Plant Soil (2015) 386:303–316
DOI 10.1007/s11104-014-2256-9

REGULAR ARTICLE

Aspects of soil lichen biodiversity and aggregation interact to influence subsurface microbial function

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Received: 4 February 2014 / Accepted: 28 August 2014 / Published online: 18 September 2014

Abstract
Background and aims Many previous studies have evaluated aboveground–heterotrophic belowground interactions such as plant-soil feedbacks, plant-mycorrhizal fungi associations or plant-actinorhizal symbioses. However, few studies have used biocrusts, which are specialized soil communities of autotrophic cyanobacteria, mosses, lichens and non-photosynthetic fungi and bacteria that are prevalent in drylands worldwide. These communities largely influence ecosystem functioning, and can be used as a model system for studying above-belowground interactions. In this study, we evaluated how biocrusts affect the functional diversity and biomass of microbial diversities beneath biocrusts.
Methods We performed two microcosm experiments using biocrust-forming lichens where we manipulated their biotic attributes to test independently the effects of species richness (from two to eight species), composition, evenness (maximal and low evenness) and spatial pattern (clumped and random distribution) on the microbial catabolic profile and microbial functional diversity.
Results Microcosms with a random pattern had a higher microbial catabolic profile than those with a clumped pattern. Significant richness × evenness × pattern and richness × evenness interactions were found when analyzing microbial catabolic profile and biomass, respectively. Microcosms with a random pattern, intermediate number of species, and maximal evenness level had higher microbial catabolic profile. At the maximal evenness level, assemblages had higher microbial catabolic profile and microbial biomass when they contained four species. The richness × evenness × pattern interaction was the most informative predictor of variations in microbial catabolic profile.
Conclusions Our results indicate that soil microorganisms are influenced by biocrusts, just as they are influenced by plants, and highlight the importance of higher order interactions among species richness, evenness, and spatial pattern as drivers of microbial communities. The

Responsible Editor: Ute Skiba.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-014-2256-9) contains supplementary material, which is available to authorized users.

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results also emphasize the importance of studying several biotic attributes simultaneously when studying biocrust-soil microorganism interactions, as in nature, community properties do not exert their influence in isolation.

Keywords Richness · Evenness · Spatial pattern · Microbial biomass · Basal respiration · Microbial communities and biological soil crusts

Introduction

Soil organisms are intimately linked to the plant community. Not only do plants (in a broad sense, inclusive of terrestrial photo-autotrophs outside of kingdom *Plantae*) provide resources such as carbon and shelter to the community, but their tissues also act as a host for many soil organisms, such as herbivores, pathogens, and symbionts (Wardle et al. 2004; Donoso et al. 2013). A large body of literature has studied specific types of autotrophic aboveground–heterotrophic belowground interactions, such as plant-soil feedbacks (Bever 2002; Kardol et al. 2006; Vivanco and Austin 2008), plant-mycorrhizal fungi associations (van der Heijden et al. 1998; Johnson et al. 2005; Antoninka et al. 2009) or plant-actinorrhizal symbiosis (Tjepkema et al. 2000; Schwintzer and Tjepkema 2001). These interactions have also been addressed in drylands (McHugh and Gehring 2006), which collectively constitute ~41 % of the terrestrial surface in the form of arid, semi-arid, and sub-humid deserts, steppes, woodlands and grasslands (Maestre et al. 2012a). Biological soil crusts (biocrusts hereafter) are a highly specialized community of autotrophic cyanobacteria, mosses, and, lichens, and non-photosynthetic fungi and bacteria that usually cover the soil surface of the open spaces located between plant patches in drylands worldwide (Belnap and Lange 2003). These communities have been seldom studied as the autotroph component of aboveground–belowground interactions, despite that they can be of particular utility in determining which of several properties of autotrophic communities exert the greatest influence on belowground heterotrophs (Bowker et al. 2010).

Biocrusts exert a strong influence on ecosystem multifunctionality (i.e. ability of communities to simultaneously sustain multiple ecosystem functions (Bowker et al. 2010, 2011, 2013; Maestre et al. 2012b). They contribute to carbon and nitrogen fixation (Lange et al. 1992; Belnap 2002), confer resistance to erosion (Chaudhary et al. 2009), and modulate soil nutrient mineralization, total C released by soil respiration and the water runoff-infiltration balance (Castillo-Monroy et al. 2010, 2011a; Eldridge et al. 2010). In addition, they exert a strong influence on communities such as vascular plants (Green et al. 2008), microfauna (Neher et al. 2009), fungi (Bates et al. 2010) and bacteria (Yeager et al. 2004; Castillo-Monroy et al. 2011b).

Recent research suggests that biocrusts may serve as a useful model system for evaluating biodiversity-ecosystem functioning relationships in soils (Bowker et al. 2010; Maestre et al. 2012b). Biocrusts may have similar value as a model for plant-soil interactions, as their visible components are taxonomically well-defined and the manipulation of their attributes (e.g., biodiversity, spatial pattern and composition) in experiments is fully feasible (Maestre et al. 2012b, Bowker et al. 2014). The biodiversity and ecological importance of biocrust-associated bacteria and fungi is well documented (e.g. Billings et al. 2003; Yeager et al. 2004, Gundlapally and Garcia-Pichel 2006; Bates et al. 2010), as are patterns of microbial community abundance, distribution, or composition (Steven et al. 2013) and temporal patterns (Johnson et al. 2012) in biocrusts and underlying soils. However, only a few studies have examined how particular attributes of the autotrophic components of biocrusts, such as their abundance or biodiversity, influence the abundance and/or functioning of the associated microbial communities (Castillo-Monroy et al. 2011b; García-Palacios et al. 2011; Maestre et al. 2012b; Bowker et al. 2013). In this study we evaluated, for the first time and using a manipulative experimental approach, how different biodiversity components (species composition, richness, and evenness) and the spatial pattern of biocrust autotrophs affect the catabolic profile, functional diversity, biomass and basal respiration of the soil microbial communities beneath them.

We previously showed that species richness, composition and spatial pattern affected multiple ecosystem functions such as organic C, total N, N availability, β-glucosidase activity, although the magnitude and direction of their effects varied with the particular function, experiment and soil depth considered (Maestre et al. 2012b). Here we present new measurements from this experiment including microbial catabolic profile, microbial functional diversity, basal respiration and active microbial biomass, and test the hypothesis that soil microbial functional diversity is influenced by the attributes of biocrust-forming autotrophs.

Material and methods

Experimental design

Two manipulative microcosm experiments were conducted at the plant growth facilities of the Rey Juan Carlos University (Móstoles, Central Spain, 40° 20′ 28″ N, 3° 52′58″ O, 650 m a.s.l.) under natural light, temperature and rainfall conditions between June 2006 and December 2008. Soil and biocrust-forming lichen species for these experiments were collected from gypsum outcrops located in the surroundings of the University. The species used in the experiments were selected among the pool of the ten most common lichen species found in gypsum outcrops from Central Spain (Maestre et al. 2008; Castillo-Monroy et al. 2010): *Acarospora nodulosa* (Dufour) Hue., *Collema crispum* (Hudson) Weber., *Diploschistes diacapsis* (Ach.) Lumbsch, *Squamarina lentigera* (Weber) Poelt., *Fulgensia subbractaceata* (Nyl.) Poelt., *Lepraria membranaceum* (Dickson) Vainio., *Psora decipiens* (Hedw.) Hoffm., *Cladonia convoluta* (Lam.) Anders., *Squamarina cartilaginea* (With.) P. James. and *Toninia sedifolia* (Scop.) Timdal.

We created microcosms with lichen colonies placed over the surface of soil, with a constant lichen cover of 60 %, but imposed variation in species richness, evenness, spatial patterning (aggregated vs. random), and different composition (Fig. 1; Maestre et al. 2012b for details). The first experiment (hereafter Composition experiment) was designed to independently test for the effects of species richness (Ri), species composition (Co), and spatial pattern (Pa) on microbial catabolic profile and functional diversity. Four Co levels were nested within two Ri levels (four and eight species). Each combination of Co and Ri was established under two Pa: clumped and random. Each combination of Ri (2), Co (4) and Pa (2) was replicated six times for a total of 96 microcosms (Appendix A). The second experiment (hereafter Evenness experiment) was set up to independently test for the effects of Ri (two, four and eight species), species evenness (Ev) (maximal evenness vs. communities with a geometric distribution of abundances among species; Wilsey and Polley 2004) and Pa (clumped and random). Each combination Ri (3), Ev (2) and Pa (2) was replicated six times for a total of 72 microcosms (Appendix B).

Characterization of microbial communities and activity

At the end of the experiment, a composite soil sample (0–2 cm depth) from the portion of the microcosm covered by lichens was obtained. The lichens were removed prior to sampling, and the samples were stored in plastic bags at −80 °C until analyses.

We analyzed soil heterotrophic microbial functional diversity using MicroResp™ (Campbell et al. 2003). This is a whole-soil method based on community level physiological profiles obtained by testing different carbon sources that vary in structural complexity and that

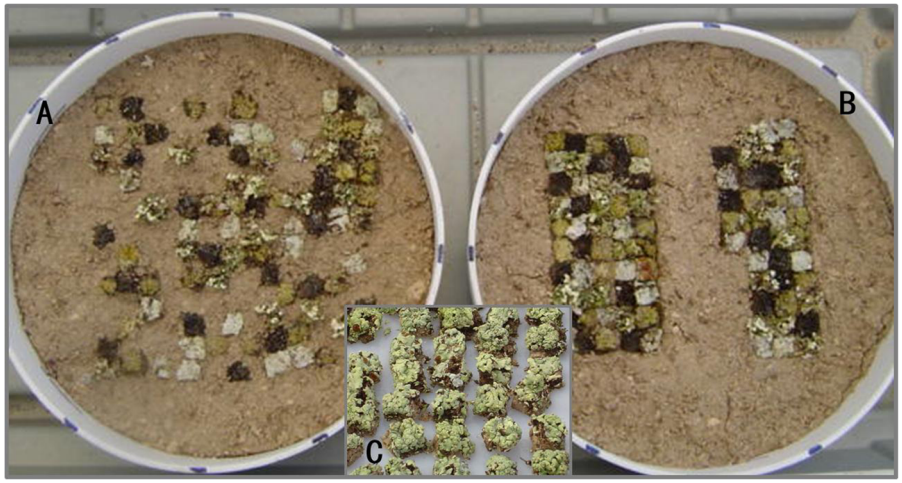

Fig. 1 Artificial biocrust microcosm created using the "mosaic" technique. Examples of microcosms with a random (**a**) and clumped (**b**) spatial pattern and intact lichen pieces collected from the field, cut into homogeneous 0.5 cm-side square fragments (**c**). See Maestre et al. 2012a for details

provide information about the ability of the microbial communities to catabolize different C sources (Oren and Steinberger 2008a). We modified the protocol of the MicroResp™ system to include C-compounds produced by lichens (e.g., phenolic acids). We used amino acids (L-alanine, L-cysteine-HCl and N-acetyl-glucosamine), carbohydrates (D-fructose, D-galactose, D-glucose and L-arabinose), carboxylic acids (citric acid, L-malic acid, oxalic acid and γ amino butyric acid), phenolic acids (orcinol and anthraquinone) and fatty acids (Tween 80 and dextrin). In functional terms, the substrate utilization rates of the carbon sources correspond to the catabolic attributes of different soil microbial functional groups (Zak et al. 1994). Even if we cannot assess microbial communities in relation to taxonomic or phylogenetic diversity (Øvreås 2000), we can use MicroResp™ data to interpret changes in microbial functional diversity (Oren and Steinberger 2008a; García-Palacios et al. 2011). Prior to MicroResp™ analyses, defrosted soils were introduced into deep well plates and pre-incubated for 5 days at 25 °C. The moisture within the plates was corrected to 40 % water holding capacity to condition the soils and reestablish active microbial populations. The plates were then incubated for 6 h and read at 595 nm. The results were calculated on the basis of the 16th substrate (water), which represents the basal respiration, as explained in García-Palacios et al. (2011). Although potential changes in microbial communities may have occurred due to freeze-thaw cycles, samples are still comparable because all the soils used in this study were subjected to the same storage conditions.

Microbial catabolic profile was characterized as the data matrix of the respiration of the carbon sources used. Using these data, we also calculated the Shannon-Weaver index as $H' = -\sum Pi\ (\ln Pi)$, where Pi is the activity of a particular carbon source/the sum of activity of all carbon sources (Yu et al. 2012). Thus, high H′ values would indicate a greater capacity of the microbial community to catabolize a gradient of C recalcitrance.

Control wells were amended with water only, which provided basal respiration and glucose-induced respiration; this was assumed to be proportional to active microbial biomass (Oren and Steinberger 2008b, Ben Sassi et al. 2012). The metabolic quotient (qCO_2) was also calculated as the ratio between basal respiration and microbial biomass (Oren and Steinberger 2008b). All dependent variables used in the entire study were measurements from the MicroResp assay.

Statistical analyses

We analyzed the results of the two experiments separately. To evaluate the effects of the different factors evaluated on univariate (H′, basal respiration, microbial biomass, qCO_2) and multivariate (microbial catabolic profile) response variables obtained from MicroResp, we used a three-way nested ANOVA/MANOVA model in the Composition experiment (Ri and Pa were considered fixed factors, while Co was a random factor nested within Ri), and a factorial ANOVA/MANOVA model in the Evenness experiment (Ri, Pa and Ev were considered fixed factors). In the Composition experiment, species richness was tested against composition, species composition(richness) and the spatial pattern × composition(richness) interaction were tested against the error term, and spatial pattern and the spatial pattern × species richness interaction were tested against the spatial pattern × composition(richness) interaction (Doncaster and Davey 2007). In the Evenness experiment, all the factors were tested against the error term. The semiparametric PERMANOVA approach (Anderson 2001) was preferred to traditional ANOVA because our response variables did not follow ANOVA/MANOVA assumptions (normality and homogeneity of variances). All PERMANOVA analyses were conducted using the Euclidean distance and 9999 permutations of the raw data. To aid in the interpretation of the effects of the factors when analyzing multivariate MicroResp data (microbial catabolic profile), we also conducted a principal coordinate analysis (PCO; Anderson et al. 2008) using the Euclidean distance.

We also used structural equation models (SEM; Grace 2006), which can be helpful in determining the relative influence of multiple factors on the response variables. SEMs have a variety of uses, including improved causal inference from observational data, and partitioning of direct and indirect effects (Shipley 2000; Grace 2006). They are also useful for answering questions related to relative importance of factors because effect sizes can be described in a common currency, the path coefficient. A path coefficient expressed the strength of the effect of one variable upon another. In their standardized format, ranging from 0 to 1, path coefficients are equivalent to partial correlation coefficients or regression weights. Associated with each path coefficient estimate is a probability test that estimates the probability that the path estimate is equal to zero.

SEMs can evaluate the interrelationships among multiple variables, but does not directly analyze multivariate responses. To express the microbial catabolic profile data in a format compatible with this technique, we conducted some data reduction using a Principal Components Analysis (PCA) on the correlation matrix of the different carbon sources used in the MicroResp analyses. In both Experiments, the first component explained a large proportion of the variation, (65 and 69 %, respectively), thus it was used as a parsimonious summary of these data.

Our SEMs are constructed similarly to a multiple regression with experimental factors exerting an influence on the PCA components. However, they differ from multiple regressions in several ways; i) spatial pattern and evenness were coded as 0 or 1; ii) composition was treated using a composite variable. Composite variables have multiple uses, but here they function to sum together the effects of the levels of a categorical variable, which are represented by dummy variables. This use of the composite variable is a graphical and numerical interpretational aid that does not alter the underlying model (Grace 2006). It simply sums together the effects of multiple conceptually related variables upon another, collapsing them into a single path coefficient; iii) we constructed an interaction term for the Ri effect in the Evenness experiment. First we viewed a plot of means and confidence intervals of the microbial PCA axis as a function of this interaction term. It was clear that the samples driving this interactive effect were those with a random spatial pattern, high evenness, and intermediate richness. Thus the simplest method to express this was to create another binary coded variable, wherein samples with this treatment combination were coded as "1" and the rest were coded as "0"; iv) richness and composition were explicitly allowed to covary because they are correlated due to nesting in the Composition experiment; and v) the Ri × Ev × Pa term was allowed to covary with the main effects in the Evenness experiment, because it is mathematically derived from them.

An overall goodness-of-fit test is usually performed with a SEM. It tests whether the proposed structure of the model is a reasonable representation of the causal relationships underlying the correlations among variables. In our case, the causal structure of the system is known with certainty because most variables are experimental factors which can affect a response, but cannot be affected by a response. Nevertheless, we verified model fit, using the traditional χ^2 goodness-of-fit test to assure that we had not misspecified the models. Unlike most probability tests, this goodness-of-fit tests the probability that the model fits the data (or more precisely, that the covariance matrix implied by the model structure is similar to the covariance matrix derived directly from the data). Upon verifying fit, we obtained estimates of path coefficients and associated probability levels. PERMANOVA, SEM, and PCA analyses were conducted using PERMANOVA + for PRIMER statistical package (PRIMER-E Ltd., Plymounth Marine Laboratory, UK), Amos 18.0 statistical software (SPSS Inc., Chicago, IL, USA), and JMP 4.0 (SAS Institute Inc., Cambridge, Ma, USA), respectively. The raw MicroResp data from our experiments are available from figshare (Castillo-Monroy and Maestre 2014; see appendices I and J for treatment summaries).

Results

We did not find any significant effect of attributes of biocrust on the soil microbial catabolic profile, H′, microbial biomass, basal respiration and metabolic quotient when analyzing data from the Composition experiment (Fig. 2, Appendices C, E). However, we found a significant effect of Pa ($F_{1,60}$ = 3.111, P= 0.042) on microbial catabolic profile in the Evenness experiment; randomly patterned samples had higher microbial catabolic profile that those with a clumped pattern. Significant Ri × Ev × Pa ($F_{1,60}$ = 2.915, P= 0.025) and Ri × Ev ($F_{1,60}$ = 3.114, P=0.021) interactions were also found when analyzing microbial catabolic profile in the Evenness experiment (Fig. 3, Appendices D, F). A significant Ri × Ev interaction was also found when analyzing microbial biomass from this experiment ($F_{1,60}$ = 5.248, P=0.008). We investigated these interactions by conducting separate PERMANOVAs for each Ev level. At the maximal evenness level, assemblages had higher microbial catabolic profile and biomass when they contained four species (Appendix H). At this evenness level, a significant effect of Pa was also found ($F_{1,30}$ = 3.889, P=0.027), with randomly patterned microcosms having higher microbial catabolic profile than those with a clumped pattern. Overall,

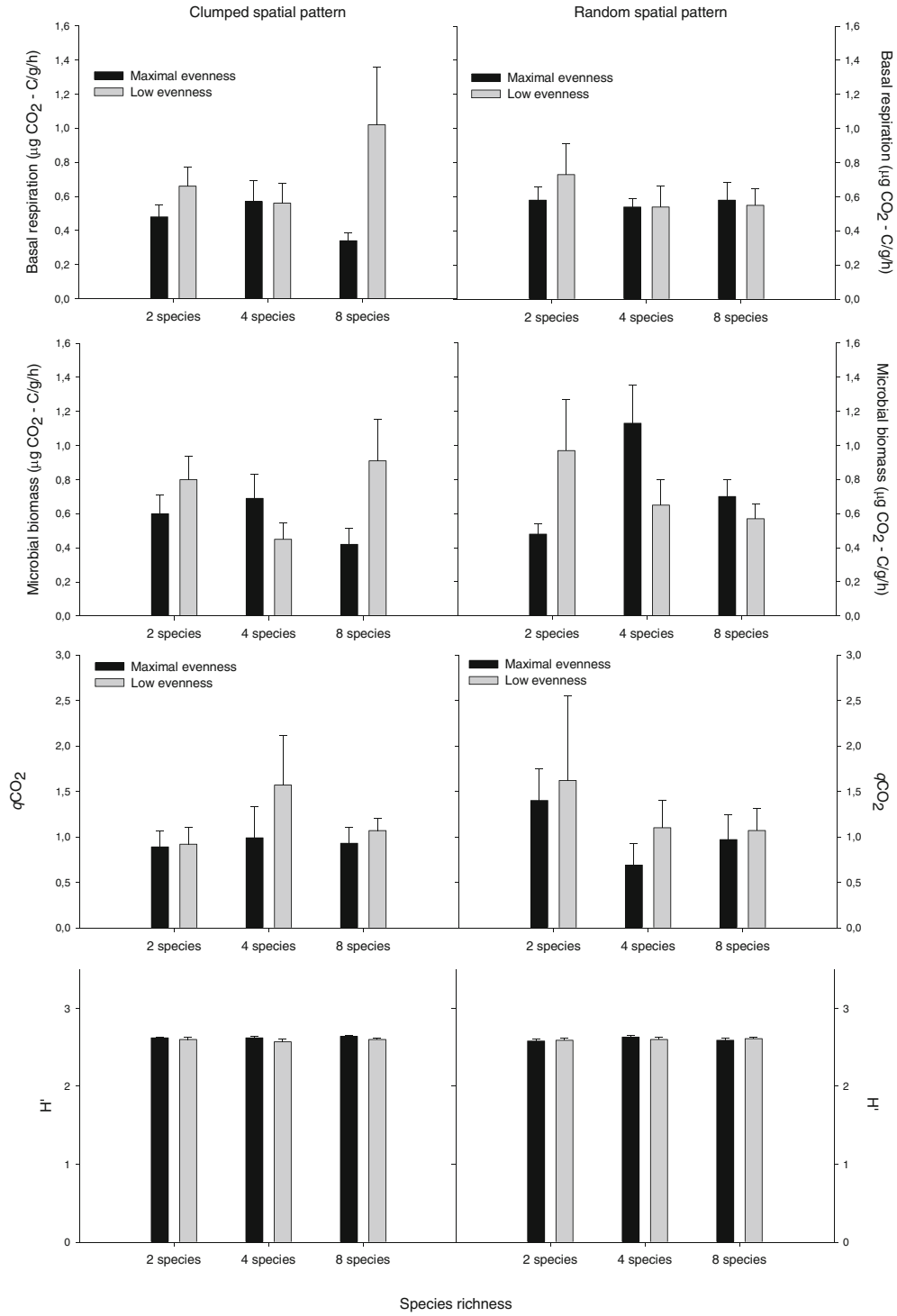

Fig. 2 Soil variables measured in the Composition experiment. Data represent means ± SE (n=6). qCO_2 = metabolic quotient, H′ = Shannon-Weaver index. A to D denote the four composition levels evaluated

microcosms with a random pattern, four species and maximal evenness level had higher microbial catabolic profile. No significant main effects or interactions were found when analyzing basal respiration

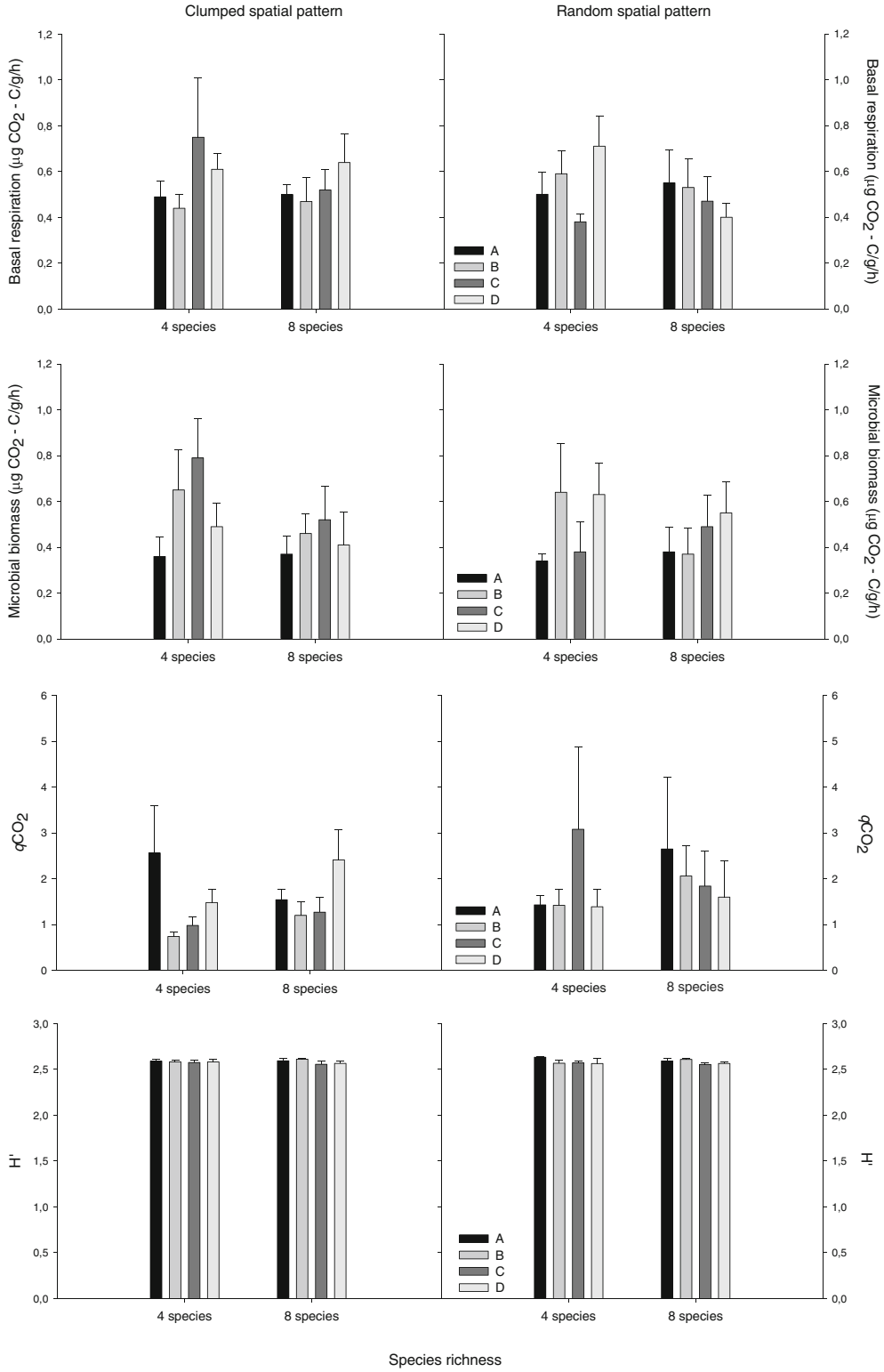

Fig. 3 Soil variables measured in the Evenness experiment. Data represent means ± SE, n=6 in all cases. q CO_2 = metabolic quotient, H′ = Shannon-Weaver index

and metabolic quotient from the Evenness experiment (Appendix D, F).

None of the biocrust community factors evaluated affected the H′, in either experiment (Figs. 2 and 3; Appendices E, F). However when we analyzed H′ for the five carbon groups independently, we found that microcosms with a random Pa had higher H′ values of amino acid than those with a clumped Pa ($F_{1,60} = 5.021$, P=0.028; Fig. 4). Among the five carbon groups, carbohydrates had the highest H′ values followed by carboxylic, amino acid and then fatty and phenolic acids, regardless the factor evaluated (Fig. 4).

Our SEMs provided estimates on the relative effects of the attributes of biocrust upon the microbial catabolic profile (Table 1). We did not find significant main or interactive effects in the Composition experiment, but were most successful modeling the Evenness experiment, explaining nearly 20 % of the variance found in this response variable (Fig. 5). In this experiment, the Ri × Ev x Pa interaction (r=0.35, P<0.002) was the most informative predictor of variation in microbial catabolic profile. However, we did not find a significant effect of the Ri x Ev interaction on this variable (Fig. 5), despite that this interaction was significant when we analyzed the data using PERMANOVA ($F_{1,60} = 3.114$, P=0.021). No direct effects of biocrust attributes were detected in either experiment (Fig. 5). Together, the SEM and PERMANOVA results suggest that the most important

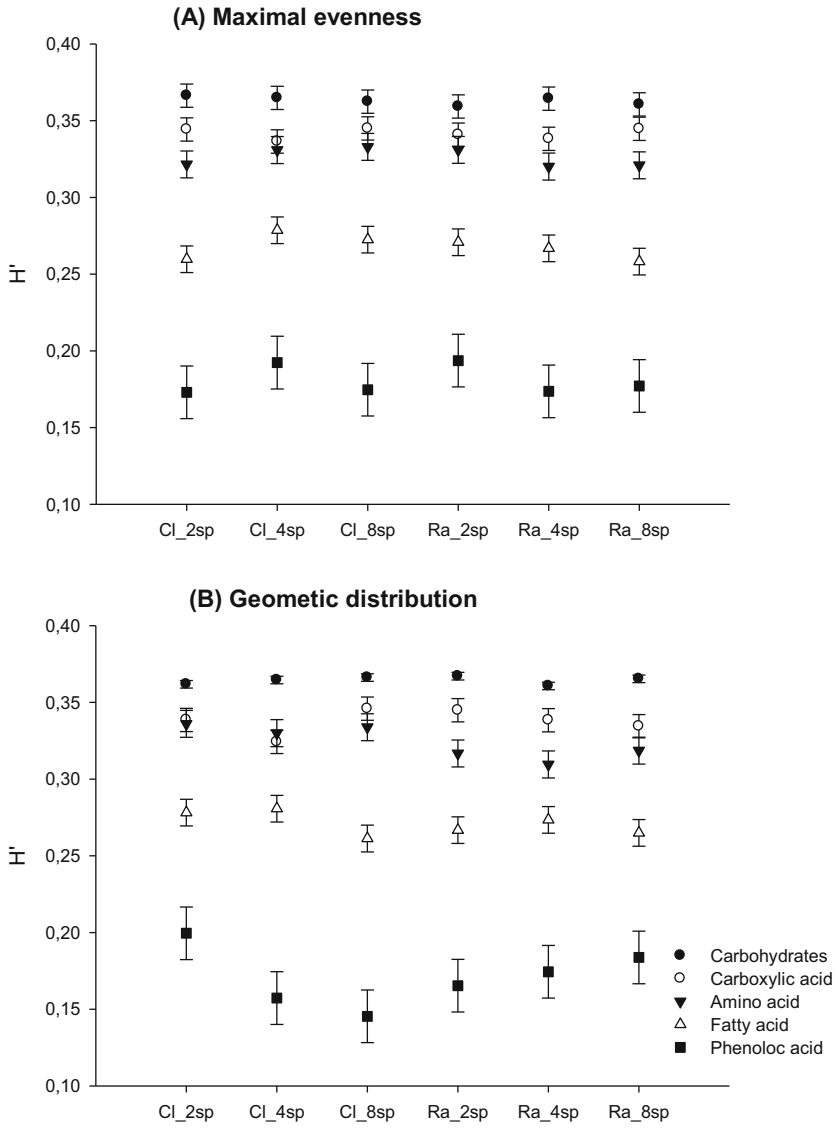

Fig. 4 Shannon-Weaver index (H′) of five carbon groups obtained in the Evenness experiment. Cl_2sp, Cl_4sp and Cl_8sp refer to microcosms with clumped spatial pattern and two, four and eight species, respectively. Ra_2sp, Ra_4sp and Ra_8sp refer to microcosms with random spatial pattern with two, four and eight species, respectively. Microcosms with maximal evenness (**a**), Microcosms with a geometric distribution of abundances among species (**b**). Data are means ± SE (n=6)

Table 1 Complete results of structural equation models tabulated by experiment and response variable

Experiment	Response	Richness	Evenness	Composition	Spatial pattern	Ri × Ev × Pa interaction	Ri × Ev interaction	R^2
E1	Microbial catabolic profile	−0.12		−0.22	−0.02			
			–			–	–	0.06
P		0.243		0.078	0.829			
E2		−0.09	0.11		0.09	0.35	0.10	
	Microbial catabolic profile			–				0.20
P		0.371	0.407		0.437	**0.025**	0.523	

Columns tabulate path coefficients (λ) and associated P-values for the four community properties (Spatial pattern (Pa), Richness (Ri), Evenness (Ev), Composition). For the subsurface measurements, the influence of the surface value of the same variable is also listed. All paths in each structural equation model are associated with probability tests, which test the probability that the path estimate differs from zero. P values below 0.05 are in bold. R^2 refers to proportion of variance explained in the listed response. All the structural equation models conducted fitted the data adequately (χ^2 <0.80, P>0.97, in all cases). E1 = Composition experiment, E2 = Evenness experiment

factor in the Evenness experiment was the Ri × Ev x Pa interaction, and apparent effects of other interaction terms and main effects were in fact mostly driven by this interaction. All the structural equation models conducted fitted the data adequately (χ^2<0.80, P>0.97, in all cases).

Discussion

Our understanding of how vascular plants, and their interactions with each other, govern the functioning of their associated belowground communities has advanced rapidly over the last decades (see Wardle et al. 2004; Bardgett and Wardle 2010 for reviews). It is now widely understood that biotic interactions between above- and belowground communities play fundamental roles in modulating ecosystem functioning (Wardle et al. 2004; De Deyn and Van der Putten 2005). Recent research on this topic has also highlighted the connections between attributes of aboveground communities, such as species diversity and composition, and belowground communities. For example, Antoninka et al. (2009) suggested that arbuscular mycorrhizal fungi influenced the community composition of plants and other root fungi, as well as soil fungi and bacteria. Johnson et al. (2005) found that the diversity and composition of plant communities regulated mycorrhizal

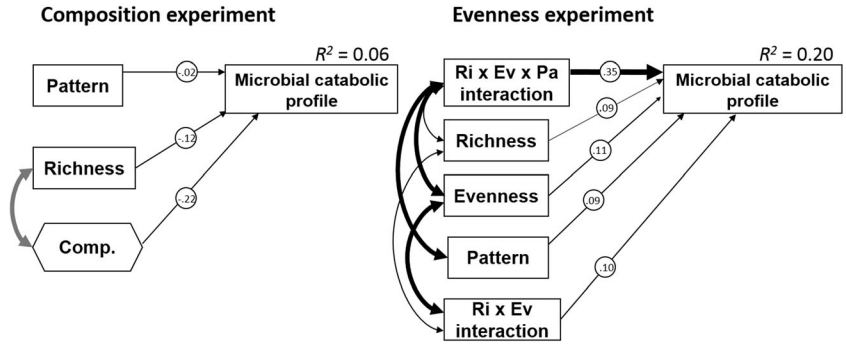

Fig. 5 Structural equation models of the effects of the biocrust attributes on microbial catabolic profile. Boxes represent measured variables, and the hexagon represents a composite variable. Directed arrows represent an effect of one variable upon another (paths), gray double headed arrow indicate correlation among variables with no direction specified. The numbers on the arrows are path coefficients, analogous to regression weights and indicative of the effect size of the relationship. The widths of the arrows are proportional to the strengths of the path coefficients. As in other linear models, R^2 signifies the proportion of variance explained and appears above the response variable in two models. Goodness-of-fit statistics for the both models are as follows: < 0.80, P>0.97. Comp. = composition, Ri = Richness, Ev = Evenness, Pa = Spatial pattern. All the structural equation models conducted fitted the data adequately (χ^2 P>0.05, in all cases)

communities in forests and grasslands. However, although the biocrusts represent the dominant soil surface system in drylands, covering up to 70 % of their surfaces (Belnap and Lange 2003), very few studies so far have evaluated how attributes of biocrusts affect soil microbial communities (Castillo-Monroy et al. 2011b, García-Palacios et al. 2011, Yu et al. 2012). The results of our experiments indicate that soil microorganisms are influenced by biocrusts, and highlight the importance of higher order interactions among species richness, evenness, and spatial pattern as drivers of the functioning of microbial communities.

Most of the research on the relationships between biodiversity and ecosystem functioning conducted to date has focused on only one community attribute at a time, such as species richness (Hooper et al. 2005; Cardinale et al. 2011), species composition (Petersen et al., 2012), evenness (Wilsey and Polley 2004; Downing 2005) or spatial pattern (Maestre et al. 2005). While the need of understanding the relationships among different biodiversity attributes as determinants of ecosystem functioning has been already highlighted (Loreau et al. 2001; Maestre et al., 2012a), few studies so far have focused on studying how these factors determine associated soil microorganisms (but see, Johnson et al. 2005; Antoninka et al. 2009). Furthermore, and to our knowledge, only one previous study has evaluated how multiple attributes of biocrusts simultaneously influence microbial communities (Castillo-Monroy et al. 2011b).

We did not find significant effects of species richness on any of the microbial attributes measured. These results were unexpected because autotrophic richness would be expected to influence heterotrophic functional diversity through its effects on substrate type and quantity. Further, autotrophic richness was previously found to be related to different soil functions under biocrusts, (e.g. Bowker et al. 2010, 2013; Maestre et al. 2012b), and one might hypothesize this effect to be partially mediated by the catabolic profile of heterotrophs. Our results might suggest that autotrophic richness does not strongly influence community composition of microbes in the biocrust system. In agreement with our findings, Castillo-Monroy et al. (2011b) did not find a significant effect of biocrust richness on the abundance and/or richness of soil bacteria. Results from that study indicate that the link between richness of biocrusts-forming lichens and their associated belowground communities was not very strong, but that particular lichen species, such as *Collema crispum* and *Toninia sedifolia*, exerted important effects on particular bacterial taxa. On the other hand, it is also possible that autotrophic richness does affect the heterotrophic species composition, but not enough to change the microbial catabolic profile, possibly due to functionally redundancy. It is well known that soil microorganisms comprise an incredibly large number of species, and because there are large numbers of trophically equivalent organisms, many species may be functionally redundant (Setälä et al. 2005). In the same set of experiments used here, Maestre et al. (2012b) reported that biocrust richness had higher relative importance compared to other attributes evaluated in determining soil nutrient stocks and cycling. It appears from the current study that this effect of autotrophic richness was not attributable to the catabolic activity of soil microbes. The results of Castillo-Monroy et al. (2011b) also suggested that functional profile of biocrusts were more clearly attributable to the richness of autotrophic biocrust components rather than that of the heterotrophic ones.

In the Evenness experiment, microcosms with a random spatial pattern had higher microbial catabolic profile than those with a clumped spatial pattern. Each lichen species is expected to exert a zone of influence in the vicinity of the thallus, and the microbial community would be likely to respond differently to the influences of different species due to the variation in chemical constituents of the lichens, something that has already been observed with rock-colonizing lichens (Bates et al. 2010). A random, rather than aggregated spatial pattern would tend to isolate different zones of influence, where a single species is able to exert its own unique set of influences upon the microbial community. This assortment of unique influences might increase microbial niche space, leading to a higher catabolic activity across the entire sample. Also, it is likely that the distribution of resources under the random spatial pattern of lichens would be heterogeneous, with isolated areas being resource rich; thus, enabling the acquisition of resources for soil microorganisms without intense competition. Our results highlight the importance that the spatial pattern of biocrusts-forming lichens may have for the associated microbial communities, and add to recent observational and experimental studies showing how the spatial pattern of primary producers can affect that of consumers and the ecosystem processes that depend on them (e.g. Maestre et al. 2005, 2012b; Pringle et al. 2010).

The H′ index (our surrogate of microbial functional diversity) of amino acids were higher in microcosms with random patterns. This was not a surprising result, as certain biocrust constituents, such as cyanobacteria and some bacterial species, have been reported to be able to liberate quantities of free amino acids into the external environment (Antarikanonda 1984; Gundlapally and Garcia-Pichel 2006). Although we did not measure cyanobacteria in our microcosms, free-living cyanobacteria are commonly associated with biocrusts-forming lichens such as those used in our experiment (Maestre et al. 2006). Phenolic acids are produced by lichens (Lawrey 1995), and thus the addition of them to soil can stimulate those microbial populations using them as a carbon source (Blum 2011). However, the H′ values of phenolic acids were lower than those of other carbon sources (Fig. 4), suggesting that microbial communities were not able to efficiently catabolize the phenolic compounds added in our experiment as well as we expected. This could be due to the fact that most of the lichen substances are insoluble or poorly soluble in water (Huneck and Yoshimura 1996). Additionally, only some microorganisms have the capacity to utilize phenolic compounds as a carbon source (Kefeli et al. 2013), and they could be absent in our microcosms.

Microbial communities in our microcosms were able to metabolize carbohydrates more efficiently than other carbon sources, which meant a higher respiration rate compared to other carbon sources (Fig. 4). Thus, microbial communities underneath lichens could be using substances produced by those lichens, since they, through their photobiont (i.e. alga or cyanobacterium), produce carbohydrates via photosynthesis which then serve as food for their fungus (Nash 1996). Besides, carbohydrates serve as major source of carbon for the growth of many microorganisms (Wright 1984; Goldman et al. 1987). These results were fully expected since carbohydrates are the most generally utilized carbon source by soil microbes (Stevenson 1993).

Species evenness did not significantly affect any of the variables evaluated. This result contrasts with Bowker et al. (2010) and Wilsey and Polley (2004), who found significant effects of species evenness on individual ecosystem functions driven by biocrusts and plants, respectively. It is not surprising that our result are different from those above mentioned since it is usual to find discrepancies in experimental and observational studies when evaluating the same variables or treatments. However, we can interpret our result as that in uneven communities where one species attains dominance, many individual zones of influence of a lichen thallus may be essentially interchangeable because they are associated with the same species, and thus the influence of rare lichens species on microbial niche space in the whole sample may be harder to discern. In an even community, each lichen exerts its unique set of influences equally strongly across the whole sample. Another hypothesis is that natural communities are either even or not due to specific reasons which shape community structure, such as a legacy of species interactions. On contrast, an artificially even community is not shaped by these forces. Thus experimentally-induced evenness may not have the same effects as naturally-occurring evenness as studied in Bowker et al. (2010).

In the Evenness experiment, when the effects of the different factors were evaluated using a semiparametric approach, it was found that significant species richness × evenness interactions modulated microbial catabolic profile and biomass. The same interaction was found when analyzing soil organic C in this experiment (Maestre et al. 2012b). Thus, our results suggest that the higher the active microbial biomass, the higher availability of carbon on soils provided by the fixation of atmospheric CO_2 by lichens, at least within the range explored in our experiments.

A third-order interaction among species richness, evenness and spatial pattern was a major determinant of variations in microbial catabolic profile in our Evenness experiment (Fig. 5). This result highlights the importance of studying several biotic attributes simultaneously to create a realistic image of natural conditions, as community properties do not exert their influence in isolation in the field. However, understanding why intermediate richness might, in concert with maximal evenness and random spatial patterning, promote microbial catabolic activity is not easy. Using a miniature model of plant-soil interactions in constructed biocrusts, we asked the question: What biotic attributes of autotrophic communities structure the communities of soil heterotrophic microbes? A major finding of our study is that interactive effects of multiple biotic attributes may dictate the structure of soil microbial communities. Therefore, there is a clear need for multi-factor crossed experiments to arrive at generalities regarding how aboveground communities influence microbial communities and the soil functions that depend on them.

Biocrusts are an ideal system to evaluate such questions, as we can experimentally manipulate multiple biotic attributes within a community at low cost and with low space requirements, and their increased use with this aim will undoubtedly increase our understanding of their role as drivers of soil functioning.

Acknowledgments We thank David Elliott and two anonymous reviewers for comments on a previous version of this manuscript. We also thank A. Escudero, I. Martínez, P. Alonso, E. Polaina, S. Soliveres, M. D. Puche, Y. Valiñani, C. Escolar, E. Barahona, S. Beltran de Guevara, C. Iriarte, J. Margalet, C. Díaz, R. Sendra, B. Paredes, L. Giménez-Benavides, Y. Cabrea, I. Conde and I. Pardo for their invaluable help during the development of this work. APCM was supported by a Studentship from the Fundación BBVA (BIOCON06/105 grant). This research was funded by the British Ecological Society (Early Career Project Grant 231/607) and by the European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013)/ ERC Grant agreement n° 242658 (BIOCOM).

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