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Relationships between biological soil crusts, bacterial diversity and abundance, and ecosystem functioning: Insights from a semi-arid Mediterranean environment

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Abstract

Questions: To what degree do biological soil crusts (BSCs), which are regulators of the soil surface boundary, influence associated microbial communities? Are these associations important to ecosystem functioning in a Mediterranean semi-arid environment?

Location: Gypsum outcrops near Belmonte del Tajo, Central Spain.

Methods: We sampled a total of 45 (50 cm × 50 cm) plots, where we estimated the cover of every lichen and BSC-forming lichen species. We also collected soil samples to estimate bacterial species richness and abundance, and to assess different surrogates of ecosystem functioning. We used path analysis to evaluate the relationships between the richness/abundance of above- and below-ground species and ecosystem functioning.

Results: We found that the greatest direct effect upon the ecosystem function matrix was that of the biological soil crust (BSC) richness matrix. A few bacterial species were sensitive to the lichen community, with a disproportionate effect of *Collema crispum* and *Toninia sedifolia* compared to their low abundance and frequency. The lichens *Fulgensia subbracteata* and *Toninia* spp. also had negative effects on bacteria, while *Diploschistes diacapsis* consistently affected sensitive bacteria, sometimes positively. Despite these results, very few of the BSC effects on ecosystem function could be ascribed to changes within the bacterial community.

Conclusion: Our results suggest the primary importance of the richness of BSC-forming lichens as drivers of small-scale changes in ecosystem functioning. This study provides valuable insights on semi-arid ecosystems where plant cover is spatially discontinuous and ecosystem function in plant interspaces is regulated largely by BSCs.

Introduction

Considerable effort has been invested during recent decades into elucidating links between community composition, species diversity and ecosystem functioning (Pimm 1984; Loreau et al. 2002; Hooper et al. 2005). Many studies have examined the role of community attributes, such as the number, relative abundance and identity of species forming a community, as drivers of ecosystem functioning (reviewed by Kinzig et al. 2002; Loreau et al. 2002; Hooper et al. 2005). However, the nature of the relationships between community properties and ecosystem functioning is not always consistent because it depends on multiple factors, and requires the study of multiple communities, including below-ground and soil surface boundary communities (Belnap et al. 2003; Bardgett et al. 2005; Bowker et al. 2010a). This boundary reflects the intersection between the atmosphere and the soil, and its characteristics contribute to transmission and transformation of materials and energy between them

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(Belnap et al. 2003). The soil surface boundary has special properties in arid and semi-arid environments because most of the area in these ecosystems is unvegetated (Turner et al. 2001; Belnap et al. 2003; Strayer et al. 2003). Below-ground communities may affect above-ground components by regulating nutrient flows, litter decomposition and root herbivory in a variety of ecosystems (Bonkowski & Roy 2005; Reed & Martiny 2007). The influence of soil biota on plant community composition has also been examined (Wardle et al. 1999; Porazinska et al. 2003; De Deyn et al. 2004). However, to our knowledge, no study has attempted to partition the relative functional contributions of semi-arid biotic communities regulating soil surface boundaries and closely associated below-ground microbiota.

In arid and semi-arid regions, the distribution of vegetation exhibits marked patchiness, with discrete patches of vascular plants and "open" areas devoid of vascular vegetation (Valentin et al. 1999). In the latter, the soil surface boundary is often occupied and regulated by biological soil crusts (BSCs). These crusts are usually composed of mosses, lichens and cyanobacteria, among other organisms. Biological soil crusts normally constitute a thin mantle that penetrates the upper millimeters of the soil, but their impact on ecosystem functioning can be quite strong because most inputs to and losses from semi-arid soils must pass through the boundary created by them (Belnap & Lange 2003). Among other functions, BSCs control the local hydrological cycle (Belnap et al. 2005), stabilize the soil against erosion (Belnap & Gillete 1998; Reynolds et al. 2001) and modulate carbon (Maestre & Cortina 2003; Thomas et al. 2008) and nitrogen (Castillo-Monroy et al. 2010; Delgado-Baquerizo et al. 2010) cycles. Despite the large body of literature on BSCs, many aspects of their ecology remain little studied, especially those related to their associated below-ground heterotrophic microbial populations. Most studies on this topic have evaluated the effects of broad types of BSC (e.g. crust dominated by lichens and mosses versus crust dominated by cyanobacteria) on particular groups of microorganisms (e.g. García-Pichel et al. 2001; Yeager et al. 2004; Johnson et al. 2005; Gundlapally & Garcia-Pichel 2006; Soule et al. 2009; Zaady et al. 2010). However, little is known about the effects of particular BSC attributes on soil microbial communities. Attributes of BSCs such as diversity, cover and spatial pattern have been found to affect ecosystem processes and variables related to nutrient cycling (Maestre et al. 2005; Bowker et al. 2010a), and thus it is likely that they will also affect the attributes of the microbial communities, and in turn, the functional roles played by these microbes. Biological soil crusts can also be seen as a microcosm of above- and below-ground interactions and their impact on ecosystem functioning because the photoautotrophic lichens of BSCs behave in some ways like miniature vascular plants, and the associated bacteria are not unlike rhizosphere bacteria (Bowker et al. 2010a).

In this study, we quantified the abundance and richness of both BSCs and associated bacteria using culture-independent molecular approaches and cultivation methods, and related these community attributes to several surrogates of ecosystem functioning. To our knowledge, this is the first attempt to partition the functional attributes of the autotrophic and heterotrophic components of BSCs, and to examine the effects of the autotrophic component on the heterotrophic component at a high taxonomic resolution. We addressed the following questions: (i) to what degree are the richness and abundance of soil surface boundary and below-ground communities related, and (ii) are these associations important to ecosystem functioning in a Mediterranean semi-arid environment?

Methods

Study area and sampling design

The study was conducted in gypsum outcrops near Belmonte del Tajo, Madrid province, in central Spain (40°7′3″N, 3°18′3″W, 686 m a.s.l.; 8° slope; 220° southwest aspect). The climate is Mediterranean semi-arid, with a mean annual temperature and rainfall of 14 °C and 452 mm, respectively. The studied 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%. The open areas between perennial plants are colonized by well-developed BSCs dominated by lichens such as *Diploschistes diacapsis* (Ach.) Lumbsch, *Squamarina lentigera* (Weber) Poelt, *Fulgensia subbracteata* (Nyl.) Poelt, *Toninia sedifolia* (Scop.) Timdal and *Psora decipiens* (Hedw.) Hoffm (Maestre et al. 2008).

A total of 45 (50 cm × 50 cm) plots, spread over a homogeneous area of 1.3 ha, were placed non-randomly on bare ground areas with well-developed BSCs located in the spaces between perennial plants. This non-random placement of plots is commonly followed with these organisms because of their small size and their high within-site spatial variability (Maestre et al. 2005; Bowker et al. 2006, 2010b; Martínez et al. 2006). We established a minimum separation distance between plots of 0.7 m to minimize the risk of sampling non-independent areas because of the small-scale spatial structure of the communities studied (Maestre et al. 2005). During the winter of 2005 and the spring of 2006, we surveyed the richness and abundance of BSC-forming soil lichens, which are the dominant BSC component in the study area. We used cover as a surrogate of species abundance because of the

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inherent difficulties associated with the definition of individuals in soil lichens, and because cover is a good estimator of biomass in these organisms (Bowker et al. 2008). For the estimation of lichen cover, each plot was divided into 100 (5 cm × 5 cm) sampling quadrats, and the cover of every lichen species was estimated. The average cover of a given species in the 100 quadrats was used as our estimate of total plot cover for that species. Species richness was estimated as the number of lichen species present in each plot.

To study soil bacterial communities, and to estimate surrogates of ecosystem functioning, we collected five soil samples in each plot using a 5-cm diameter core (0-1 cm depth). Soil sampling was conducted in all plots in late September 2006, when the soil was dry after a pronounced summer drought. Soil samples were bulked and homogenized in the field to obtain a composite sample for each plot. Soil subsamples (10 g) were stored in plastic bags at −80 °C until DNA analyses (see below). The remaining soil was air dried in the laboratory over several weeks. After drying, lichen and moss colonies were removed by hand and soils were passed through a 2-mm sieve to remove rocks, litter and remaining bryophyte or lichen fragments.

Characterization of bacterial communities

We characterized the abundance of active bacteria using a culture-based technique, and community richness and presence/absence of bacterial taxa using a culture-independent technique (PCR-DGGE). While colony-forming bacteria represent a limited fraction of the total bacterial community, culture-based methods can still provide high quality information on potential metabolic activity and on the role that heterotrophic bacteria play in biogeochemical cycles (Zaccone et al. 2002; Gundlapally & Garcia-Pichel 2006). PCR-DGGE is one of the most effective, high-throughput techniques used to estimate bacterial richness and microbial community structure in environmental samples (Gelsomino et al. 1999; Lorenzo et al. 2010; Zaady et al. 2010). However, the use of DGGE to estimate bacterial abundance and diversity is problematic because of subjectivity in comparing band intensity (Forney et al. 2004). Thus, we combined culture-based and molecular methods to complement each other and obtain a more complete picture of bacterial abundance and richness.

For estimating the abundance of culturable bacteria, we transferred 40 g of air-dried soil from each plot to dilution bottles containing 90 ml of sterile deionized water. Bottles were allowed to stand on a magnetic stirrer for 15 min and then the soil was dispersed with the magnetic stirrer bar (2.5 cm × 0.8 cm) at about 2800 rpm for 15 min. Immediately after dispersion, we made five series of ten-fold dilutions of the suspension by pipetting 1-ml aliquots into tubes containing 9 ml of sterile deionized water. Final dilutions were 10^{-6}-fold. To count total aerobic–mesophyllic–heterotrophic bacteria, 1-ml aliquot of the final three dilutions were transferred to 9-cm diameter Petri dishes (replicated twice) containing 20 ml of molten medium R2A agar (Difco, Detroit, MI, USA). Finally, we added 100 μg g^{-1} of cycloheximide to each Petri dish to prevent fungal growth. Plates were put in an incubator at 30 °C, and colonies were counted after 72 h. The isolated colonies were characterized according to development in space relative to the shape, size, elevation, area, edges, density and consistency. Bacterial abundance was defined as number of culturable bacteria per gram of soil, and bacterial richness was defined as number of different colony-forming units (CFU) per soil sample.

For assessing bacterial richness, we extracted total DNA from the frozen soil samples using the UltraClean™ Soil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. DNA was checked for quality on 1% agarose TAE-gels (Tris-acetic acid, ethylenediaminetetraacetic acid) by standard gel electrophoresis followed by SYBR green staining. We used the bacteria-specific primers EUB (5′-ACTCCTAC GGGAGGCAGAAG-3′) and EUB518 (5′-ATTACCGCGGC TGCTGG-3′) to amplify approximately 259 bp of the 16S rDNA gene (Fierer et al. 2005). A GC clamp (CGCCCG GGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGG) was attached to the 5′-end of primer EUB to improve band separation during DGGE. All PCRs were performed in 25 μl using Takara Ex Taq DNA polymerase premix PCR kits. Amplifications were performed on a Mjmini thermal cycler (BioRad, Laboratories Inc., Hercules, CA, USA) with the following cycling parameters: 94 °C for 9 min, 30-36 cycles of denaturation at 94 °C for 1 min; annealing at 53 °C for 1 min; extension at 72 °C for 1 min; and a final extension at 72 °C for 9 min. Products were checked for quality on 1% agarose gels stained with SYBR green (Øvreås et al. 1997). Fifteen microliters of the PCR products were analysed with denaturing gradient gel electrophoresis (DGGE) using a DGGE-2401 System (C.B.S. Scientific Company, Del Mar, CA, USA). Gels contained 10% polyacrylamide (37:1 acrylamide/bis-acrylamide) in 0.5 × TAE and a denaturing gradient of 35-60%, where 100% denaturing is defined as containing 7 M urea and 40% (v/v) formamide (Muyzer & Smalla 1998). Gels were run 20 min at 20 V and 16 h at 80 V with constant temperature of 60 °C, subsequently stained with a 1:10 000 dilution of SYBR gold (Molecular Probes) in 0.5 × TAE for 30 min and photographed under ultraviolet light. Gel digital images were analysed with the software GelCompar II version 4.0 (Applied Maths, Kortrijk,

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Belgium). Each detected band was defined as a bacterial strain (Appendix S1), and the number of bands was defined as the genotypic richness of each sample (hereafter "bacterial richness").

Measuring ecosystem functioning

Soil enzymes strongly influence the functioning of soil ecosystems, as they catalyze several important reactions involved in the decomposition of organic matter and in the cycling of key nutrients such as N and P (Dick 1994; Makoi & Ndakidemi 2008). Thus, we measured the activity of three soil enzymes related to the carbon (β-glucosidase), nitrogen (urease) and phosphorus (phosphatase) cycles as surrogates of ecosystem functioning. Urease activity was determined as the amount of NH_4^+ released from 0.5 g of soil after incubation for 90 min with 6.4% urea at 30 °C in phosphate buffer (pH 7), as described in Nannipieri et al. (1980). Phosphatase activity was measured by determination of the amount of *p*-nitrophenol (PNF) released from 0.5 g soil after incubation at 37 °C for 1 h with the substrate *p*-nitrophenyl phosphate in MUB buffer (pH 6.5; Tabatabai & Bremner 1969). The activity of β-glucosidase was assayed according to Tabatabai (1982), following the procedure for phosphatase, but using *p*-nitrophenyl-β-D-glucopyranoside as substrate and trishydroxymethyl aminomethane instead of NaOH when preparing the buffer.

Statistical analyses

We evaluated the relationships between the richness and abundance of both BSCs and bacteria and the different surrogates of ecosystem functioning using path analysis (Shipley 2002) based upon partial Mantel statistics (Smouse et al. 1986). We based the analysis on Mantel statistics rather than univariate correlation statistics because this approach compares the correlation between two or more matrices composed of multiple variables. Path analysis allows us to move beyond bivariate correlation statistics and partition multiple pathways that one variable may have upon another and calculate direct and indirect effects and their total. A path coefficient, ranging from 0 to 1 and analogous to a regression weight or partial correlation coefficient, is estimated and describes the strength of each pathway. We proposed an *a priori* model in which the richness and abundance of the lichen component of the BSCs were inter-correlated and exerted direct effects on the same properties of the soil bacteria and on ecosystem functions (Fig. 1). The bacterial richness and abundance also exerted direct effects upon ecosystem functions. The variables in this model were a mixture of univariate (lichen richness, lichen abundance, bacterial abundance) and multivariate matrices (bacterial richness, ecosystem functions). The crust abundance matrix contained the average of the cover of all lichens per plot. The crust richness matrix contained total lichen species richness. The bacterial abundance matrix contained total number of culturable bacteria per gram of soil. The bacterial richness matrix contained total number of bacterial CFUs and the number of strains estimated from the DGGE analysis. The ecosystem function matrix contained the activities of the three soil enzymes analysed.

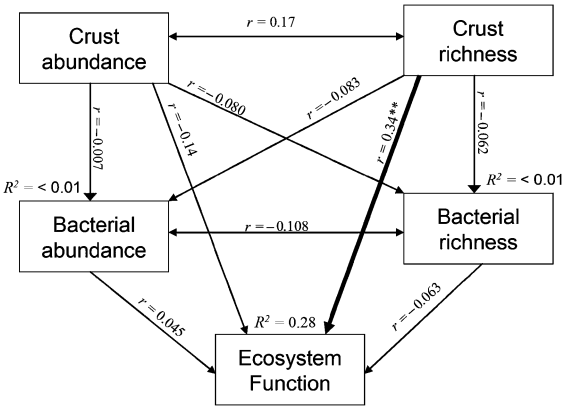

Fig. 1. Final partial Mantel path analysis. Boxes represent matrices of conceptually related variables. Numbers adjacent to arrows are partial Mantel coefficients, analogous to regression weights among matrices rather than univariate variables, and indicative of the effect size of the relationship. Width of arrows is proportional to path coefficients. $^*P < 0.05$, $^{**}P < 0.01$.

We used a partial Mantel test approach to estimate path coefficients, using the Euclidean distance to construct our distance matrices. Our *a priori* model was saturated, meaning that there was a direct uni- or bi-directional relationship between pairing of variables. This precludes an overall test of fit, but still allows for partitioning effects among multiple pathways. Partial and bivariate Mantel statistics were obtained in R 2.6.2 (http://www.r-project.org/), using the ECODIST package (Goslee & Urban 2007). Indirect, and total effects of variables, and R^2 of endogenous variables were calculated by hand according to McCune & Grace (2002).

In addition to our Mantel test-based path analysis, we also conducted a more detailed analysis on the effects of community structure in BSC-forming lichens upon the presence and absence of underlying soil bacterial taxa. We also used the Mantel test as an overall test of correlation between an untransformed matrix containing the abundance of lichens by species, and a presence/absence matrix of all bacterial strains encountered. We used the Bray-Curtis distance in these analyses because zeros were very common in the data.

We also used an ordination-based approach to determine if particular lichen species have an especially

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important effect on the bacterial community. Because there are many possible pair-wise correlation tests among pairs of lichen and bacterial species, this approach is more efficient than conducting univariate tests. We created an NMDS ordination of the lichen data, again using Bray-Curtis distance. We created a joint biplot wherein vectors are added to the ordination representing the correlation of presence or absence of bacterial strains to the lichen ordination. To reduce the number of bacterial strains to only those that were most sensitive to lichens, we used an R^2 cutoff of 0.15 (corresponding to a P value of about 0.001) to choose the bacterial vectors that were added to the biplot. These selected vectors are henceforth called "sensitive" bacteria or bacterial taxa. For each of these cases, one at a time, we rotated the lichen ordination to maximize correlation with this sensitive bacterial vector. In this procedure, the first axis of the ordination is realigned so that it parallels the bacterial vector being considered. After the rotation, we obtained the Pearson correlations between the various lichen species and this axis. This exercise was repeated for 11 sensitive taxa. We analysed the resultant sets of correlation statistics in two ways using one-sample t-tests. First, we simply tested whether these correlations of a given lichen species tended to depart from a mean of zero; this test helps us identify whether a lichen species tends to exert negative (if mean correlation is distinct from zero and negative) or positive (if mean correlation is distinct from zero and positive) effects on the sensitive bacteria. Second, we repeated the one-sample t-test for each lichen species, instead using the absolute value of its correlations with sensitive bacteria. This test is focused on the magnitude of correlations rather than the sign; it helps identify species that tend to exert strong effects, whether they are negative, positive, or a mixture. The ordination-based approach was conducted using the PC-Ord package v. 4.01 (MjM Software Design, Gleneden Beach, OR, USA).

Results

Our path model was able to explain 28% of the variance in the ecosystem functioning matrix. The greatest effect upon this matrix was the BSC richness matrix ($r = 0.34$). Biological soil crust richness and abundance matrices were positively and negatively correlated, respectively, with ecosystem functioning. Bacterial richness and abundance had only weak effects on ecosystem functioning, and were negatively and positively correlated, respectively (Fig. 1).

The total effect of BSC richness on ecosystem functioning was higher than all other variables in the model ($r = 0.30$). The total effect of bacterial richness and BSC abundance was negatively related to ecosystem functioning in both cases, albeit quite weakly ($r = -0.079$ and -0.006, respectively). However, total effects of bacterial abundance and BSC richness were both positively related to ecosystem functioning ($r = 0.029$ and 0.30, respectively; Table 1).

Table 1. Final results of partial Mantel path analysis showing the direct, indirect and total effects that one variable may have upon another.

Predictor	Response variable	Direct effect	Indirect effect	Total effect
Crust abundance	Bacterial abundance	−0.012	0.00	−0.012
Crust abundance	Bacterial richness	−0.090	0.00	−0.090
Crust abundance	Ecosystem functioning	−0.068	0.062	−0.006
Crust abundance	Crust richness	0.17	0.00	0.17
Crust richness	Bacterial abundance	−0.078	0.00	−0.078
Crust richness	Bacterial richness	−0.068	0.00	−0.068
Crust richness	Ecosystem functioning	0.32	−0.023	0.30
Bacterial abundance	Ecosystem functioning	0.023	0.006	0.029
Bacterial abundance	Bacterial richness	−0.10	0.00	−0.10
Bacterial richness	Ecosystem functioning	−0.075	−0.004	−0.079

The matrices containing lichen abundance for each species and bacterial presence/absence were essentially uncorrelated ($r = -0.01$, $P = 0.75$). However, there were a few bacterial species whose presence was sensitive to the lichen community structure, and some lichens had an effect on these species disproportionate to their abundance (Appendix S2). Despite its low abundance in the study area (Appendix S3), *Collema crispum* exerted the strongest average effect on the sensitive bacterial taxa (mean $|r| = 0.55$). *Toninia sedifolia* was also rather influential, considering its abundance (mean $|r| = 0.43$). Several of the dominant lichen species also affected the sensitive bacteria, including *Diploschistes diacapsis*, *Fulgensia subbracteata*, *Psora decipiens* and *Squamarina lentigera* (mean $|r| = 0.40$). Several species tended to exhibit antibacterial characteristics (e.g. *Toninia* spp., *Collema crispum, Squamarina cartilaginea, Lepraria crassissima* and *Endocarpon pusillum*), whereas *D. diacapsis* showed a mixture of negative and positive effects on the sensitive bacterial taxa. No lichen species consistently had positive effects on sensitive bacteria.

Discussion

Effects of BSC-forming lichens on composition of the bacterial community

The links between above- and below-ground diversity in the studied ecosystem were not very strong. However, we found that the presence of some bacterial species was

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sensitive to the structure of the lichen community. The effects of BSC-forming lichens on sensitive bacteria were often disproportionate to their abundance and frequency. For example, *Toninia sedifolia* was present in just 5% of the plots, but exerted a significant negative effect on the sensitive bacterial taxa, while *Cladonia convoluta*, present in 97% of the plots, was not a major player in shaping the bacterial composition, and exerted both positive and negative effects on sensitive bacteria, with a mean effect near zero. These results agree with those of Stark & Hyvärinen (2003), who found that the leaching of *Cladonia* sp. did not change the amount of microbial biomass in soil. In the same direction, Stark et al. (2007) found no significant effects of *Cladonia stellaris* on soil microbial respiration. However, the antibacterial activity of extracts of *Cladonia* sp. has been revealed in the laboratory (Rankovic et al. 2009) and in the field (Akpinar et al. 2009). *Squamarina lentigera,* a species with similar chemical characteristics to *Cladonia* sp. (Nimis & Martellos 2004; see Appendix S4), is very frequent in the sampled plots (present in 79% of plots), and consistently exerted negative effects on the bacterial community studied. Interestingly, *Diploschistes diacapsis* – a ubiquitous (frequency of 100%) and often very abundant species at our study site – did not have consistent positive or negative effects, but rather a mixture of the two, on the sensitive bacteria. This agrees with Saenz et al. (2006), who found that *D. scruposus* only had a small inhibitory effect on soil bacteria in southern Spain. *Collema crispum* is a nitrogen-fixing lichen that is very frequent (present in 79% of the sampled plots) but not very abundant (average cover is 23%) in our study area (Appendix S3). Like many N-fixers, *Collema* spp. are early successional lichens capable of colonizing nutrient-poor and degraded sites (Lange et al. 1998). Accordingly, *C. crispum* has been positively associated with bare soil cover in gypsum outcrops of central and SE Spain (Martínez et al. 2006). In our study area, this species does not seem to facilitate the occurrence of other lichen species (Maestre et al. 2009). Here, we found that *C. crispum* had a negative effect on several sensitive bacterial species. Similar results were found by Martínez et al. (2006), who observed that this lichen was negatively associated with soil respiration in a gypsum site close to our study area. Overall, these results suggest that, although *C. crispum* is a good colonizer, it does not facilitate the establishment of other lichen and bacterial species in gypsum soils.

No lichen species had consistent positive effects on bacterial strains, but species such as *C. crispum, Endocarpon pusillum, Fulgensia subbracteata, Lepraria crassissima, Squamarina cartilaginea, Toninia sedifolia* and *T. tininiana* tended to have negative effects on soil bacteria. To our knowledge, this is the first field study reporting such multiple negative effects of co-occurring BSC-forming lichens in a field study. Our experimental design and measurements cannot provide a detailed understanding of the processes underlying these relationships, which may be mediated by multiple mechanisms. One of them is the chemical interactions mediated by secondary metabolites, which is common in BSC-forming lichens such as those that have been studied (Fahselt 1994). Most of the lichens mentioned above contain secondary compounds (Appendix S4), which are believed to have antimicrobial and allelopathic properties, among other functions (Lawrey 1989; Fahselt 1994; Gauslaa 2004), but the ecological roles of these substances are largely unknown, and could be multi-faceted. Several studies have shown the negative influence of many lichen substances on bacteria and fungi in the laboratory and the field (Sedia & Ehrenfeld 2003; Tay et al. 2004; Akpinar et al. 2009). Recent studies have also found a strong reduction in both the available nitrate and potential nitrification rate beneath BSC-forming lichens in different environments (including gypsum ecosystems close to our study area; Castillo-Monroy et al. 2010; Delgado-Baquerizo et al. 2010), suggesting that microbial communities may be inhibited under these organisms (Sedia & Ehrenfeld 2005). Additional research is needed to further study the detailed mechanisms involved in the relationships found, but our results suggest that the particular traits and chemical ecology of the species forming the BSC community are crucial to define their effects on the soil bacterial community.

Bacterial and ecosystem functioning at the soil surface boundary

To our surprise, we did not find any effects of either bacterial abundance and/or richness on the surrogates of ecosystem functioning evaluated. We cannot fully explain these results, which can be driven by several alternative mechanisms. Perhaps the most common view amongst soil ecologists is that since the soil microbiota comprises an incredibly large number of species, and because there are large numbers of trophically equivalent organisms, most species must be functionally redundant (Setälä et al. 2005). Thus, the soil bacterial community in our study area may have greater functional redundancy than that of BSC-forming lichens. However, it is also likely that the different spatio-temporal scales at which BSC and bacterial communities affect the studied soil variables could have influenced our results. In addition, the methods employed to characterize the bacterial community may fail to detect changes in those bacterial groups directly involved in the soil enzymes measured. On the other hand, recent studies have shown that ammonia-oxidizing archaea are more abundant than ammonia-oxidizing

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bacteria in semi-arid soils (Leininger et al. 2006; Adair & Schwartz 2008), and the methods used were not able detect archaea species because of the general primers for eubacteria that were employed. Furthermore, molecular methods based on DNA cannot discriminate between active and dormant organisms (Bridge & Spooner 2001), lowering the resolution of the analysis to detect any relationship between bacterial richness and enzyme activity.

Overall, in our model, the richness of BSC-forming lichens was an important determinant of below-ground ecosystem functioning. As found in previous studies conducted with BSCs (Bowker et al. 2010b), we saw linear relationships between BSC richness and ecosystem functioning. This suggests that the relationship is predominantly consistent with the rivet hypothesis (i.e. each species has a unique role in an ecological system, and that the loss of species contributes toward the collapse of the system, therefore diminishing its function) rather than the redundancy hypothesis (i.e. species are functionally redundant, and to some degree the loss of some species may be compensated by other species) (Naeem et al. 2002). The sampling effect, whereby more diverse assemblages of species are more likely to contain a more highly functional species (Hooper et al. 2005), is inflated in randomly assembled experimental systems, but is unlikely to be strong in our data. Thus, we propose that the patterns we observed are due to complementarity in mechanistic contributions to a particular function.

Conclusion

This study provides valuable insights about the functioning of semi-arid ecosystems where plant cover is spatially discontinuous and ecosystem function in plant interspaces is regulated by BSCs. These communities are crucial for the functioning of arid and semi-arid ecosystems, since they modulate changes in ecosystem functioning below ground and, as shown by our results, the soil bacterial community. The composition of BSCs had a direct effect on the structure of below-ground bacterial communities, affecting sensitive bacterial strains. The BSC-forming lichens exerted greater direct effects than soil bacteria on the surrogates of ecosystem functioning. Further, there may be fundamental differences regarding complementarity in these two communities, with the bacteria possibly being functionally redundant and the lichens being comparatively functionally singular. Thus, the explicit consideration of particular attributes (abundance/composition/richness) must occupy a major role when studying the effects of BSCs on microbial communities and ecosystem functioning. The study of diversity–ecosystem functioning relationships in plant communities has been a core research topic for vegetation scientists during the last decade. Most of the empirical research carried out on this topic has focused on grasslands and shrublands (Loreau et al. 2002; Hooper et al. 2005; Montès et al. 2008). This limits our ability to make confident generalizations on the functional role of biodiversity, and to extrapolate the results obtained so far to other communities (Giller et al. 2004). By focusing on BSCs, our results contribute to fill current gaps in our knowledge; they also add to the still scarce literature dealing with these organisms (Maestre et al. 2005, 2010; Bowker et al. 2010a), and provide new empirical evidence showing the importance of BSC richness as a driver of ecosystem functioning in semi-arid environments.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Appendix S1. Example DGGE profile. Each lane (1–7) represents a soil sample. Each band represents a bacterial strain. The number of bands was defined as the bacterial richness of each sample. Each strain was labelled with a number follow by a sequential letter (e.g. 1A, 1B . . . 2A, 2B . . . 3A, 3B . . . etc.). Lane M contains the DGGE marker.

Appendix S2. Results of effects of community structure in BSC-forming lichens upon the presence and absence of underlying soil bacterial taxa. NMDS: non-metric multidimensional scaling analysis was performed; Bact: different bacterial strains found in our samples; Acno: *Acarospora nodulosa*; Plsq: *Placidium squamulosum*; Plpi: *Placidium pilosellum*; Cla: *Cladonia convoluta*; Coll: *Collema crispum*; Dd: *Diploschistes diacapsis*; End: *Endocarpon pusillum*; Fg: *Fulgensia subbracteata*; Lepra: *Lepraria crassissima*; Psgl: *Psora globifera*; Psde: *Psora decipiens*; Pssa: *Psora savizcii*; Sqca: *Squamarina cartilaginea*; Scle: *Squamarina lentigera*; Toal: *Toninia albilabra*; Tose: *Toninia sedifolia*; Toto: *Toninia toniniana*.

Appendix S3. Frequency and cover of lichens forming biological soil crusts in the study area. Cover data represent means ± SD ($n = 45$). Data from Maestre, F.T., Escolar, C., Martinez, I. and Escudero, A. 2008. Are soil lichen communities structured by biotic interactions? A null model analysis. *Journal of Vegetation Science* 19: 261–266.

Appendix S4. Lichen substances identified in biological soil crust-forming lichens found in our study area.

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