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Differences in thallus chemistry are related to species-specific effects of biocrust-forming lichens on soil nutrients and microbial communities

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Summary

1. It is well-known that vascular plants have species-specific effects on soil properties. However, little is known on how individual species forming biocrusts, communities dominated by lichens, mosses and cyanobacteria that are prevalent in many ecosystems world-wide, affect microbial communities and soil variables related to nutrient cycling.

2. We evaluated the relationship of six biocrust-forming lichens (*Buellia epipolia, Diploschistes diacapsis, Fulgensia subbracteata, Psora decipiens, Squamarina cartilaginea* and *Squamarina lentigera*) with microbial abundance and multiple variables associated with soil nitrogen (N), carbon (C) and phosphorus (P) cycling and storage. We also evaluated whether the composition of lichen tissues (contents in C, N, P and polyphenols) is related to the C, N, P availability and microbial abundance in soils. Finally, we assessed what lichen species positively and negatively relate to soil fertility compared to bare ground areas without biocrusts.

3. We found contrasted C, N, P availability and soil microbial abundance under the different biocrust-forming lichens. Interestingly, inorganic P and amino acids were the most important factors differentiating lichen microsites. These differences in nutrient availability seem to be related to the C, N and P composition of the lichen tissues. For example, soils under *D. diacapsis* and *P. decipiens*, which had the lowest and highest C, N and P contents in their tissues, respectively, had the lowest and highest nutrient availability, respectively. We also found contrasted soil microbes abundance under the different soil lichens. For instance, *F. subbracteata* and *D. diacapsis* were negatively related to the abundance of bacteria compared to bare ground areas.

4. Our results support the idea that, as found with vascular plants, biocrust-forming lichens have species-specific effects on soil microbial communities and C, N and P cycling. Thus, continuing considering biocrusts as a unique entity will only add confusion to our knowledge of how they control nutrient availability and microbial abundance in the ecosystems where this key community is prevalent.

Key-words: *amoA* genes, bacteria, carbon cycle, drylands, fungi, nitrogen cycle, phosphorus cycle

Introduction

Understanding how individual plant species affect soil properties and ecosystem functioning has been a main ecological research topic over the decades (e.g. Charley & West 1975; Bardgett *et al.* 1999; Wardle *et al.* 2004). Many studies have shown that vascular plants have species-specific effects on soil variables linked to ecosystem processes such as soil respiration (Castillo-Monroy *et al.* 2011a; Vesterdal *et al.* 2012) and nitrogen (N) mineralization, and

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on associated services such as carbon (C) storage (Vesterdal *et al.* 2012; Aponte, García & Marañón 2013). While these studies have substantially advanced our knowledge of the effects of particular vascular plant species on ecosystem functioning, and thus of how changes in species composition may affect it, our understanding of how non-vascular plants affect soil variables and ecosystem processes is much more incomplete (Castillo-Monroy *et al.* 2011b; Miralles *et al.* 2012, 2013). Communities dominated by mosses, lichens, microalgae and cyanobacteria (biocrusts hereafter) constitute a key biotic component from boreal, arctic, temperate and dryland ecosystems world-wide, where they play major roles in controlling ecosystem structure and functioning (Belnap & Lange 2003; Lindo & Gonzalez 2010).

Biocrust communities largely determine a wide range of hydrological and nutrient cycling processes in drylands, including run-off and infiltration, C fixation, soil respiration, and N mineralization, fixation and gaseous losses (see Eldridge & Green 1994; Belnap 2003; Maestre *et al.* 2011 for reviews). They also affect the abundance of soil micro- and macroorganisms. For example, the degree of biocrust development affects the abundance and/or activity of both broad (e.g. fungi; Bates *et al.* 2010) and functional [e.g. N-fixing cyanobacteria and ammonia-oxidizing archaea (AOA) and bacteria; Belnap 2002 Marusenko *et al.* 2013] groups of micro-organisms, as well as that of soil fauna such as nematodes (e.g. Darby, Neher & Belnap 2007). These studies highlight the importance of biocrusts as drivers of nutrient cycling and microbial abundance in drylands. However, most of them have considered biocrusts as a 'black box', which integrate the effect of different biocrust types and species on the nutrient cycles and microbial communities as a unique entity. Much less information is currently available on the potential impacts of individual species on soil functioning and microbial communities within biocrusts. Recent studies have shown that biocrust-forming lichens and mosses can have differential effects on soil nutrient cycles and microbial activity. For example, Miralles *et al.* (2012, 2013) showed that soils under late-successional lichen species such as *Diploschistes diacapsis* and *Lepraria crassissima* have a higher soil respiration, enzyme activities and inorganic N concentrations than those located under cyanobacteria. Similarly, Concostrina-Zubiri *et al.* (2013) showed that *D. diacapsis* enhanced the concentration of macro [C, N and phosphorus (P)]- and micro (iron and copper)-nutrients compared to bare ground areas. Other studies have indirectly evaluated how particular lichens affect microbial communities. Castillo-Monroy *et al.* (2011b) showed that lichen species such as *Squamarina cartilaginea* and *Fulgensia subbracteata* can have a negative impact on the abundance of soil bacteria, while co-occurring *D. diacapsis* showed a mixture of negative and positive effects on particular bacterial taxa. None of these studies have, however, evaluated how co-occurring biocrust-forming species simultaneously affect both microbial communities and multiple soil variables related to C, N and P cycling (but see Bowker *et al.* 2011), nor have explored how important effect traits (*sensu* Cornelissen *et al.* 2007), such as tissue chemical composition, drive observed species-specific effects on these variables.

Advancing our understanding of species-specific effects of biocrust constituents on soil nutrient availability and microbial communities is crucial to improve our knowledge of the ecological role of biocrust constituents. It is also of paramount importance to accurately predict how climate change-induced changes in the abundance of particular biocrust constituents (Escolar *et al.* 2012; García-Pichel *et al.* 2013) will impact ecosystem processes linked to nutrient cycling and storage (Reed *et al.* 2012; Maestre *et al.* 2013). Furthermore, evaluating such effects is fundamental to advance key topics that are poorly studied in cryptogamic communities in comparison with their vascular counterparts, such as biodiversity–ecosystem functioning relationships (Bowker *et al.* 2011; Maestre *et al.* 2012a) or the role of functional traits on ecosystem functioning (Cornelissen *et al.* 2007).

We assessed the effects of six frequent biocrust-forming lichens from central Spain (*B. epipolia, D. diacapsis, F. subbracteata, Psora decipiens, S. cartilaginea* and *Squamarina lentigera*) on 24 soil variables linked to N, C and P cycling (such as dissolved organic and inorganic N, carbohydrates, phenols, dissolved organic P and inorganic P) and on the abundance of broad (bacteria and fungi) and functional [ammonia-oxidizing bacteria (AOB) and archaea] groups of micro-organisms. Additionally, we evaluated the relationships between the composition of lichen tissues (contents in C, N, P and polyphenols) and the availability of C, N, P and microbial abundance in soils and assessed the most important factors characterizing the microsites created by the different lichen species. We hypothesize that: (i) as found with different plant species (e.g. Bezemer *et al.* 2006), biocrust-forming lichens will have species-specific effects on soil nutrients and microbial abundance; (ii) differences in the chemistry of lichen thalli (C, N, P and polyphenols) will determine these species-specific effects; and (iii) changes in soil fertility compared to bare ground areas (i.e. areas without visible biocrust components) will be found among the lichen species studied (Castillo-Monroy *et al.* 2011b; Miralles *et al.* 2012, 2013).

Materials and methods

STUDY SITE AND EXPERIMENTAL DESIGN

This study was conducted in the Aranjuez Experimental Station, located in the centre of the Iberian Peninsula (40°02′N–3°32′W; 590 m a.s.l.). Its climate is Mediterranean semi-arid, with a mean annual temperature and rainfall of 15 °C and 349 mm, respectively. Perennial plant cover is lower than 40% and is dominated by the perennial grass *Stipa tenacissima*. Open areas between plant patches contain a well-developed biocrust community dominated by lichens such as *D. diacapsis, S. lentigera* and *F. subbracteata* (see Maestre *et al.* 2013 for a full species checklist). The pH in our study sites ranged between 7·05 and 7·23 under *D. diacapsis* and *F. subbracteata*, respectively. The soil has a fine texture dominated

by the presence of gypsum and is classified as Gypsiric Leptosol (IUSS Working Group WRB 2006). The sand and silt contents in our soils ranged from 61·3% to 63·7% and from 30% to 32% in places with low and high biocrust cover, respectively (Castillo-Monroy *et al*. 2010). We do not expect high carbonates in the soils of our study site, which are characterized by their high gypsum content. For example, inorganic total C ranged between 0·5% and 11·4% of the soil total C under *D. diacapsis* and *F. subbracteata*, respectively.

Five soil samples (0–4 cm depth), as well as their respective lichen thalli, were randomly collected in Autumn 2012 within a 50 m × 50 m area under each of the most abundant biocrust-forming lichens presents in our study site (Castillo-Monroy *et al*. 2010): *B. epipolia*, *D. diacapsis*, *F. subbracteata*, *P. decipiens*, *S. cartilaginea* and *S. lentigera* (n = 5 per species, 30 samples in total). To minimize potential variations in soil characteristics not due to the lichen species sampled, all the samples were collected from areas that had a minimum surface of 4 cm × 4 cm covered by each species (Concostrina-Zubiri *et al*. 2013). Similarly, we collected five soil samples (0–4 cm depth) in randomly selected bare ground areas without visible biocrust constituents. A minimum separation distance between samples, and between these and plant patches, of 1 m was maintained. The spatial autocorrelation of soil nutrients associated with both vascular plants and biocrust-forming lichens in our study site occurs at spatial scales smaller than 60 cm (Delgado-Baquerizo *et al*. 2013c), and with this separation distance, we aimed to remove potential sources of non-independence between samples. After field sampling, the lichen thalli and plant roots were carefully separated from the soil, which was sieved (2 mm mesh) and separated into two fractions. One fraction was immediately frozen at −80 °C for quantifying the amount of the abundance of broad and functional groups of micro-organisms in our samples. The other fraction, as well as the lichen thalli, was air-dried and stored for 1 month before C, N and P analyses. Previous studies have found that air drying and further storage of dryland soils does not appreciably alter variables such as those we studied (Zornoza *et al*. 2006, 2009). It is also important to note that the soil was also dry (gravimetric soil moisture at 0–5 cm = 1·1%; n = 35) when we conducted our sampling. Thus, the potential bias induced by our drying treatment is expected to be minimal.

MEASUREMENT OF C, N AND P VARIABLES IN LICHEN AND SOIL

We measured in all the soil samples 20 variables linked to the stocks and cycling of C, N and P [organic C, dissolved organic C (DOC), hexoses, pentoses, phenols, microbial biomass C, activity of B-glucosidase, basal respiration, total N, dissolved organic N (DON), ammonium, nitrate, proteins, amino acids, potential net mineralization and nitrification rates, microbial biomass N, activity of phosphatase, dissolved inorganic (DIP) and organic P (DOP)]. All these variables are either measurements of specific ecosystem processes (e.g. N mineralization rate) or key properties (e.g. organic C, total N, inorganic P and soil enzymes), which together constitute a good proxy of nutrient cycling, biological productivity and build-up of nutrient pools (Reiss *et al*. 2009; Jax 2010; Maestre *et al*. 2012b). Similarly, we studied both broad (total bacteria and fungi) and functional genes (AOA and bacteria) because of the importance of microbial communities as drivers of nutrient cycling and organic matter decomposition (Schlesinger & Bernhardt 2013). In particular, AOA and AOB are well-known to carry out the first step of nitrification (Verhamme, Prosser & Nicol 2011). We also determined the concentration of C, N, P and polyphenols in lichen thalli. We measured polyphenols because they are often related to the antimicrobial capacity observed in lichen species similar to those studied here (e.g. Saenz, Garcia & Rowe 2006). A detailed explanation on the rationale of the selected variables is available in the supplementary information of this manuscript (Appendix S1, Supporting information).

The concentration of soil organic C and lichen total C was determined as described in Anderson & Ingramm (1993). Soil and lichen total N was measured with a CN analyzer (Leco CHN628 Series; Leco Corporation, St Joseph, MI, USA). Lichen polyphenols and total P were determined as described in Covelo, Avila & Gallardo (2011). β-Glucosidase and phosphatase activities were measured as described in Maestre *et al*. (2012b). The concentration of DIP and DOP was measured from $NaHCO_3$ 0·5 M soil extracts, as described in Tiessen & Moir (1993). Similarly, the concentration of ammonium, nitrate, DON, amino acids, proteins, hexoses, pentoses and phenols was measured from K_2SO_4 0·5 M soil extracts in a ratio 1:5, according to Chantigny *et al*. (2006) and Delgado-Baquerizo, Covelo & Gallardo (2011). The concentration of DOC was also measured from these extracts by using a TOC analyzer (TOC–Vsch, Shimadzu, Kyoto, Japan). Air-dried soil samples were rewetted to reach 80% of their water-holding capacity and incubated in the laboratory for 14 days at 30 °C to measure potential net mineralization and nitrification. These variables were estimated as the difference between initial and final dissolved organic N (DIN, sum of ammonium and nitrate) and nitrate concentrations, respectively (Allen, Grimshaw & Rowland 1986). Finally, we measured the basal respiration of our soils as described in Campbell *et al*. (2003). Prior to measuring this respiration, the soil was pre-incubated for 5 days at 25 °C and 40% of water-holding capacity to re-establish active microbial populations (Delgado-Baquerizo *et al*. 2013a).

The C and N of microbial biomass were determined using the fumigation-extraction method of Brookes *et al*. (1985). Non-incubated and incubated soil subsamples were fumigated with chloroform for 5 days. Non-fumigated replicates were used as controls. Fumigated and non-fumigated samples were extracted with K_2SO_4 0·5 M in a ratio 1 : 5 and filtered through a 0·45-μm Millipore filter (Merck Millipore, Billerica, MA, USA). The C and N concentrations of microbial biomass were estimated as the difference between total N and DOC of fumigated and unfumigated digested extracts, respectively, and then divided by a Kn (fraction of biomass N extracted after the CHC_{13} treatment) of 0·54 (Brookes *et al*. 1985).

ASSESSMENT ON MICROBIAL ABUNDANCE

Soil DNA was extracted from 0·6 g of defrosted soil samples using the Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the instructions provided by the manufacturer. Using these extractions, we quantified the amount of bacteria, fungi, AOA and AOB using quantitative PCR (qPCR); qPCRs were conducted in triplicate using 96-well plates on an ABI 7300 Real-Time PCR (Applied Biosystems, Foster City, CA, USA) following Delgado-Baquerizo *et al*. (2013b). The *amoA* genes of AOB and AOA were amplified using the primer sets *amoA1F – amoA2R* and *Arch-amoAF – Arch amoAR*, respectively, as described in Rotthauwe, Witzel & Liesack (1997) and Francis *et al*. (2005). Similarly, total bacterial 16S and fungal 18s rRNA genes were amplified with the *Eub 338-Eub 518* (Lane 1991) and *ITS1-5.8S* primer sets (Vilgalys & Hester 1990), respectively, as described in Maestre *et al*. (2013). Efficiencies for all quantification reactions were higher than 90%, with R^2 values ranging from 0·90 to 0·99.

STATISTICAL ANALYSES

To test our first hypothesis (i.e. species-specific effects on soil nutrients and microbial abundance), we searched for differences among the different lichen species studied on the 24 soil variables

evaluated by conducting a semiparametric multivariate ANOVA (PERMANOVA, Anderson 2001), with lichen species as a fixed factor. PERMANOVA allows the testing of the simultaneous responses of a multivariate data set to one or more factors in an ANOVA experimental design on the basis of any distance measure using permutation methods. We used PERMANOVA because our data did not meet MANOVA assumptions (normality and homogeneity of variances). Differences among species in the 24 soil and microbial variables measured were evaluated using pairwise *post hoc* tests in PERMANOVA (Anderson 2001). To visualize the differences among species, and to aid in the interpretation of PERMANOVA results, we also conducted a principal component analysis (PCA) with the 24 soil and microbial variables measured. Before carried out PERMANOVA and PCA analyses, the different variables measured were standardized by using the *z*-score (Kreyszig 1978). PERMANOVA analyses were carried out using 9999 permutations (permutation of raw data) and the Euclidean distance with the PERMANOVA+ for PRIMER statistical package (PRIMER-E Ltd., Plymouth Marine Laboratory, Ivybridge, UK). PCA analyses were carried out using SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA).

We then assessed which soil variables, among the 24 measured, were the most important drivers of the differences among the lichen species studied. To do this, we conduct a classification random forests analysis (RF, Breiman 2001). Random forest is a novel machine-learning algorithm that extends standard classification and regression tree (CART) methods by creating a collection of classification trees with binary divisions (Wei *et al.* 2010). Unlike traditional CART analyses, the fit of each tree is assessed using randomly selected cases (1/3 of the data), which are withheld during its construction (out-of-bag or OOB cases). The importance of each predictor variable is determined by evaluating the decrease in prediction accuracy (i.e. increase in the mean square error between observations and OOB predictions) when the data for that predictor are randomly permuted. This decrease is averaged over all trees to produce the final measure of importance (Wei *et al.* 2010). This accuracy importance measure was computed for each tree and averaged over the forest (5000 trees). In RF, the different soil variables were included as predictor of the different microsites conformed by the different lichen species (response variables). These analyses were conducted using the RANDOMFOREST package (Liaw & Wiener 2002) for the R statistical software, version 3.0.2 (http://cran.r-project.org/). The significances of the model and the cross-validated R^2 were assessed with 5000 permutations of the response variable using the A3 (Fortmann-Roe 2013) R package. Similarly, the significance of the importance measures of each predictor (here soil variables) on the response variable (lichen species) was assessed by using the RFPERMUTE (Archer 2013) package for R.

To test our second hypothesis (i.e. whether changes in the chemistry of biocrust-forming lichens determine the concentration of soil microbial communities and C, N and P variables), we evaluated the relationships between the concentration of C, N, P and polyphenols in lichen thalli and in the soil, and between the former and the soil microbial variables measured, using Spearman correlation analyses. Finally, to test our third hypothesis (differences in soil fertility compared to bare ground areas will be found among the lichen species studied), we estimated the effects of different lichen species on soil fertility (C, N and P variables) by calculating the RII index as (Sli − Sbg)/(Sli + Sbg), where Sli and Sbg are the values of a given soil variable under the lichen thalli and in bare ground areas, respectively (Armas, Ordiales & Pugnaire 2004). The RII index was calculated separately for each variable and lichen species studied, using as replicates for Sli the values obtained under each lichen thalli sampled ($n = 5$), which were compared in all cases with the average of the five replicates obtained from bare ground areas. Values of the RII index range from −1 to +1, with positive values indicating increases in the variable studied under the canopy of lichens compared to bare ground areas and negative values the opposite. To test whether RII values differed significantly from zero, we assessed their 95% bootstrap confidence interval by using the BOOTES (Kirby & Gerlanc 2013) R package. Differences among lichen species in the RII indexes and the concentration of C, N, P and polyphenols in their thalli were also evaluated by using one-way PERMANOVA, with lichen species as a fixed factor. These analyses were carried out using 9999 permutations (permutation of raw data) and the Euclidean distance with the PERMANOVA+ for PRIMER statistical package.

Results

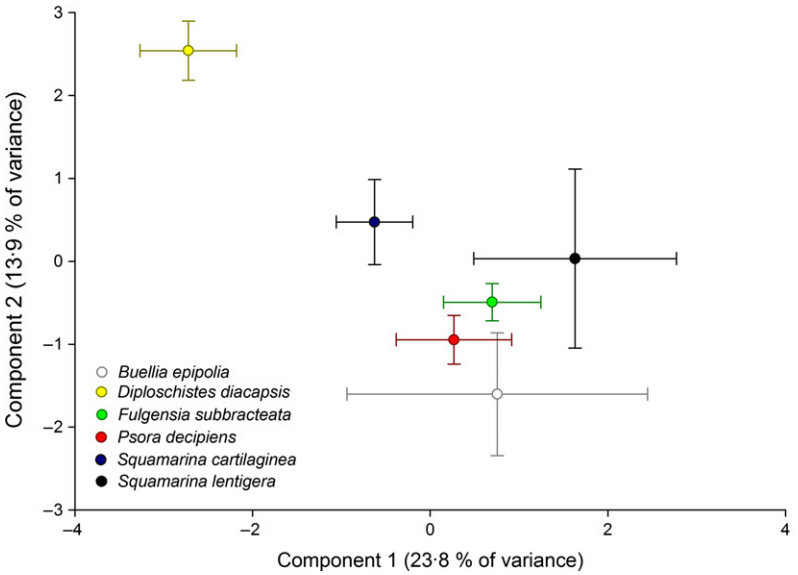
SPECIES-SPECIFIC EFFECTS ON SOIL NUTRIENTS AND MICROBIAL COMMUNITIES

We found that diverse lichen species differentially relate to the soil variables evaluated (PERMANOVA; $P < 0{\cdot}001$; Pseudo-F = 2·39; d.f. = 5; Fig. 1). *Diploschistes diacapsis* showed clear differences with the other species studied, which were driven mostly by the lowest soil C, N and P availability, and by the highest contents of phenols, DON, fungi, mineralization rates and basal respiration found under its thallus ($P < 0{\cdot}01$; Table S1; Fig. 1). Soils under *S. cartilaginea* had intermediate contents of C, N and P, phenols and total fungi, being these values lower than those from *P. decipiens* and *F. subbracteata* soils ($P < 0{\cdot}05$; Table S1; Fig. 1). *Squamarina cartilaginea* and *S. lentigera* had similar values of phenols, DON, N mineralization rates, total fungi and basal respiration under their thalli, albeit higher C, N and P contents were found in the former. Soils from *P. decipiens*, *F. subbracteata*, *B. epipolia* and *S. lentigera* showed the highest C, N and P contents (Fig. 1). Similarly, soils from *B. epipolia* tended to have lower values of DON, total fungi, phenols, mineralization rates and basal respiration than those from species such as *P. decipiens* and *F. subbracteata* ($P < 0{\cdot}10$; Table S1; Fig. 1).

Dissolved inorganic P, amino acids, proteins, phenols and soil respiration were the most important variables determining the differences among the studied lichens (Fig. 2). The rest of the soil variables characterizing each lichen species followed the next order: total N > AOA > microbial biomass C > bacteria > microbial biomass N > hexoses > phosphatase. Other soil variables evaluated had a negligible effect on the differences found among the lichens studied.

LICHEN CHEMISTRY EFFECTS ON SOIL VARIABLES

Species-specific differences in thallus chemistry (C, N, P and polyphenols) were highly related to the content of C, N, P and microbial abundance in the soil. For example, *D. diacapsis*, which had the lowest C, N and P contents, also had the lowest soil nutrient availability (Fig. 3; Table 1; Table S2 for raw data). Similarly, soils under *P. decipiens* and *F. subbracteata*, which had the highest N and P contents in their thalli, had two of the highest N and P concentrations (Fig. 3; Table 1; Table S2). The highest N and P availability found under these species



	Component 1	Component 2
Organic C	0·68 (<0·001)	
DOC	0·56 (0·001)	
Hexoses	0·69 (<0·001)	
Pentoses	0·52 (0·003)	
Phenols		0·49 (0·007)
BM-C		
B-glucosidase	0·53 (0·003)	
Basal respiration		0·46 (0·015)
Total N	0·64 (<0·001)	
Ammonium	0·84 (<0·001)	
Nitrate	0·38 (0·037)	−0·46 (0·003)
DON	0·62 (<0·001)	0·49 (0·006)
BM-N	0·43 (0·018)	
Amino acids	0·56 (0·001)	
Proteins		−0·70 (<0·001)
Nitrification	0·53 (0·004)	0·51 (0·004)
Mineralization	0·46 (0·011)	0·51 (0·004)
DIP	0·56 (0·001)	
Phosphatase	0·59 (0·001)	
Total fungi		0·46 (0·011)

Fig. 1. Results of the principal component analysis showing the effects of the lichen species studied on the soil C, N, P and microbial variables measured. Significant ($P < 0{\cdot}05$) Spearman correlations among the original variables and the ordination components (PC1 and PC2) are shown next to them. Values represent means ± SE ($n = 5$). DIP, dissolved inorganic phosphorus; MB-C, microbial biomass carbon; MB-N, microbial biomass nitrogen; DOC, dissolved inorganic carbon; DON, dissolved organic nitrogen.

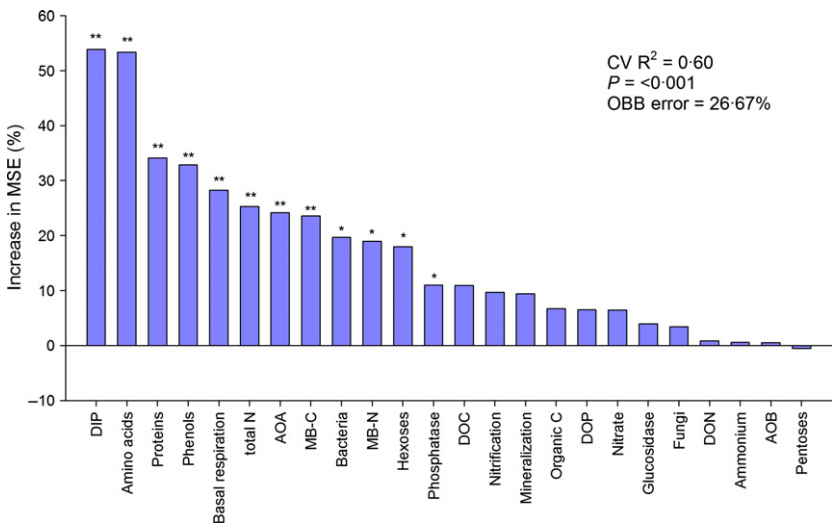


Fig. 2. Random forest mean predictor importance (% of increase of mean square error) of soil variables studied as drivers of the different biocrust-forming lichens microsites in this study. This accuracy importance measure was computed for each tree and averaged over the forest (5000 trees). Significance levels are as follows: $*P < 0{\cdot}05$ and $**P < 0{\cdot}01$. DIP, dissolved inorganic phosphorus; AOA, ammonia-oxidizing archaea; MB-C, microbial biomass carbon; MB-N, microbial biomass nitrogen; DOC, dissolved inorganic carbon; DOP, dissolved organic phosphorus; DON, dissolved organic nitrogen; AOB, ammonia-oxidizing bacteria.

seem to be negatively related to the abundance of soil bacteria (Fig. 3; Table 1). Additionally, soils under *D. diacapsis*, which had the highest polyphenol content, were also those with the highest phenol concentrations (Fig. 3; Table S2). As a whole, the concentration of C in lichen thalli was positively related to that of soil organic C, DOC, total N and ammonium, and with the activity of phosphatase, but was negatively related to the abundance of AOA (Table 1). The concentration of N in lichen thalli was positively related to that of soil organic C, hexoses, total N and ammonium, and with the activity of β-glucosidase and microbial biomass N, and was negatively related to the abundance of bacteria (Table 1). The concentration of P in lichen thalli was positively related to that of soil hexoses, pentoses, ammonium, DIP and amino acids, and with both nitrification and mineralization, but was negatively related to the soil basal respiration (Table 1). In addition, the concentration of polyphenols in lichen thalli was positively related to that in the soil and to basal respiration and negatively related to the content of soil amino acids and DIP, and to AOA (Table 1).

SPECIES-SPECIFIC EFFECTS ON SOIL FERTILITY COMPARED TO BARE GROUND AREAS

We found both positive and negative effects of biocrust-forming lichens on nutrient availability and microbial abundance relative to bare ground areas, which varied with the species

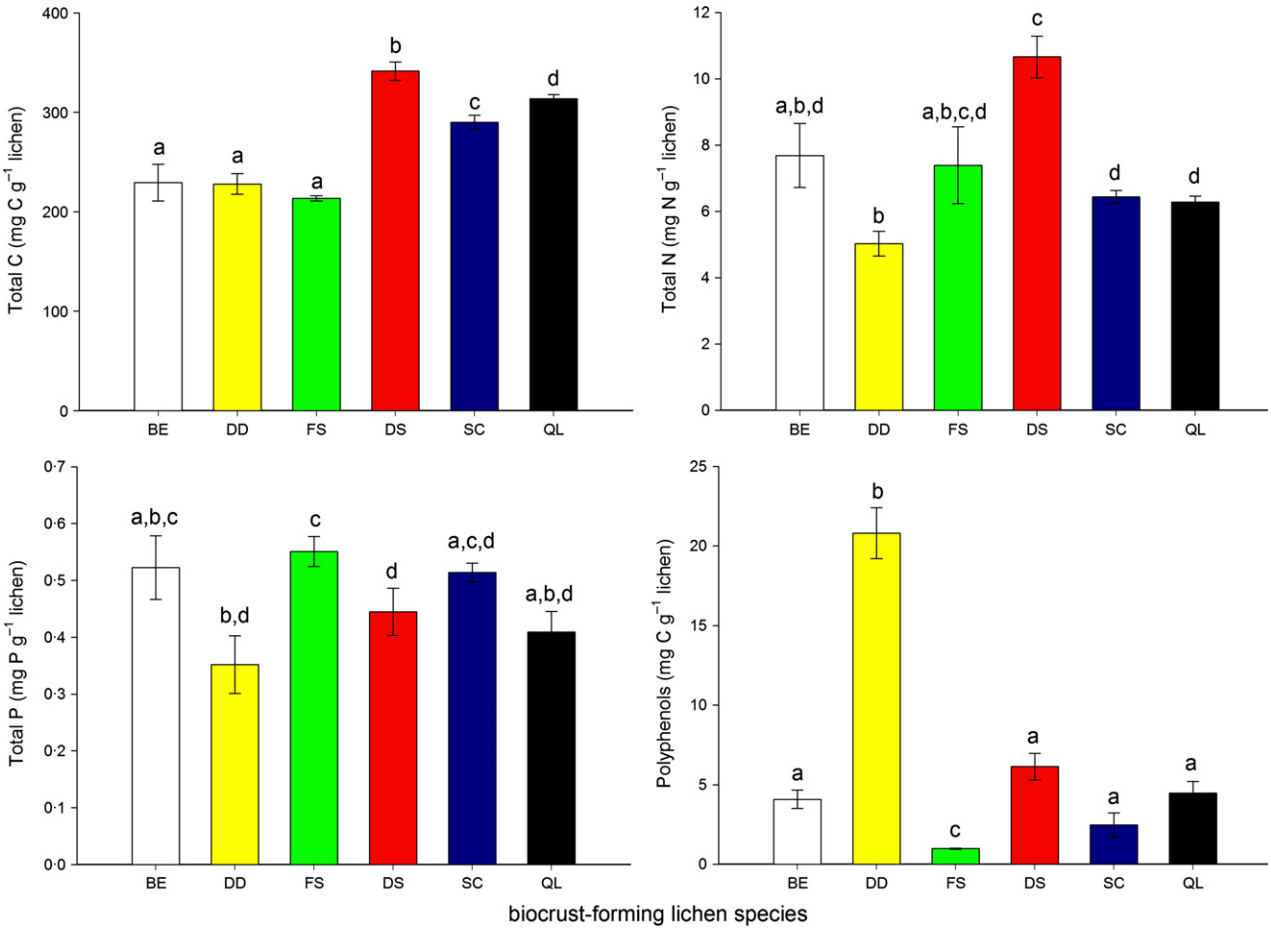


Fig. 3. Concentration of lichen C, N and P and polyphenols for the different biocrust-forming lichen species studied. Values represent means ± SE (n = 5). Different letters indicate significant differences among the lichen species studied ($P < 0{\cdot}05$, *post hoc* test after PERMANOVA analyses). Species abbreviations as follows: *Buellia epipolia* (BE), *Diploschistes diacapsis* (DD), *Fulgensia subbracteata* (FS), *Psora decipiens* (PS), *Squamarina cartilaginea* (SC) and *Squamarina lentigera* (SL).

and soil variable considered (Figs 4 and S1). *Diploschistes diacapsis* and *P. decipiens* reduced the concentration of amino acids when compared to bare ground areas, while the rest of lichen species increased it (Fig. 4). Similarly, *B. epipolia* and *P. decipiens* had the highest positive effects on the microbial biomass N (Fig. 4), while *D. diacapsis* showed the highest negative effect on this variable (Fig. 4; Table S3). Interestingly, all the lichen species in this study had a negative effect on the concentration of soil nitrate and microbial biomass C (Figs 4 and S1). *Fulgensia subbracteata* and *P. decipiens* had the highest positive and negative effects on the abundance of AOA, respectively (Fig. 4). Similarly, *B. epipolia* and *D. diacapsis* had the highest positive effects on soil bacteria, while *F. subbracteata* and *P. decipiens* presented the highest negative effects on these micro-organisms (Fig. 4).

Discussion

Our results suggest that biocrust-forming lichens have species-specific effects on soil nutrient availability and both broad (i.e. total bacteria) and functional (i.e. AOA) microbial groups. These effects seem to be linked to the C, N and P content of the lichen thalli. The availability of N and P was the most important factor characterizing the soil microsites created by the different lichens studied. Even when most of these species had a positive effect on fertility and microbial abundance, we found that species such as *D. diacapsis* negatively affected the concentration of microbial biomass N, while others such as *P. decipiens* reduced the abundance of total bacteria and AOA micro-organisms. We acknowledge that the observational nature of our study does not allow us to establish causal relationships. However, our previous experimental work has demonstrated how model communities formed with the lichen species studied can alter the nutrient content and microbial activity of gypsum soils such as those studied here (Maestre *et al.* 2012a; Castillo-Monroy *et al.* 2015). These experimental results, together with the characteristics of our survey, provide a sound basis for inferring from our results that the relationships found are likely due to the effect of the lichens themselves.

SPECIES-SPECIFIC EFFECTS ON SOIL NUTRIENTS AND MICROBIAL COMMUNITIES

Biocrust-forming lichens differentially related to multiple soil variables linked to C, N and P cycling and storage,

Table 1. Correlation coefficients (Spearman's ρ) between the lichen chemical composition (C, N, P and polyphenols) and the concentration of soil C, N, P and microbial variables in this study

	Lichen C	Lichen N	Lichen P	Lichen polyphenols
Organic C	0·57 (**0·001**)	0·43 (**0·017**)	0·14 (0·433)	0·04 (0·808)
DOC	0·41 (**0·023**)	0·29 (0·119)	0·28 (0·124)	0·15 (0·414)
MB-C	0·28 (0·132)	0·15 (0·403)	0·07 (0·693)	−0·12 (0·532)
Hexoses	0·02 (0·889)	0·45 (**0·011**)	0·71 (**< 0·001**)	−0·32 (0·080)
Pentoses	0·04 (0·830)	0·12 (0·506)	0·35 (0·055)	0·02 (0·918)
Phenols	−0·06 (0·751)	−0·31 (0·090)	−0·13 (0·465)	0·56 (**0·010**)
Glucosidase	0·24 (0·189)	0·38 (**0·039**)	0·14 (0·455)	0·15 (0·409)
Basal respiration	0·22 (0·230)	−0·23 (0·206)	−0·51 (0·004)	0·69 (**< 0·001**)
Total N	0·50 (0·005)	0·54 (**0·005**)	0·18 (0·318)	−0·01 (0·992)
Ammonium	0·39 (**0·034**)	0·55 (0·002)	0·42 (**0·022**)	−0·04 (0·806)
Nitrate	0·27 (0·137)	0·36 (**0·050**)	0·25 (0·176)	−0·04 (0·806)
DON	0·05 (0·777)	0·09 (0·630)	0·34 (0·066)	0·19 (0·291)
MB-N	0·15 (0·428)	0·41 (**0·024**)	0·18 (0·318)	−0·03 (0·873)
Amino acids	−0·06 (0·757)	0·07 (0·691)	0·36 (**0·049**)	−0·40 (**0·028**)
Proteins	0·26 (0·150)	0·34 (0·064)	0·02 (0·899)	−0·28 (0·131)
Nitrification	−0·01 (0·963)	0·10 (0·590)	0·37 (**0·045**)	−0·09 (0·632)
Mineralization	−0·03 (0·856)	0·09 (0·627)	0·41 (**0·025**)	−0·15 (0·429)
DIP	0·08 (0·674)	0·12 (0·522)	0·38 (**0·036**)	−0·49 (0·006)
DOP	−0·07 (0·712)	−0·05 (0·779)	0·19 (0·304)	0·14 (0·455)
Phosphatase	0·41 (**0·023**)	0·34 (0·061)	0·03 (0·854)	−0·09 (0·621)
AOB	0·01 (0·998)	−0·13 (0·475)	−0·28 (0·131)	0·14 (0·435)
AOA	−0·56 (**0·001**)	−0·14 (0·438)	0·29 (0·110)	−0·39 (**0·031**)
Total bacteria	−0·20 (0·298)	−0·42 (**0·020**)	−0·34 (0·062)	0·13 (0·478)
Total fungi	0·07 (0·695)	0·01 (0·933)	0·09 (0·619)	0·13 (0·487)

DOC, dissolved organic carbon; MB-C, microbial biomass carbon; DON, dissolved organic nitrogen; MB-N, microbial biomass nitrogen; DIP, dissolved inorganic P; DOP, dissolved organic P; AOB, ammonia-oxidizing bacteria; AOA, ammonia-oxidizing archaea.
P values below 0·05 are in bold ($n = 30$).

and on the abundance of soil microbial communities (i.e. AOA and total bacteria). Of particular interest was that available P (inorganic P) and N (amino acids, proteins and total N), the most important nutrients limiting plant productivity in terrestrial ecosystems (Schlesinger & Bernhardt 2013), were the most contrasting soil variables among the lichens studied. For example, species such as *B. epipolia* and *P. decipiens* had the best soil N availability conditions, as supported by the highest total N contents in both soil and microbial biomass. However, *F. subbracteata* and *S. lentigera* showed the highest concentration of inorganic P in the soil. After P and N, the content of phenols in soil was the most important factor differencing the lichen species studied. Species such as *D. diacapsis* and *F. subbracteata* showed the lowest and highest organic matter quality in the soil, respectively (i.e. ratio hexoses: phenols; Rovira & Vallejo 2002). A relative increase of soil phenols (derived from lignin and other polyphenolic compounds) compared to sugars can reduce the quality of soil organic matter by reducing the amount of C sources easily accessible for microbes and can promote allelopathic effects on microbes and plants (Herbert & Bertsch 1995; Zsolnay 1996; Chantigny *et al.* 2006). Similarly, soils under *D. diacapsis* and *F. subbracteata* had the highest bacterial and the lowest AOA abundances, respectively. As a whole, the high variety of soil microsites coexisting close to each other, characterized by different nutrient availabilities and both broad (i.e. total bacteria) and functional (i.e. AOA) groups of micro-organisms, together with the typical low range of spatial dependency for nutrients such as N under biocrusts in our study site (between 11·7 and 15·5 cm; Delgado-Baquerizo *et al.* 2013c), may facilitate the nearby acquisition of N and P by plant roots under well-developed biocrusts. For example, Harper & Belnap (2001) showed that plants living in areas with well-developed biocrusts usually have higher nutrient contents than those living in areas without them, although these effects may differ among different locations (DeFalco 1995). In addition, Green, Porras-Alfaro & Sinsabaugh (2008) showed that biocrusts can exchange C and N with vascular plants, which can be dispersed over approximately 1 m^2 areas at rates up to 100 cm per day during periods of active plant growth. The high spatial heterogeneity in soil nutrient availability promoted by biocrusts, and the potential for nutrient translocation between plants and biocrusts (usually linked to fungal communities; Green, Porras-Alfaro & Sinsabaugh 2008), may contribute to the coexistence of different plant species in dryland ecosystems (Harrison 1999; Casieri *et al.* 2013).

LICHEN CHEMISTRY EFFECTS ON SOIL VARIABLES

Our results indicate that species-specific changes in the content of C, N and P in lichen tissues were linked to the availability of C, N and P in the soil. Organisms have generally conservative stoichiometries and elemental

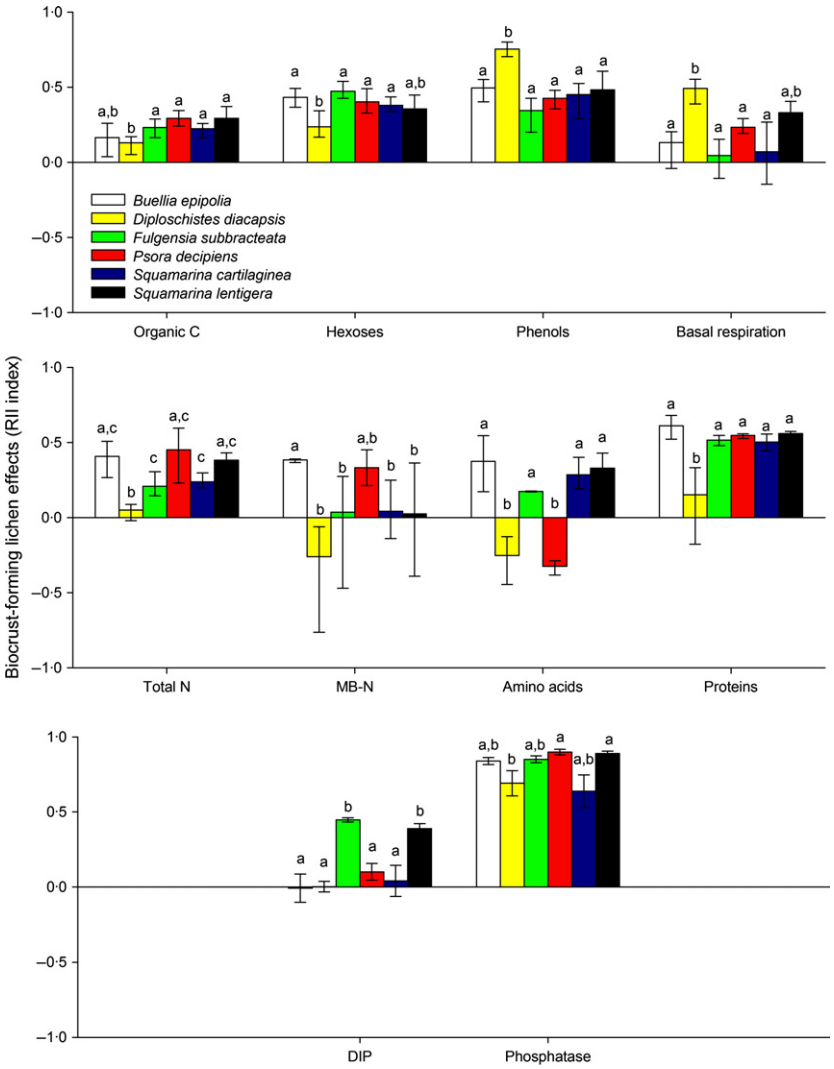


Fig. 4. Values of the RII indexes obtained for the soil C, N and P and microbial variables evaluated. Different letters indicate significant differences among the lichen species studied ($P < 0{\cdot}05$, *post hoc* test after PERMANOVA analyses). Values represent means ± 95% bootstrap confidence intervals ($n = 5$). DIP, dissolved inorganic phosphorus; MB-N, microbial biomass nitrogen. The soil studied variables that did not show significant differences among species ($P > 0{\cdot}05$) are available in Fig. S1.

compositions to catalyse metabolic reactions and synthesize structural compounds (Sterner & Elser 2002). In this sense, Bell *et al.* (2014) showed that the stoichiometry of plants is strongly linked to that of soil and soil microorganisms. Given that the nutrient content of vascular plants can increase in areas with well-developed biocrusts (Harper & Belnap 2001), an interesting question derived from this study is whether plants growing in areas dominated by biocrust-forming lichens with high nutrient contents (e.g. *P. decipiens* and *S. lentigera*) can acquire more nutrients than those from ecosystems dominated by lichens with low nutrient contents (e.g. *D. diacapsis*). This question is not trivial, since climate change will change the composition of biocrusts world-wide (Escolar *et al.* 2012; Reed *et al.* 2012), a process that will alter both C and N cycling (Maestre *et al.* 2012a,b; Reed *et al.* 2012; Zelikova *et al.* 2012).

In addition, the composition of lichen thalli was also highly related to that of phenols and microbes in soil. The high concentration of soil phenols found under *D. diacapsis* suggests that this species may have allelopathic effects that prevent other lichen species from occupying its microsite (Souza-Egipsy *et al.* 2002). However, the high basal respiration (potential respiration) found under *D. diacapsis* also indicates that the microbial communities under this lichen may be highly efficient consuming phenolic components (Yu *et al.* 2012; Miralles *et al.* 2013). The capacity to degrade recalcitrant phenol compounds may allow these microbial communities to thrive under *D. diacapsis*, despite the lowest levels of labile C under this species. In addition, phenolic components such as catechol and hydroxamate siderophores can play an important role by forming complexes with the metal cations present in the rock-forming minerals, which may give an advantage to *D. diacapsis* when acquiring micronutrients (Schatz 1963; Haselwandter & Winkelmann 2007).

Regarding microbial abundance, we observed a negative relationship between the content of C in lichen thalli, soil organic C and the abundance of AOA. In this regard, our results suggest that the relationship found between the C content of lichen thalli and soil organic C may indirectly impact on the abundance of microbial functional groups

such as AOA (Table S3). Previous studies have found that AOA are out-competed by other group of micro-organisms, such as AOB, in the most fertile microsites within a given ecosystem (Verhamme, Prosser & Nicol 2011; Delgado-Baquerizo *et al*. 2013c). In this sense, AOA may only be competitive under oligotrophic conditions because of their high adaptation to low nutrient conditions (Verhamme, Prosser & Nicol 2011; Delgado-Baquerizo *et al*. 2013b), occupying the soil microsites with the lowest nutrient content, such as those provided by *F. subbracteata*, *B. epipolia* and *D. diacapsis*. We also found a negative relationship between the concentration of N and P in lichen thalli and the abundance of soil bacteria. A possible explanation for this result is that species such as *P. decipiens* and *F. subbracteata*, which were associated with the highest contents in N and P in the soil, respectively, may produce antibacterial agents (i.e. usnic acid, Tay *et al*. 2004) to reduce the competition for N and P against soil micro-organisms (Dahlman *et al*. 2004).

As suggested by the predominant geochemical vs. biological control found in dryland ecosystems (Delgado-Baquerizo *et al*. 2013d), one could expect that abiotic variables such as pH, electrical conductivity (i.e. salinity) or total inorganic C can influence the nutrient and microbial abundance more than biocrust lichen-forming species. However, abiotic factors had a lower influence con the C, N, P and microbial variables studied than the nutrient concentration of the lichen thalli (Table 1; Table S4). Thus, and despite the correlative nature of our study and the limitations of the approach followed, our results suggest that the nutrient content of the lichen thalli, and not abiotic properties (e.g. pH, total inorganic C and electrical conductivity), is a major driver of the variability in soil nutrient availability associated with the different lichen species studied.

SPECIES-SPECIFIC EFFECTS ON SOIL FERTILITY COMPARED TO BARE GROUND AREAS

Multiple studies have suggested that biocrusts can enhance soil nutrient availability compared to bare ground areas. For example, Castillo-Monroy *et al*. (2010) found that biocrusts enhance the concentration C, N and ammonium regarding bare ground areas, showing a soil nutrient concentration of a similar order or magnitude than that found under the dominant plant species in our study site (*Stipa tenacissima*). However, we found that this assumption varied with the lichen species and soil variable considered (see also Bowker *et al*. 2011; Miralles *et al*. 2012, 2013). For example, species such as *D. diacapsis* were positively related to variables from the C cycle, but had a negligible effect on the availability of both inorganic and organic P (Fig. S1), and showed a negative relationship to some of the N cycle variables evaluated (e.g. amino acids and microbial biomass N). On the contrary, species such as *P. decipiens, F. subbracteata* and *S. lentigera* were positively related to the RII values of soil C, N and P, matching with the general idea that biocrusts increase nutrient availability compared to bare ground areas (Belnap & Lange 2003; Maestre *et al*. 2011; Concostrina-Zubiri *et al*. 2013). We observed an overall negative effect of the different lichen species studied on the microbial biomass C. This result suggests that despite the positive relationship between biocrusts and the RII index for soil organic C, this C may not be directly available for the microbes, likely because of the typical high recalcitrance of C sources under biocrust-forming lichens (i.e. *D. diacapsis*; Yu *et al*. 2012; Miralles *et al*. 2013). In addition, the negative relationship between *D. diacapsis* and the microbial biomass N suggests that this species may favour communities with low C-to-N ratio, such as those dominated by fungi (Austin *et al*. 2004; Table S2). We also found negative values of the RII index of nitrate in the different lichens studied (Fig. S1). Interestingly, the values of this index were positive for the potential nitrification rates and AOB. Since these rates are determined from soil incubation under laboratory conditions and without the presence of biocrusts, we have to consider the possibility that even when microbes under biocrusts can carry out nitrification, this process can be inhibited in the field due to the environmental conditions found under the lichen thalli (i.e. anoxic conditions; Garcia-Pichel & Belnap 1996).

The availability of inorganic P was the soil variable most affected by the lichen species studied. This result suggests that the species-specific relationships with soil nutrients observed may be especially important for controlling the availability of P in the soil surface. The availability of C and N is primarily linked to biological processes such as photosynthesis, atmospheric N fixation and subsequent microbial mineralization, which are largely influenced by biocrusts in drylands (Belnap 2002; Castillo-Monroy *et al*. 2010; Ladrón de Guevara *et al*. 2014). However, the availability of P for plant and micro-organisms is linked to the desorption and dissolution of P from soil minerals, and to a lesser extent, to the decomposition of organic matter (Vitousek 2004; Delgado-Baquerizo *et al*. 2013d). Despite the important influence of abiotic factors on the P cycle, we did not find significant relationships between key abiotic variables (e.g. pH, electrical conductivity and total inorganic C) and the soil P variables studied (Table S4). This result reinforces the idea that lichen species may control soil nutrient availability in our study site. Why did we observe then such differences among the biocrust-forming lichens studied? Species such as *F. subbracteata* and *S. lentigera* showed a much higher content of inorganic P than *B. epipolia* and *D. diacapsis*. It would be reasonable to think that *F. subbracteata* and *S. lentigera* may have a high capacity to produce compounds that, such as oxalic acid, promote the weathering of P from soil minerals (Schlesinger & Bernhardt 2013). Different lichens have been observed to be covered with a thick layer of calcium oxalate, which plays an important role protecting them against high solar radiation levels (Magnusson 1929; Syers, Birnie & Mitchell 1967), and can constitute up to 66% of

the dry weight of the thalli in species such as *Lecanora esculenta* and *Rhizocarpon numbilicatum* (Syers, Birnie & Mitchell 1967). The different capacity of the species studied to produce calcium oxalate could have an indirect impact on their capacities to modify the local P content under their thalli. Interestingly, the relationships between the P content in lichen thalli and the rest of the soil P variables were only significant and positive for inorganic P, but not for either the activity of β-phosphatase or organic P. In general, similar results are observed when we analysed the correlations between the concentration of P in lichen thalli and soil P variables separately for each of the species evaluated (Table S5). In this direction, the overall positive effect of the lichens studied on the production of extracellular enzymes such as phosphatase may play an important role in the production of inorganic P from organic P forms in our study site (Bell *et al*. 2014). Indeed, both inorganic P and activity of phosphatase were highly related to each other (Spearman's $\rho = 0{\cdot}47$; $P = 0{\cdot}008$; Table S3).

Conclusions

Our study provides, for the first time, evidence of species-specific correlations between biocrust-forming lichens and both microbial abundance and the availability of soil C, N and P under field conditions. The species-specific relationships with soil nutrients observed may be especially important for controlling the availability of P in the soil surface. Our results may improve our understanding of ecosystem functioning in biocrust-dominated ecosystems, which may be obscured when these communities are considered as a unique entity ('black box'), as commonly done in previous studies. Our findings also open the door to advance our understanding of key topics such as functional traits and biodiversity–ecosystem functioning relationships in biocrust-dominated communities.

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Data accessibility

Data available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.3m1v4 (Delgado-Baquerizo *et al*. 2014).

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Supporting Information

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

Appendix S1. Rationale on the included C, N and P and microbial variables in this study.
Fig. S1. RII indexes for the soil C, N and P and microbial variables than did not show significant differences ($P > 0{\cdot}05$) among biocrusts-forming lichens species in this study: *Buellia epipolia* (BE), *Diploschistes diacapsis* (DD), *Fulgensia subbracteata* (FS), *Psora decipiens* (PS), *Squamarina cartilaginea* (SC) and *Squamarina lentigera* (SL).
Table S1. Results of pairwise *post hoc* tests including all the C, N, P and microbial variables evaluated comparing pairs of biocrust-forming lichen species: *Buellia epipolia* (BE), *Diploschistes diacapsis* (DD), *Fulgensia subbracteata* (FS), *Psora decipiens* (PS), *Squamarina cartilaginea* (SC) and *Squamarina lentigera* (SL).
Table S2. Concentration for soil C, N and P variables and microbial abundance for bare ground areas and the different biocrusts-forming lichens species in this study: Bare ground areas (BG), *Buellia epipolia* (BE), *Diploschistes diacapsis* (DD), *Fulgensia subbracteata* (FS), *Psora decipiens* (PS), *Squamarina cartilagine*a (SC) and *Squamarina lentigera* (SL).
Table S3. Correlation coefficients (Spearman´s ρ) between the soil variables (C, N, P and microbial abundances) in this study.
Table S4. Correlation coefficients (Spearman´s ρ) between pH, electrical conductivity and total inorganic C and the concentration of the different soil variables in this study.
Table S5. Correlation coefficients (Spearman´s ρ) between the lichen chemical composition (C, N, P and polyphenols) and the concentration of soil C, N and P and microbial variables in the different biocrusts-forming lichens species in this study: *Buellia epipolia* (BE), *Diploschistes diacapsis* (DD), *Fulgensia subbracteata* (FS), *Psora decipiens* (PS), *Squamarina cartilagine*a (SC) and *Squamarina lentigera* (SL).