relation to abiotic stresses. The stress-gradient hypothesis (SGH) postulated that facilitation is dominant in harsh conditions whereas competition is more important in benign environments. However, the generality and applicability of this theory has remained controversial due to contradictory results concerning the validity of predictions and the complexity and limitations of field observations (Callaway, 2007; Brooker et al., 2008; Maestre et al., 2009a).

Co-occurrence studies may help to evaluate the effect of environmental factors on positive, neutral or negative relationships among species at the community level (Brooker et al., 2008; Hanski, 2008), particularly if environmental gradients are selected appropriately and co-occurring environmental stressors are controlled adequately (Maestre et al., 2009a). Surprisingly, the majority of such studies have focused on vascular plants, so the biotic interactions among individuals in other taxa remain poorly

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characterized (Brooker et al., 2008). This is remarkable because other organisms seem better suited to testing how interactions might vary with the environment.

In particular, lichens may provide an excellent model system for evaluating co-occurrence patterns, especially epiphytic species, for a number of reasons. First, epiphytic lichens are poikilohydric organisms and both their performance and occurrence is tightly linked to water and light availability (Barkman, 1958; Esseen and Renhorn, 1998). Second, they readily facilitate whole community studies because of their size and growth form. Third, lichen epiphyte communities provide a good model system for testing predictions of the SGH because biotic interactions are considered to be important drivers of their structure (Maestre et al., 2008). It is also easy to find sharp environmental gradients over small spatial scales in lichen communities (Esseen and Renhorn, 1998; Rheault et al., 2003). For example, most studies that have addressed edge effects in epiphytic forest lichens have found drastic community changes, i.e., species disappearances and shifts in species size or growth, along steep edge-interior environmental gradients running up to 100 m from the edge (Johansson, 2008 and references therein). Thus, an environmental gradient may act as a strong habitat filter, thereby restricting the pool of potential colonists at a specific point in the gradient and affecting co-occurrence patterns (Gotelli and Ellison, 2002).

Lichen species compete with each other for space, nutrients, light, and water (Barkman, 1958; Armstrong and Welch, 2007). However, few studies have explicitly evaluated co-occurrence patterns among lichens (Maestre et al., 2008, 2009b). Some authors have suggested that competition is important for determining the distribution of individual species and the composition of the overall community (Armstrong and Welch, 2007 and reference therein), but there are few cases where facilitation has appeared relevant (Maestre et al., 2008, 2009b). To the best of our knowledge, no previous studies have evaluated the interplay between biotic interactions among epiphytic lichens and abiotic stress over an environmental gradient.

Thus, we evaluated the relative importance of different lichen–lichen interactions in structuring epiphytic lichens in a fragmented semi-deciduous Mediterranean forest. We hypothesized that epiphytic lichens would co-occur less often than expected by chance along the edge-interior gradient, while the magnitude of facilitation would increase with abiotic stress (SGH prediction; Bertness and Callaway, 1994). Edges affect epiphytic lichens by exposing them to new gradients of light, humidity, and wind, which could affect the species composition, lichen performance, and turnover along an edge-interior gradient (Renhorn et al., 1997; Kivistö and Kuusinen, 2000; Belinchón et al., 2007). Additional nested tree-scale factors such as the location on a tree, which can be summarized as height and aspect, may modulate this gradient and either exacerbate or mitigate differences along the primary gradient, e.g., a southerly aspect and breast height is generally correlated with drier and sunnier conditions (Matlack, 1993; Chen et al., 1999). First, we tested the hypothesis that epiphytic lichens co-occur less often than expected by chance, by evaluating their co-occurrence patterns at the community level using null models (Gotelli, 2000). Second, we assessed changes in the species co-occurrence pattern as evidence of underlying environmental heterogeneity at two scales (i.e., along the edge-interior gradient and within a tree) by applying generalized linear models. In Mediterranean forests, abiotic stress is located at the forest edges and in southerly exposures, simply because irradiance exacerbates the dry conditions (Belinchón et al., 2007). Finally, we assessed the relative effects of the number of coexisting species, the cover of dominant species, and the bryophyte cover on the observed co-occurrence pattern.

2. Material and methods

2.1. Study site

The study was conducted in “La Herrería”, a well preserved forest of ca. 500 ha located in the Sierra de Guadarrama in the vicinity of Madrid (50 km distance), central Spain (40°34'N, 4°8'W, altitude 985 masl). The climate is subhumid continental Mediterranean with a mean annual temperature of 13 °C, annual precipitation of 739 mm, and a very pronounced seasonal drought during the summer. The tree species composition was dominated by *Quercus pyrenaica* with some scattered individuals of *Fraxinus angustifolia*.

The study forest stand is one of the few remaining unmanaged *Q. pyrenaica* forests in Mediterranean Spain, which is characterized by a good state of conservation (Izco, 1984; Costa Tenorio et al., 1998) and high lichen diversity (Belinchón et al., 2007). The forest was divided into two similarly sized fragments (230 ha and 258 ha) after the construction of a road (M-505) 40 years ago. The road is 20 m wide and its length within the forest is ca 3 km. These two unmanaged forest fragments have not been disturbed since the construction of the road, so we considered that the epiphytic lichen communities would have been relatively stable over this period.

2.2. Sampling design and data collection

Fieldwork was carried out in the western forest fragment (258 ha) between July 2003 and October 2004. We established ten transects perpendicular to the road in a flat area to minimize sources of environmental heterogeneity due to rough and extended terrain. Transects ran 100 m into the forest and they were at least 15 m apart to avoid the overestimation of species with patchy distributions at medium spatial scales (Dauber and Wolters, 2004). Data were also collected from seven forest plots (5 × 5 m) located along each transect. Plots were placed at 5, 15, 25, 35, 45, 55, and 100 m from the edge.

Field sampling was based on the method of Esseen and Renhorn (1998). All epiphytic lichens and bryophytes were included in the study, although bryophytes were not considered at the species level. Within each plot, the occurrence of epiphytic lichens was estimated using two trees per plot (a total of 113 trees). We selected the tree with the greatest diameter and the tree with the diameter closest to the mean tree diameter of the plot to ensure that we sampled good representatives of the most developed epiphytic communities. On some occasions this was impossible because there was only one tree per plot. During lichen sampling, four 20 × 20 cm squares were placed on the bark of each selected tree, i.e., a total sampling effort of 452 squares. This sample size was haphazardly established based on our own field experience with these communities with consideration for two contrasting requirements, i.e., to achieve an accurate representation of the community and to ensure that all species in the sample were genuinely interacting. The number of individuals and their sizes appeared to guarantee this condition. None of the sampling quadrats were fully covered by the thallus of a single species (see Appendices 2 and 3). In order to capture the greatest possible contrast in epiphytic lichen community composition and structure, these plots were placed non-randomly in different locations on a tree. Squares were placed at breast height (BH) and the tree base, in northerly and southerly aspects. Thus, eight squares per plot were surveyed. A digital image was captured of each sampling square at 50 cm distance from the tree bark. The epiphytic lichen cover and the density of each lichen species were calculated using an image analyzer program (Micro-Image v. 4.5). In the field, we also estimated the cover of all species in the square to avoid minor crustose species going unnoticed

during the image analysis. We measured the lichen thallus coverage (cm^2) for the projected area of each thallus within sampling squares by calibrating the quadrat sample, which more accurately recorded the coverage values for each species.

2.3. Assessment of lichen–lichen interactions

We constructed 28 presence-absence matrices for our co-occurrence analysis. Each presence-absence matrix represented one of the seven distances (5, 15, 25, 35, 45, 55, and 100 m) and a combination of the four different square locations (North-Base, North-BH, South-Base, and South-BH). In the matrix, each row represented a different lichen species while each column represented different sampling squares (two trees per plot \times 10 transects, when it was possible to have two trees per plot).

Following Gotelli (2000) we estimated three indices to quantify the patterns of lichen community structure, i.e., the number of checkerboard species pairs (CHECKER), the C-score (C-score), and the number of species combinations (COMBO). Gotelli and Ulrich (2011) recommend using a wide array of null models when basic assembly rule information is unknown. All the indices measured species co-occurrence, although they evaluated different aspects of the co-occurrence pattern. For example, the C-score gives an idea of species segregation (less species co-occurrence) or aggregation (more species co-occurrence), which is useful for distinguishing between facilitation and competition, while it also has the additional advantage that it is insensitive to noise in the data whereas COMBO may reflect the existence of forbidden species combinations (see Gotelli, 2000). The first index was calculated by counting the number of unique pairs of species that never co-occurred. The C-score was 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 , while S is the number of squares where both species occur. This score was then averaged for all possible pairs of species in the matrix. The number of species combinations was counted by checking the columns of each matrix for specific combinations. In a competitively structured community, there should be more checkerboard species pairs, a higher C-score, and a lower number of species combinations than expected by chance (Diamond, 1975; Gotelli, 2000; Gotelli and Ellison, 2002; Gotelli and McCabe, 2002). If facilitation is prevalent, there should be fewer checkerboard species pairs, a lower C-score, and a higher number of species combinations than expected by chance.

We used two null models for each comparison: (1) fixed–fixed method and (2) fixed–equiprobable (Gotelli, 2000). In the fixed–fixed null model, the row and column sums of the original matrix were preserved, so each random community contained the same number of species in each sample unit (column total) while each species occurred at the same frequency as that in the original community (row total) (Connor and Simberloff, 1979). In the fixed–equiprobable null model, only the frequency of each species was fixed while the total number of species supported by a single square was reshuffled randomly.

Therefore, the fixed–fixed null model retained differences in species richness among sites, whereas the fixed–equiprobable model allowed any number of species, from zero to the total number of species recorded. The performance of both null models using the indices employed in this study has been tested extensively and both have good statistical properties (low Type I error and good power in detecting non-randomness; Gotelli and Entsminger, 2003). They also share two characteristics that make them appropriate for the objective of this study (Gotelli, 2000): (1) they maintain the observed species occurrence frequencies, which is a conceptually satisfying assumption that corresponds to a colonization model where species colonize a given plot randomly with

respect to each another; and (2) species occurrences are random with respect to each another, which is an appropriate null model for detecting patterns caused by species interactions. Therefore, the fixed–equiprobable null model assumed that all sampling units were equivalent, whereas the fixed–fixed model preserved the differences among sampling units in terms of the number of lichen species they contained (Gotelli and Rohde, 2002). The fixed–fixed model appeared to be especially suited to island lists whereas the fixed–equiprobable model appeared to be more applicable to sample lists, such as our data set. However, it was not easy to separate both types so we preferred to include both alternatives.

We determined the outcome of the fixed–fixed and fixed–equiprobable null models and three indices (CHECKER, C-SCORE and COMBO) by incorporating different levels of randomness, as recommended by Gotelli (2000). The advantage of this approach is that it can explore the effects of changing model assumptions on the results obtained in a basic comparison of any model conformation using real data (Gotelli, 2000).

2.4. Data analysis

To make the results comparable, we calculated the standardized effect size (SES) for each matrix as $(I_{\text{obs}} - I_{\text{sim}})/S_{\text{sim}}$, where I_{obs} is the observed value for a given index of the lichen community structure, while I_{sim} and S_{sim} are the mean and standard deviation, respectively (Gotelli and McCabe, 2002). The mean and standard deviations of these indices were calculated from 10,000 null randomization matrices.

A one-sample t -test was used to test the null hypothesis that the mean SES measured for the 28 presence-absence matrices did not differ from zero (Gotelli and Rohde, 2002). We performed six such tests (three indices using two null models) and we applied the Bonferroni-type correction for multiple tests ($P < 0.05/k$, where k is the number of independent tests). Co-occurrence analyses and t -tests were conducted using Ecosim 7.72 (Gotelli and Entsminger, 2006) and SPSS 17.0 (SPSS Inc. Chicago, IL), respectively.

These co-occurrence indices were considered as community attributes, which could be used by any modeling approach. To evaluate the effects of distance from the edge and tree scale predictors (aspect of the sampling square and relative height on the trunk of the square) on species interactions (co-occurrence indices), we produced generalized linear models by applying the GENMOD procedure in SAS (v. 9.0). For the C-score fixed–fixed, C-score fixed–equiprobable, and CHECKER fixed–equiprobable indices, we used a normal distribution with an identity link function. For the CHECKER fixed–fixed, COMBO fixed–fixed, and COMBO fixed–equiprobable, we used a Poisson distribution with a log link function. We fitted each model using all applicable link functions and selected the one that minimized the deviance of the model (Herrera, 2000). The model was produced using the distance from the edge, height of the square (BH, base), and the aspect of the square (northerly and southerly) as independent variables.

We also used Spearman's rank correlation coefficient test to explore relationships among the co-occurrence indices and species richness at the square level, bryophyte coverage, and the coverage of the most abundant species, i.e., *Melanelia glabra*, *Parmelina tiliacea*, *Physcia aipolia*, *Physconia distorta*, *Physconia venusta*, and *Ramalina farinacea* (SPSS 17.0; SPSS Inc. Chicago, IL).

3. Results

A total of 57 epiphytic lichen species were recorded in the 452 lichen squares surveyed (App. 1). The mean number of species per 400 cm^2 square was 6.7, with a maximum of 15 species in a square. The mean total cover in the squares was almost 70%, while the

mean size per individual in the most common species was around 6 cm². The most common species (listed above) appeared in approximately 45% of the squares sampled (more than 200 sampled squares), whereas others were rare (<2% of sampled squares), such as *Fuscopannaria ignobilis*, *Leptogium furfuraceum*, *Lobaria pulmonaria*, *Mycobilimbia berengeriana* and *Waynea adscendens*.

The SES of the number of checkerboard species pairs did not differ significantly from zero with the fixed–equiprobable null model (Table 1, Fig. 1a). With the fixed–fixed null model, the corresponding SES value was higher than expected, even after Bonferroni correction (Table 1, Fig. 1a). In this case, more than 14% of matrices had a lower co-occurrence than expected by chance (Table 2). The mean SES obtained with the co-occurrence index C-score was significantly higher than expected with the fixed–fixed null model algorithm, whereas the SES of the C-score was significantly lower than expected with the fixed–equiprobable null model (Table 1; Fig. 1b). This effect was only significant for the fixed–fixed null model after Bonferroni correction, where the null hypothesis accepted almost 30% of the data (Table 1 and 2). The SES value for the number of species combinations (COMBO) did not differ significantly from zero with both of the null models we employed (Table 1, Fig. 1c).

The co-occurrence patterns among the 28 environmental scenarios/matrices varied significantly. Some of the 28 conditions (edge distance × trunk position) contained species aggregation and segregation, whereas others did not differ from random. The generalized linear models showed that the distance from the edge was positively correlated with the number of checkerboard species pairs (fixed–fixed null model), indicating a higher level of species segregation in the forest interior (Table 3). However, the fixed–equiprobable null model was not significant (Table 3). Both C-score null models were not correlated with the independent variables (distance from the edge, aspect, and height of the square). The mean SES value of the number of species combinations was correlated with the aspect of the square when using the fixed–equiprobable null model, indicating the segregation of epiphytic lichens at south aspect (Table 3).

Finally, we found no significant correlation when we evaluated the relationships between the SES values and the coverage of the six most frequent species in the community, when compared with richness and bryophyte coverage in the corresponding matrixes (Table 4).

4. Discussion

Our results showed that lichens co-occurred less often than expected by chance in Mediterranean epiphytic communities, although this segregation pattern was not very strong. The results

of our null models showed that species were organized into checkerboard distributions, which agreed with the basic predictions of Diamond (1975) assembly rules model. However, the magnitude of the species co-occurrence was not affected by the total number of coexisting species, the coverage of dominant species, or the coverage of bryophytes.

After comparing our results with the null model analyses of other communities dominated by different taxa, we found that the SESs for epiphytic lichens were relatively small and closer to those of other poikilothermic organisms (reptiles, amphibians, invertebrates, and fish), rather than mammals, birds, ants or vascular plants (Gotelli and McCabe, 2002; Gotelli and Rohde, 2002). The epiphytic lichen assemblages contained species with different ecological requirements that belonged to different functional groups, as suggested by the high diversity of morphologies, reproductive modes, or types of photobionts. For example, one group of species had a preference for the forest interior. Some of these species contained cyanobacteria as a photosynthetic partner, so they required some degree of shade for their development and stability in humid conditions (e.g., *Collema fragrans*, *Leptogium furfuraceum*, *Lobaria pulmonaria*, and *M. berengeriana*). However, another group of species preferred edge habitats, e.g., *Physcia aipolia*, *Physconia distorta*, and *Physconia enteroxantha*. Fruticose species were mainly associated with the upper part and northerly aspects of tree trunks, whereas foliose species were found in the most exposed sites, such as southerly exposures and at BH. Crustose species were found mainly on the lower part of the trunks with a northerly exposure. Thus, the assemblages represented in the matrices did not correspond to true ecological guilds (Simberloff and Dayan, 1991), so all of these analyses potentially suffered from the so-called “dilution effect” (Diamond and Gilpin, 1982) and our tests for segregation patterns may have been too conservative. For example, generally speaking, crustose species are generally less competitive than foliose species, which are less aggressive than fruticose, and these represent the general stages of succession (Topham, 1977). As a consequence of these life history characteristics, niche overlap should be limited and the overall strength of co-occurrence patterns in this community may be weak (Hart and Marshall, 2009). Thus, coexistence in our communities was probably determined by a strong environmental filter via propagule anchorage mechanisms and/or emergence, and growth requirements.

For a long time, non-random co-occurrence patterns have been interpreted as a result of interspecific competition (Diamond, 1975; Tilman, 2007; Hanski, 2008). However, the most popular conceptual model is now that proposed by Bertness and Callaway (1994), which was derived from Grime's hypothesis that the relative importance of competition in plant communities increased in higher primary productivity conditions (Callaway, 2007). Bertness and Callaway postulated that competitive interactions would be most important when abiotic stresses did not limit the ability of plants to acquire and exploit resources, which implied higher productivity. In the case of lichens, it has been argued that competitive interactions among species arise as a result of competition for space (Shimizu, 2004; Maestre et al., 2008) and because of differences in the environmental conditions (Yarranton, 1972). Our results showed that species segregation, which is a surrogate of competition, was higher as the distance from the edge increased and in sunnier southerly aspects. The current paradigm arose from the SGH so the conditions should be less stressful (Bertness and Callaway, 1994), which from a lichenological point of view seems counterintuitive, at least in relation to the sunnier southerly orientations because water conditions are limiting in Mediterranean climates. However, this pattern cannot be attributed solely to competitive interactions. It is known that

Table 1

Summary of the null model tests for deviations of epiphytic lichens co-occurrence matrices from randomness. Significant *P*-values (after Bonferroni correction for multiple testing) are in bold. CHECKER = number of species pairs forming a checkerboard distribution; COMBO = number of species combinations. Fixed–fixed (ff) = null model in which matrix row and column sums are preserved; Fixed–equiprobable (fe) = null model in which the rows are fixed and the column total of the matrix are allowed to vary freely. SES: standardized effect size.

Index	Null model	Average SES	<i>t</i>	<i>p</i>
CHECKER	ff	0.81	2.85	0.008
CHECKER	fe	−0.10	−0.33	0.744
C-score	ff	0.89	4.00	0.000
C-score	fe	−0.76	−2.35	0.026
COMBO	ff	−0.73	−1.33	0.194
COMBO	fe	−0.48	−1.29	0.206

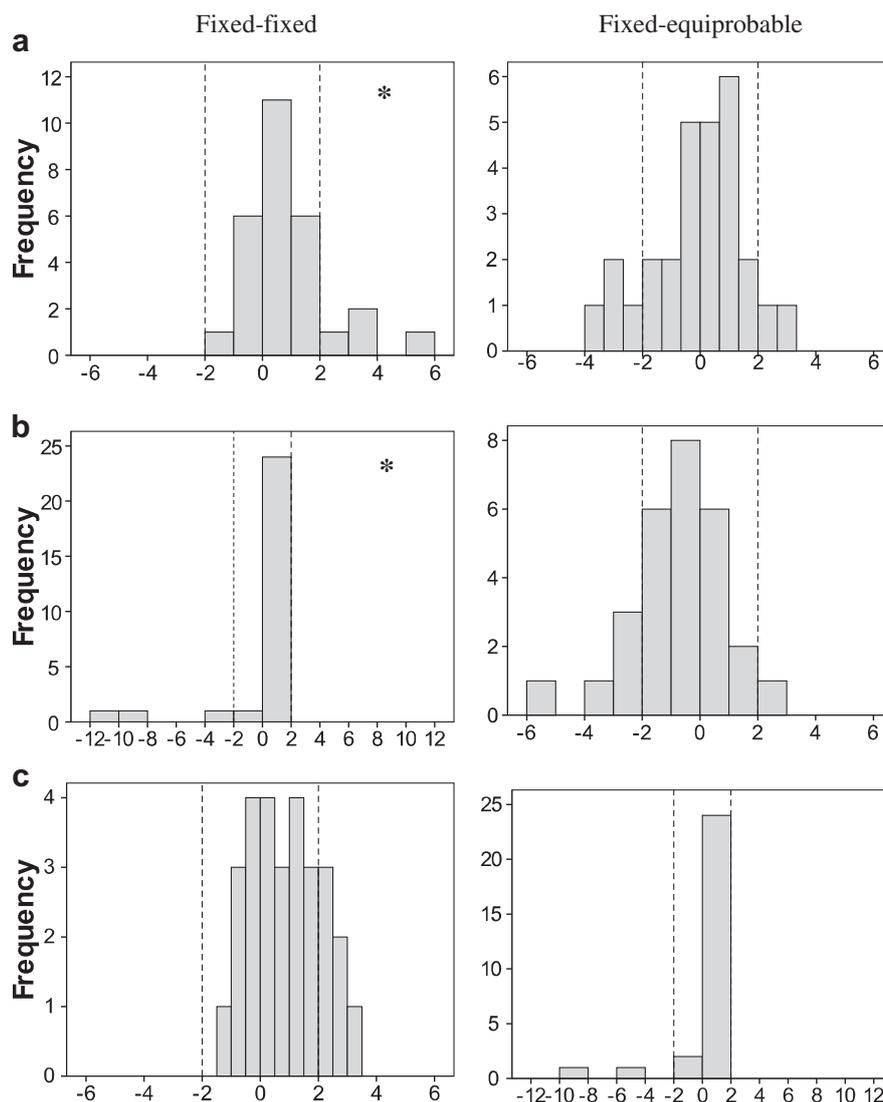


Fig. 1. Histograms for standardized effect sizes (SES) measured in presence-absence matrices of epiphytic lichens. Each observation is an SES for a different epiphytic lichen presence-absence matrix. The dashed vertical lines indicate ± 2 standard deviations, the approximate boundaries for statistically significant patterns. The null hypothesis is that the mean size equals 0. (a) Number of checkerboard species pairs; (b) C-score; (c) Number of species combinations. In all figures, the asterisk indicates the tail for which species co-occurrence would be less than expected by chance, indicating competitive structuring. See Table 1 for observed means.

differences in the species composition and lichen performance along the edge-interior gradient are related to environmental variation at different scales in both Mediterranean forests (Burgaz et al., 1994; Belinchón et al., 2007) and boreal forests (Renhorn et al., 1997; Kivistö and Kuusinen, 2000; Will-Wolf et al., 2006). These relatively harsh physical conditions (e.g., forest edge, southerly aspect, or BH) may act as a strong habitat filter, thereby

Table 2
Null model comparisons of Standardized Effect Size (SES) among indices. Values represent the times in which segregation or aggregation occurred more frequently than expected by chance. Differences were considered significant if observed values differed from zero (Table 1). CHECKER = number of species pairs forming a checkerboard distribution; COMBO = number of species combinations.

	Fixed-fixed		Fixed-equiprobable	
	Segregation	Aggregation	Segregation	Aggregation
CHECKER	14.3%	3.6%	10.7%	17.8%
C-score	28.6%	–	10.7%	25%
COMBO	–	7.14%	–	7.14%

restricting the pool of potential colonizers and affecting the overall co-occurrence pattern (Gotelli and Ellison, 2002). For example, in our study area, changes in the forest environment at the two spatial scales studied (stand and tree) along the edge-interior gradient had

Table 3
Results from GENMOD examining the influence of stress variables on co-occurrence indices. Chi-square values of the variables and sign of the coefficients are indicated (** $P < 0.001$; * $P < 0.05$; ns: non significant). CHECKER = number of species pairs forming a checkerboard distribution; COMBO = number of species combinations. Fixed-fixed (ff) = null model in which matrix row and column sums are preserved; Fixed-equiprobable (fe) = null model in which the rows are fixed and the column totals of the matrix are allowed to vary freely.

	CHECKER		C-score		COMBO	
	ff	fe	ff	fe	ff	fe
Distance to edge	+11.65**	+0.45 ns	+3.48 ns	+0.07 ns	–0.09 ns	–2.88 ns
Height of the square	+1.91 ns	–0.17 ns	–0.01 ns	–1.28 ns	+0.09 ns	–1.10 ns
Aspect of the square	+0.24 ns	–0.25 ns	+0.55 ns	–0.43 ns	–0.85 ns	–7.82*

Table 4

Correlation matrix (Spearman's coefficient) for community traits and co-occurrence indices. CHECKER = number of species pairs forming a checkerboard distribution; COMBO = number of species combinations. Fixed–fixed (ff) = null model in which matrix row and column sums are preserved; Fixed–equiprobable (fe) = null model in which the rows are fixed and the column totals of the matrix are allowed to vary freely. SES: standardized effect size. * $p < 0.05$; $n = 28$.

	Richness	Bryophyte cover	<i>Melanelia glabra</i>	<i>Parmelina tiliacea</i>	<i>Physcia aipolia</i>	<i>Physconia distorta</i>	<i>Physconia venusta</i>	<i>Ramalina farinacea</i>
CHECKER ff	−0.053	0.178	−0.020	−0.211	−0.257	−0.359	−0.162	−0.041
CHECKER fe	0.016	−0.004	0.152	0.137	−0.153	−0.166	0.143	0.001
C-score ff	0.171	0.065	0.031	−0.007	−0.191	−0.086	0.276	−0.206
C-score fe	−0.014	−0.196	0.222	0.326	0.117	0.034	0.166	−0.076
COMBO ff	0.119	−0.103	0.018	−0.113	0.098	−0.066	−0.097	−0.248
COMBO fe	−0.098	−0.317	0.198	−0.052	0.314	0.209	−0.024	−0.282

direct consequences on the composition of the epiphytic lichen communities (i.e., different lichen groups in relation to their characteristics and position on a tree). Therefore, if some species responded differentially to the microhabitat then subtle “habitat checkerboards” may lead to species segregation, which is not necessarily related to competition (Gotelli and McCabe, 2002).

It is well-known that the relationship between light and moisture is critical for the dynamics of epiphytic lichens (Barkman, 1958; Palmqvist and Sundberg, 2000). Light and water availability in Mediterranean forests are recognized as highly heterogeneous in space and time (Marañón et al., 2004; Gómez-Aparicio et al., 2005). Water is the main limiting ecological factor in Mediterranean forests (Marañón et al., 2004; Valladares, 2004). In contrast, light is rarely a limited resource because canopies are not sufficiently closed to filter a significant fraction of the light from the ground in forests (Valladares, 2004). However, this scheme is more complex and Pintado et al. (1997) suggested that light efficiency during brief periods of suitable moist conditions was also a crucial factor in most Mediterranean habitats. Indeed, lichen metabolism is regulated primarily by water availability (Lange et al., 1986; Green et al., 1994), but it appears to be limited by the available irradiance when wet and active (Nash, 1996; Renhorn et al., 1997). Mediterranean areas are characterized by relatively low rainfall, high temperatures, and a severe summer drought, so the water-light micro-environment conditioned by the forest canopy is essential for lichens (Burgaz et al., 1994; Aragón et al., 2010). As noted above, a southerly aspect had a negative effect on the number of species combinations in the fixed–equiprobable null models. Thus, aspect appeared to be critical for light (Matlack, 1993; Chen et al., 1999). *Q. pyrenaica* is a semi-deciduous tree with leaves throughout most of the year (Costa Tenorio et al., 1998) and this characteristic may reduce the irradiance in the understory, thereby making a southerly aspect more suitable for lichen development by providing an equilibrium level of water-light availability. This interpretation of environmental conditions may also match the predictions of the SGH because southerly exposures in the forest interior should be particularly suitable for promoting lichen performance, thereby reducing any stress limitations.

The increased competition with the distance from the edge is also related to the balance between light and water in boreal and temperate forests (Esseen and Renhorn, 1998; Kivistö and Kuusinen, 2000; Rheault et al., 2003; Boudreault et al., 2008), as well as Mediterranean forests (Belinchón et al., 2007). The impact of edges on epiphytic lichen composition is mainly associated with microclimatic changes (i.e., decreasing humidity and high light levels at the forest edge), which can affect the metabolic activity of lichens (Gauslaa and Solhaug, 2000; Palmqvist and Sundberg, 2000). As suggested above, light does not appear to be a limiting factor in Mediterranean forests compared with boreal or temperate forests (Valladares, 2004), whereas humidity appears to be critical in these systems, especially during the summer drought when the physiological activity of these poikilohydric organisms is severely limited (Pintado et al., 1997). The forest interiors of semi-deciduous

stands are not considered to be closed canopies, so medium light irradiance may not be functionally important (Percy et al., 1994; Valladares, 2004). Thus, epiphytic lichens in Mediterranean forests may have an adequate light-water trade-off, even in the forest interior (see Belinchón et al., 2007).

The co-occurrence patterns were also unaffected by the size of the species pool, the dominant species coverage, and the bryophyte coverage, although we previously detected significant shifts in composition along the primary edge gradient (see Belinchón et al., 2007). Species richness did not vary significantly along the edge-interior gradient (see Belinchón et al., 2007), but it is expected that a few species could dominate in each location due to environmental filtering (possibly because of their greater capacity for capturing and accumulating resources). The remaining species would be poorer competitors in these conditions and they have to survive in an environmental matrix that is moulded by interactions among the dominant species (Keddy, 2005). For example, Maestre et al. (2009b) found that segregated co-occurrence patterns in biological soil crust-forming lichens were mainly promoted as the *Diploschistes diacapsis* coverage increased, which was the most abundant lichen in the community. Indeed, there is evidence from studies of ant communities that the dominant species may control the spatial occurrence of other species (see Ward and Beggs, 2007). Thus, the lack of any bryophyte coverage effect may be surprising, because previous studies have suggested their influence on epiphytic lichen establishment and performance (i.e., *Lobarion* spp.; see Barkman, 1958; Öckinger et al., 2005; Belinchón et al., 2009). Lichens and bryophytes are often ecologically equivalent, occupying many of the same niches on trees and soil (Barkman, 1958). Both are found in a range of micro-environmental conditions, suggesting that although both epiphytic groups prefer different light and moisture micro-habitats, they can still survive in less preferred conditions (Barkman 1958; Sedia and Ehrenfel, 2003). In the study area, we found that lichen and bryophyte coverage had opposite relationships to light and moisture availability (see Belinchón et al., 2007). Thus, the low overlap in habitat use between lichens and bryophytes may explain the lack of change in the co-occurrence patterns in our study.

In summary, our findings suggest that the net outcome of biotic interactions was weak and it tended to be related to environmental conditions at the tree scale and the distance from the forest stand to the edge. Our co-occurrence analysis supported the predictions of Grime (1979) because epiphytic lichens were competitively structured and we found no evidence of facilitation in our environmental framework. This result did not agree with our previous study of biological soil crusts (Maestre et al., 2008, 2009b), where the co-occurrence analyses matched the predictions of Bertness and Callaway's (1994) hypothesis that facilitation would be dominant under stressful conditions. Thus, positive interactions (i.e., facilitation) increased in stressful conditions, depending on the abiotic stress estimator and the spatial scale employed.

It is well-known that microclimatic variations (such as light and moisture) are very important in determining the presence of

epiphytic lichens in forests and community shifts along primary edge gradients (e.g., distance from the edge and the aspect on trees; see Belinchón et al., 2007 for more details). Our results also showed that they are critical for the establishment of relationships among species. There is a general lack of knowledge about the influence of other biotic community attributes on co-occurrence patterns, but we found that the co-occurrence patterns were unaffected by the pool of species, the dominant epiphytic lichens, or bryophyte coverage.

Finally, we conclude that the integration of biotic and abiotic factors in co-occurrence studies may facilitate a better evaluation of community structure and a better knowledge of how communities are formed. Our results fill a gap in the knowledge of epiphytic lichen ecology, but our understanding of interactions in lichen communities and pair-wise lichen interactions remains extremely limited.

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