BIOCRUST COVER AND SUCCESSIONAL STAGES INFLUENCE SOIL BACTERIAL COMPOSITION AND DIVERSITY IN SEMIARID ECOSYSTEMS

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ABSTRACT

Biocrusts are an important constituent of landscape in drylands, which enrich the upper millimeters of the soil with organic matter and initiate the biogeochemical cycles. However, little is known about the influence of the biocrust on soil bacterial community structure and diversity. Different biocrust types representing a successional gradient were studied. This gradient, from the earliest to the latest successional stages, consisted in incipient cyanobacterial biocrust < mature cyanobacterial biocrusts < biocrust dominated by the *Squamarina lentigera* and *Diploschistes diacapsis* lichens < Biocrust characterized by the *Lepraria isidiata* lichen. Moreover, in each biocrust type, four different percentage of biocrust-cover were also selected. The soil diversity gradually increased with biocrust successional stage and percentage of biocrust-cover. The biocrusts-cover had an important role in the total abundance of bacteria generally increasing in soils colonized by the highest percentages of cover. Biocrust successional stage was the most important factor significantly influencing in 108 soil bacteria genera, whereas biocrust-cover showed significant differences in only 10 genera. Principal Component Analysis showed contrasting microbial composition across the biocrust successional gradient. Some bacterial taxa

were dominant in the soil colonized by different biocrust types. Thus, *Leptolyngbya sp.*, *Rubrobacter*, *Solirubrobacter*, *Geodermatophilus*, etc., were more abundant in incipient cyanobacteria; *Nostocales*, *Chroococcidiopsaceae*, *Coleofasciculaceae* etc., in soils colonized by mature cyanobacterial biocrusts; *Truepera*, *Sphingobacteriaceae*, *Actinophytocola*, *Kribella*, etc., in soils colonized by *D. diacapsis* and *L. isidiata* and *Bryobacter*, *Ohtaekwangia*, *Opitutus*, *Pedosphaeraceae*, etc., in soils colonized by *L. isidiata*. Several soil bacteria taxa showed significant correlations ($p<0.05$) with chemical soil properties (pH, total nitrogen, total organic carbon, available phosphorous and electrical conductivity). We discuss the role of biocrusts influencing these variables by their effects on chemical soil parameters, and also in soil moisture and the presence of certain metabolites secreted by biocrusts, which could favor a more selective environment for certain bacteria.

Keywords: microbial communities, lichen, cyanobacteria, Illumina MiSeq, chemical soil properties, drylands.

1. INTRODUCTION

Biocrusts constitute one of the most important and extensive dryland landscapes worldwide (Belnap and Eldridge, 2003), and have developed multiple essential roles in ecosystem functioning (Belnap and Lange, 2003; Li, 2012; Weber et al., 2016). Study of the microbial communities associated with biocrusts has recently attracted great interest, due to their possible synergistic relationships (Grube and Berg, 2009). Some authors have shown that the size and activity of microbial communities in biocrusts and immediately underlying soils is higher than in bare soils (Miralles et al., 2012a). The microbial species composition in biocrusts encompasses widely diverse bacterial taxa (Cardinale et al. 2008; Grube et al. 2009; Hodkinson and Lutzoni 2009; Bates et al. 2011; Hodkinson et al. 2012; Moquin et al., 2012; Aschenbrenner et al., 2016; Zhang et al., 2016; Liu et al., 2017). This great diversity in the composition of microbial communities in lichen biocrusts could be merely opportunistic because of the immediately surrounding soil environment (Cardinale et al., 2006), or due to the presence of certain groups of bacteria

associated with different types of biocrusts with species-specific patterns (Grube et al., 2009). Some studies have provided data supporting that hypothesis, showing that lichen species appear to be the strongest predictor of community composition, and that highly-structured lichen-associated bacterial communities reflect different functional roles in symbiosis with the lichens (Grube et al., 2009; Bates et al., 2011). Biocrusts composed of lichens and cyanobacteria certainly secrete a wide variety of secondary metabolites, such as oxalates, and acid compounds, such as gyrophoric acid, lecanoric acid, usnic acid, and so on (Cockell and Knowland, 1999; Wynn-Williams and Edwards, 2000; Dickensheets et al., 2000; Edwards, 2007; Miralles et al., 2012b), which could have significantly different (acidotolerant) bacterial communities than species without such compounds. On the contrary, hydrophilic parts of the lichens composed of a large amount of extracellular polysaccharides, could also promote the growth of anaerobic bacteria (Grube and Berg, 2009). Moreover, some of the secondary metabolites produced by lichen-dominated biocrusts have antimicrobial activity (Francolini et al., 2004; Boustie and Grube, 2005; Hodkinson et al. 2012; Kosani´c and Rankovi´c, 2015), providing a selective environment in which some complex bacterial communities thrive (Cardinale et al., 2008; Grube et al., 2009; Grube et al., 2015). Differences in chemistry, structure and growth of lichens dominating biocrusts (i.e. crustose, foliose or fruticose lichens) also create a wide diversity of ecological niches for additional microorganisms (Grube and Berg, 2009; Grube et al., 2009; Hodkinson et al., 2012). Therefore, the presence of selective bacteria communities in biocrusts and the leaching of certain secondary metabolites from the biocrusts to the immediately underlying soil could influence and select the composition of the soil microbial communities. However, there are hardly any studies focused on analyzing specific microbial communities of soils which could be influenced by biocrusts.

Biocrusts could also indirectly influence the microbial communities in the immediately underlying soil due to changes in it as it is colonized by the biocrusts (Pointing and Belnap, 2012; Miralles et al., 2012a). Several studies have shown that soil microbial communities are governed by physical and chemical properties of soils, such as pH, soil organic carbon or amount of salts in the soils (Lauber et al., 2009; Goldfarb et al., 2011; Griffiths et al., 2011; Kuramae et al., 2012; Canfora et al., 2014; Sánchez-Marañón

et al., 2017). As biocrusts colonize the soil surface, changes in physical, chemical and biochemical soil properties occur in the underlying layers (Pointing and Belnap, 2012; Miralles et al., 2012a, c). Biocrust fungal and cyanobacterial filaments stabilize the soil by penetrating the soil surface and providing particle cohesion (Pointing and Belnap, 2012). They favor soil aggregation and porosity by increasing water infiltration and retention (Belnap, 2006), and increase carbohydrate-C, polyphenol-C and labile-C content in underlying soils (Miralles et al., 2013). They also influence the nitrogen cycle, including N fixation (Elbert et al., 2012) and nitrification (Castillo-Monroy et al., 2010), drive soil hydrolytic enzyme activity (Miralles et al., 2012c) and reduce bulk density by increasing porosity (Miralles et al, 2011). These changes in soil properties are in turn closely related to the different biocrust types, which are often related to successional stages. Thus, biocrusts release organic acid secondary metabolites in abundance (Elix and Stocker-Wörgötter, 2008), and some of their polysaccharides are characteristic of certain lichen groups (Carbonero et al., 2002). Late successional stages of biocrusts predominated by lichens produce higher labile-C, carbohydrate and polyphenol contents than early successional biocrusts (Miralles et al., 2013), and show high N-cycle enzyme activity that hydrolyzes low-molecular-weight substrates, indicating a gradual replacement of atmospheric N_2-fixing organisms by other autotrophs which are not (Miralles et al., 2012a). At the same time, provision of those nutrients by biocrusts as well as the differences in carbon sources and N dynamics between late and early successional biocrusts, could be shaping the bacterial community in the immediately underlying soil on a very fine scale. Nevertheless, very little is known about the taxonomic composition of bacterial communities in the geological substrate colonized by biocrusts, or which specific groups of bacteria could be associated with different types of biocrusts, and even less about how different soil bacterial communities are affected by the successional stages and cover of the biocrusts, or the factors that determine these changes in their soil microbial communities. We hypothesized that biocrust-cover and its successional stage may affect the total abundance of soil bacteria, diversity and specificity of the soil bacterial taxa associated with the each biocrust. The development of the biocrust would also have a positive effect on soil microbial communities. Thus, the composition of the soil microbiota would depend on the type and cover of biocrust.

101 The aim of this study was to explore the changes in bacterial communities in soils colonized by four
102 biocrust types related by their succession, each representing a further step in their natural succession, and
103 different percentages of biocrust cover in the Tabernas Desert, a typically crusted location in semiarid
104 southeast Spain. The detailed objectives were to study: (i) total abundance of soil bacteria, diversity and
105 taxonomic composition at phylum and genus levels associated with biocrust types at their different
106 successional stages, and each of them associated in turn with different biocrust covers, (ii) the
107 relationships between soil bacterial taxa associated with each biocrust type and key chemical soil
108 parameters, and (iii) dominant soil bacteria taxa associated with the different biocrust types.
109

110 ## 2. MATERIALS AND METHODS

111 ### 2.1. Site description

112 The study was conducted at an experimental site (El Cautivo) located in the Tabernas Desert
113 (Almería, SE Spain), a Neogene-Quaternary depression characterized as one of the most extensive
114 badlands in Spain. This basin is located in the interior of the Betic System, delimited by the Filabres
115 Range to the north, the Alhamilla Range to the southeast, the Gador Range to the southwest and the Sierra
116 Nevada Range to the west. The altitude in the study site ranges from 240 to 385 m.a.s.l. The main
117 geological materials in the basin are Neogene marine sediments, most of which are calcaric-gypsiferous
118 mudstones and calcaric sandstones. The climate is semiarid Thermo-Mediterranean with hot dry summers
119 and mild temperatures throughout the rest of the year (the mean annual temperature is 18ºC, with an
120 absolute maximum of 45°C and absolute minimum of −5.5°C; Lázaro et al., 2004). The mean annual
121 rainfall is 235 mm, most of which falls in winter. Most rainfall events are of low magnitude (less than
122 10% over 20 mm), with occasional high-intensity events associated with thunderstorms, but low-intensity
123 rainfall events lasting several hours are frequent as well. The main types of soil at the study site are
124 Epileptic and Endoleptic Leptosols, Calcaric Regosols and Eutric Gypsisols, according to the World
125 Reference Base for Soil Resources (FAO-ISRIC-ISSS, 1998). Soil pH is basic with very high calcium

carbonate content, and the main soil texture fractions are silt followed by sand and clay (Chamizo et al., 2012). The study area landscape consists of asymmetric NW–SE valleys comprising a mosaic of zones with vascular plants, biocrusts and bare substrate. The NE-facing hillslopes often have two parts: Near the top, with gradients of nearly 30°, they are carpeted by Endoleptic Regosols (FAO–ISRIC–ISSS, 1998) or Lithic–xeric Torriorthent (Soil Survey Staff, 1999). The soils are densely covered by lichens (mostly *Squamarina lentigera* (Web.) Poelt., *Diploschistes diacapsis* (Ach.) Lumbsch, *Lepraria isidiata* (Llimona) Llimona & Crespo and *Fulgensia fulgida* (Nyl.) Szatala) and cyanobacteria. Near the bottom of the NE-facing hillslope, with gentle slope gradients, the soils are relatively thick, formed by Haplic Calcisols (FAO–ISRIC–ISSS, 1998) or Xeric Haplocalcid (Soil Survey Staff, 1999) and covered by patches of vascular plants in which annuals and perennials are very often spatially discriminated (with predominance of *Stipa capensis* Thunb., *Helianthemum almeriense* Pau, *Hammada articulate* (Moq.) O. Bolós & Vigo, *Artemisia barrelieri* Besser, and *Salsola genistoides* Poiret), while biocrusts cover interplant spaces. The SW-facing slopes are steeper (slope gradients up to 70°) with poorly developed soils, Epileptic Regosols (FAO–ISRIC– ISSS, 1998) or Lithic Torriorthent (Soil Survey Staff, 1999), and very often bare, or with very sparse individuals of very few species of perennial and annual plants (*S. genistoides* and *Moricandia foetida* Bourgeau ex Cosson) and very occasional small biocrust patches (Lázaro et al. 2000).

2.2. Experimental design

The selected study area is a representative semiarid Mediterranean ecosystem characterized by its abundant biocrust coverage of over 50% of the surface. The biocrusts selected for this study were the most common types representing ecological successional stages (Lázaro et al., 2008), from earliest to latest, as follows: (1) Incipient cyanobacterial biocrust (Incipient, IC) < (2) Mature cyanobacterial including early lichens (Cyanobacterial, MC) < (3) Biocrust dominated by the lichens *Squamarina lentigera* and *Diploschistes diacapsis* (Squamarina-Diploschistes, SD) < (4) Biocrust characterized by the *Lepraria isidiata* (Lepraria, LI) lichen. In each biocrust type, four points representing four cover levels

were selected along a gradient of biocrust cover. The four coverage levels were defined by the following cover ranges: less than 20%, 20-50%, 50-80% and over 80%. Three replicates were taken from every cover level of each biocrust, according to a factorial design totalling 4 x 4 x 3 = 48 samples. The samples were taken from the upper soil horizon to a depth of 3 cm; lichen thalli were extracted previously to keep the soil right below them. The samples were sieved to 2 mm and part of them was reserved for chemical analysis and the other part was frozen to -80ºC for biological analysis.

2.3. Chemical soil properties

Basic soil chemical property analyses were performed following standard procedures. Electrical conductivity (EC) of aqueous extract 1/5 (w/v) was measured using a digital conductivity meter (Basic 30, Crison, Carpi, Italy), and pH was determined in an aqueous solution 1/2 (w/v) in a micropH 2002 Crison pHmeter (Crison, Barcelona, Spain). Soil Organic Carbon (SOC) was measured via wet oxidation with potassium dichromate and 590-nm absorbance determined with a spectrometer (Barahona et al, 2005). Total nitrogen content (TN) was determined using a Variomax CN analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) and assimilable phosphorus (AP) was determined by the Watanabe and Olsen (1965) method.

2.4. DNA extraction and qPCR

Deoxyribonucleic acid (DNA) was extracted from 0.5 g soil aliquots using a PowerSoil® DNA Isolation kit following the manufacturer's protocol (Mo-Bio Laboratories, Carlsbad CA). DNA size and quality were verified by electrophoresis in 1% (w/v) agarose gel, and its concentration and purity were estimated spectroscopically using Nanodrop 2000. Quantitative polymerase chain reaction (qPCR) analysis was performed using iTaq™ (BioRad, Los Angeles) universal SYBR® Green supermix (5 mM dNTPs, 5 mM $MgCl_2$, 1 mM SYBR® I and 1 U/uL hot-start Taq polymerase). qPCR parameters consisted of initial denaturation at 95°C for 10 min, followed by 40 95°C cycles for 15 s and 60°C for 1 min. Triplicate PCR reactions (analytical replicates) were performed with a total 20-µL volume

containing 5 µL × iTaq™ universal SYBR® Green supermix, 2 µL of each primer (10µM) and 10 ng of template DNA. Specific primers from conserved regions of the bacterial 16S rRNA gene (Steven et al., 2014) were used for quantitative PCR analysis. Results were expressed as number of copies of bacterial rRNA gene per gram of soil.

2.5. High-throughput sequencing and bioinformatics analysis

Illumina MiSeq platform (Reagent Kit v3 -2x300 cycles) was used for pair-ended sequencing of amplicon libraries of 16S V4-V5 rRNA gene. The 16S rRNA data was processed with MOTHUR Software v.1.39.5 (Schloss et al., 2009), following the MiSeq SOP. Identification and exclusion of chimeric readings used Chimera UCHIME. Alpha-diversity was examined using operational taxonomic units (OTU) defined at 3% dissimilarity with the distance-based greedy clustering algorithm (DGC) implemented in VSEARCH. The rarefaction curves were calculated with increments of 100 sequences computed at 97% similarity. The Good's coverage index and the number of observed OTUs (Sobs) were calculated, as well as Shannon and InvSimpson diversity indices, Chao richness index and Evenness Pielou (J') index. Finally, the taxonomy was estimated using the RDP Bayesian classifier with an 80% homology level in the fixrank option, using the Silva v.132 database as a reference. The results are expressed as relative abundance of each taxon in the sample, with respect to its total number of valid sequences. Taxa with total abundance over 0.1% of the total number of sequences of all the samples, were retained for statistical analysis.

2.6. Statistical analysis

The relative abundance of bacterial genera (variables) in the soil samples was analysed to assess the significance of changes in the soil microbial composition along both gradients of biocrusts (cover and successional). Permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001) was used to check the effects of two factors, biocrust successional stage and biocrust cover, and their interaction. The first factor encompasses four biocrust types representing a gradient of successional

stages, from latest to earliest as follows: LI > SD > MC > IC. The second factor encompasses four different cover levels of each biocrust type/stage. PERMANOVA analysis uses permutation tests to find the P values, does not rely on the assumptions of traditional parametric ANOVA, and can handle experimental designs such as the one employed here (Anderson, 2001). The similarity matrix of the samples was carried out using the Bray Curtis dissimilarity index for multivariate PERMANOVAs. Two-way Univariate Permutational Analysis of Variance (PERANOVA) with Euclidean distances was also performed to check the effects of the both factors mentioned on each individual variable. PERMANOVA and PERANOVA analyses were carried out using PERMANOVA+ for the PRIMER statistical package (PRIMER-E Ltd., Plymouth Marine Laboratory, Ivybridge, UK) and R software (R Foundation for Statistical Computing, Vienna, Austria). Pairwise comparisons were also performed for chemical soil properties and diversity parameters.

A multivariate method based on Principal Component Analysis (PCA) was used to analyse contrasting microbial compositions of soils under different biocrust types representing a successional gradient and the relationships between the variables (abundance of bacterial genera) and the affinity of the soil microbial communities with each biocrust type. The PCA displayed sample scores (soils colonized by different biocrust types) in the space defined by the original variables (bacterial genus) on new axes represented by principal components that explain the most variability between the variables. PCA was conducted using Statgraphic Centurion XVII (Statpoint Technologies, Inc., Warrenton).

Box Plots were performed for the different biocrust types. The middle line in the boxes represents the median and the asterisk the mean. Boxes include 50% of the data between the first and third quartiles (interquartile range), and whiskers include values that deviate from the first and third quartile by a distance less than 1.5 times the interquartile range. Values with a deviation over 1.5 times the interquartile range are represented as circles.

To study the relationships between the abundance of each bacterial taxon and chemical soil properties, the Pearson's correlation coefficient (r) was calculated, as well as its degree of significance (p).

Box plot and Pearson's correlation analyses were done using R software (R Foundation for Statistical Computing, Vienna, Austria).

3. RESULTS

3.1. Chemical properties in soils colonized by different biocrusts

In general, desert soils colonized by biocrusts were poor in SOC, TN and AP, with a slightly alkaline pH. Biocrust types significantly influenced SOC and pH while biocrust cover significantly influenced SOC, TN and EC, but the interaction between both factors did not significantly influence the chemical soil parameters (Supplementary Table 1). Soils colonized by lichen-dominated biocrusts showed significantly higher SOC, pH and EC than in cyanobacteria-dominated biocrusts (Table 1). The increasing percentage of biocrust cover increased the SOC and TN content in LI, SD and MC. On the contrary, the soils with the lowest percentage of biocrust cover showed higher EC in LI, SD and IC (Table 1).

3.2. Bacterial abundance, alpha-diversity and richness of bacterial communities in soils colonized by different biocrust types and covers

Rarefaction curves (Supplementary Fig. 1) did not show any apparent saturation in this survey, which covered 87% to 97% of within-community (alpha) diversity (Table 2). Sequencing the 16S rRNA gene (V4-V5) amplicons with the Illumina MiSeq system resulted in a total of 2129955 sequences after eliminating those nonaligned and chimeras with an average length of 413bp. High-quality sequences from each soil sample were subsampled to 22373 sequences prior to calculating alpha-diversity parameters. Biocrust types significantly influenced all diversity indices, but biocrust cover and interaction between the two factors did not significantly influence the diversity indices (Supplementary Table 2). In general, the pattern shown by Sobs, Chao, InvSimpson, Shannon, and Evenness (J') indices were similarly significantly higher in soils colonized by LI, followed by SD, and finally MC and IC, which were not significantly different from each other (Table 2). The Good's coverage index was significantly higher in

LI than in MC and IC, but SD was not significantly different from the rest of the biocrusts types. Neither were any significant differences found between biocrust covers (Table 2). The total abundance of bacteria estimated by qPCR of the 16S rRNA gene (number of copies per gram of soil) was the highest in IC followed by MC, SD and LI (Figure 1). The total abundance of bacteria decreased progressively from the highest percentage of biocrust cover to those with the lowest biocrust cover in LI, SD and IC. Nevertheless, in the MC biocrusts, the total abundance of bacteria per gram of soil was greater at the sampling points with an intermediate cover percentage (Figure 1).

3.3. Taxonomic composition of microbial communities in soils colonized by different biocrust types and covers

Metagenome analysis revealed the same dominant phyla at different proportions in soils under different biocrust types (Fig. 2). The Bayesian classifier with SILVA reference identified 29 phyla. The most abundant phyla in LI were *Proteobacteria*, *Bacteroidetes* and *Planctomycetes* follow by *Actinobacteria* and *Acidobacteria*, whereas in SD *Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Acidobacteria* were the most abundant. The *Planctomycetes* phylum was much less abundant in SD biocrusts than in LI (Fig. 2). The abundance percentage of the *Cyanobacteria* phylum was very small in soils colonized by both lichenic biocrusts (2% in LI and 6% in SD). Nevertheless, *Cyanobacteria* was the most abundant phylum in MC follow by the *Bacteroidetes* and *Proteobacteria* phyla, whereas in IC, the dominant phyla were *Bacteroidetes*, *Proteobacteria*, *Actinobacteria* and *Cyanobacteria*, where this last phylum was much less abundant than in MC (Fig. 2).

Phylogenetic analysis showed a total of 136 taxa with relative abundance over 0.1% identified at the lowest classification level (subgroup-to-genus). PERMANOVA results showed that differences in soil bacterial communities between biocrusts types (different successional stages) were highly significant ($P < 0.001$), while differences between soil bacteria communities by biocrust cover or biocrust type/biocrust cover interaction were not significant ($p > 0.05$; Table 3). Nevertheless, univariate PERANOVA showed that both factors analyzed and their interaction, significantly influenced some soil bacteria classified at the

lowest classification level available (genus subgroup). However, the biocrust-successional stage was the most important factor with significant influence in 108 genera of soil bacteria, whereas biocrust-cover differences were only significant in 10 genera, and the interaction between both factors only in five genera of soil bacteria (Supplementary Table 3).

The PCA biplot of the two first principal components calculated from the taxa with highest significance in the univariate PERANOVA test (Supplementary Table 3) explained 57.52% of the total variability, ranging from 37.46% to 20.06% between the first and second components, respectively. Interestingly, the bacteria eigenvectors coincided with the biocrust types/successional stages (LI, SD, MC, IC), each forming one of four independent clusters in different regions of the factor space. The horizontal axis differentiates biocrust succession from the cyanobacteria, the first colonizers, followed by the better-developed biocrust stages from SD to LI. The vertical axis differentiates the first step of bare soil colonization by cyanobacteria from an incipient (IC) to a more developed colonization stage (MC) (Figure 3).

The bacterial genera (variables) with the largest positive loadings on PC1 (*Bdellovibrio, Lineage IIb genera, Ferruginibacter, Microscillaceae, Opitutus, Pirellula, Subgroup 6 unclass, Subgroupes 6 and 7 genera, Pedosphaeraceae, TRA3-20 genera, Planctomycetales, Bryobacter, Azospirillaceae unculture, Tepidisphaerales, Chitinophagaceae, Gemmata*) mostly influenced the scores of soils colonized by the lichen LI at their different percentages of biocrust cover (replicates of LI1, LI2, L3 and L4 in Fig. 3). On the contrary, the variables with negative loadings *(Blastocatella, Coleofasciculacea, Nostocales uncultured, Chroococcidiopsis, Rhodocytophaga)* influenced mainly the scores of soils dominated by cyanobacteria (MC1, MC2, MC3, MC4 and some replicates of IC1). The progressive increase in the genera represented on the right led to a decrease in those on the left and vice versa (Supplementary Table 4). The cluster in an intermediate position between the MC and LI clusters, grouped mainly soil samples colonized by lichens SD (samples SD1, SD2, SD3 and SD4 and some replicates of LI4). The position of the SD samples, near the origin of ordinates, although expanding slightly to the right on PC1, indicates that they are mainly influenced by the bacterial genera in the LI and MC clusters. Likewise, the genera

with loadings with opposite signs (inverse correlation) in PC2 (Fig. 3) showed differences between soils with an incipient colonization of cyanobacteria (mainly replicates IC1 to IC4) and soils colonized by mature cyanobacteria (MC), also indicating changes in the content of soil microbial communities in those soils. *Nocardioides, Beijerinckiaceae, Geodermatophilaceae, Geodermatophilus, Rubellimicrobium, Rubrobacter, Sphingomonas* and *Blastococcus* were especially abundant in IC1, IC2, IC3, IC4 (Fig. 3).

In general, the box plot analysis corroborated that the soil bacterial taxa associated with each biocrust type in PCA also showed higher relative abundance or were almost exclusive in the soils colonized by those same biocrust types (Fig. 4). Thus, some bacteria were more abundant in LI or gradually increased from the early successional (IC) to late successional stages of biocrusts (LI) (Fig. 4a). Other soil bacteria were more abundant in SD (Fig. 4b) or in soils colonized by both lichen types (LI and SD; Fig. 4c), and on the contrary, other bacteria were more abundant or almost exclusive in MC (Fig. 4d) or IC (Fig. 4f), or decreased from the latest successional to the earliest successional development stages (Fig. 4f).

3.4. Relationships between bacterial communities and edaphic properties in soils colonized by different biocrust types.

The soil parameters with the largest number of significant correlations ($p > 0.05$) with soil bacterial abundance at their lowest taxonomic level (subgroup-to-genus) were pH, SOC, TN and EC, although these correlations were not very high (r ranged from 0.30 to 0.55) (Supplementary Table 5). Soil bacteria positively correlated with pH were more abundant in LI and SD, whereas the bacteria communities correlated negatively with soil pH were more abundant in MC and IC. SOC and TN were positively correlated with the abundance of bacteria per gram of soil, and in general, the soil bacteria positively correlated with SOC and TN content were more abundant in LI, SD and MC biocrusts. On the contrary, the soil bacteria communities correlated positively and negatively with EC, as well as those soil bacteria communities correlated negatively with SOC and TN content, were ubiquitous in soils colonized by all biocrust types. EC correlated negatively with the abundance of bacteria per gram of soil (Supplementary Table 5).

4. DISCUSSION

4.1. Influence of the biocrust-cover and their successional stage in soil parameters, total abundance of soil bacteria, diversity and soil microbial composition.

The results show that the diversity of soils colonized by biocrusts was parallel to the biocrust successional stage. The Sobs, Chao, Shannon, InvSimpson and Evenness indices gradually increased from soils colonized by cyanobacteria up to soils colonized by later successional biocrusts dominated by the lichen *L. isidiata* (Table 2). Moreover, in general, the abundance of bacteria per gram of soil increased in soils with more biocrust cover in all biocrust types (Fig. 1). The increase in diversity and richness in soils colonized by the highest lichen covers (LI and SD) could be due, on the one hand, to higher TN, AP and SOC contents in the soils colonized by these biocrust types than soils colonized by cyanobacteria (MC and IC) (Table 1). The results show that SOC and TN correlated significantly positively ($p>0.05$) with bacterial abundance (Supplementary Table 5). Moreover, the soils below *L. isidiata*, *D. diacapsis* and *S. lentigera* lichens were also characterized by their higher biomass-C, carbohydrate and polyphenol contents and enzymatic activity of microbial communities (Miralles et al., 2012a, 2012c, 2013). Moreover, biocrusts colonized by lichens in the Tabernas Desert have higher soil moisture than soils colonized by cyanobacteria (Chamizo et al., 2012). Therefore, that increase in nutrients and soil moisture in soils colonized by lichens, especially in those where the biocrust cover is greater, could provide ideal conditions for establishing greater richness and diversity of soil bacteria communities.

The majority phyla in soils colonized by all biocrust types were Proteobacteria, Bacteroidetes, Actinobacteria and Acidobacteria, including the Planctomycetes phylum in LI and the Cyanobacteria phylum in MC and IC, although in the Tabernas Desert, they showed dissimilar proportions at the different successional stages of the biocrusts. The different proportions of dominant phyla could alter microbial community functions in the biocrust succession process, which in turn could promote their development (Liu et al., 2017). In general, these phyla have also been found to be predominant in

358 biocrusts and their underlying soils in other arid and semi-arid ecosystems in Nyngan, New South Wales,
359 Australia and the Colorado Plateau (Steven et al., 2013; Liu et al., 2017). The most abundant phylum in
360 MC biocrusts was Cyanobacteria, while this phylum was much less abundant in soils just below lichens
361 (Fig. 2). This low percentage of Cyanobacteria in soils colonized by lichens (6% and 2% in SD and LI
362 respectively) could be due to the changes in physical, chemical and biochemical soil properties during
363 succession, making new biocrusts more competitive by receiving more positive feedback from the new
364 soil properties, and eventually replacing the previous one (Lázaro et al., 2008). Consequently, the
365 percentage of cyanobacteria, which are primo-colonizers, could be progressively decreasing as the
366 successional state increases in the biocrust characterized by lichen *L. isidiata*. The Cyanobacteria phylum
367 was much more abundant in MC than in IC biocrusts, whereas, other bacterial groups belonging to the
368 Bacteroidetes, Proteobacteria and Actinobacteria phyla were the most abundant in the IC (Fig. 2).
369 In the Tabernas Desert, the successional biocrust stage exerted a stronger influence on more bacterial
370 genera than the biocrust-cover or the interaction between them (Table 2), although biocrust cover also
371 exerted a positive effect on the abundance of bacteria per gram of soil in all biocrusts types (Fig. 1). Our
372 results suggest that the effect of the biocrust type could influence the existence of a contrasting microbial
373 composition across a successional gradient from early successional stages of biocrusts colonized by
374 cyanobacteria (MC and IC; richer in the bacteria *Blastocatella, Nostocales-unclassified, Chroococcidiopsaceae*, etc., Fig. 3) to soils colonized by lichens *D. diacapsis* and *S. lentigera* (SD) and
375
376 late-successional biocrusts characterized by *L. isidiata* (LI; richer in the bacteria *Subgroupes 6 genera*
377 *and unclassified, Pedosphaeraceae, TRA3-20 genera*, etc., Fig. 3). The SD sample cluster in the middle
378 of PC1 and close to the ordinate axis in the biplot (Fig. 3), suggests that these soils were sharing bacteria
379 with MC and LI biocrust types. Likewise, the axis aligned along PC2 in Fig. 3 also showed changes in the
380 soil microbial communities across a successional gradient from incipient colonization of cyanobacteria
381 (IC; richer in the *Nocardioides, Geodermatophilus, Rubrobacter* … genera) to developed cyanobacteria
382 (MC; richer in *Blastocatella, Coleofasciculaceae, Chroococcidiopsaceae*, etc.). These differences in the
383 microbial community composition in soils colonized by biocrusts at different successional stages could be

384 explained by several interacting factors related to changes in soil properties due to the presence of
385 biocrusts and the ecophysiology of each biocrust type. A first explanation could be that the biocrusts can
386 substantially modify the local soil environment, affecting soil stability, nutrient content, soil texture and
387 pH (Belnap and Gardner, 1993; Belnap et al., 2003). Aggregation simultaneous with the accumulation of
388 carbon and nitrogen, begins with the higher soil moisture below the biocrusts (Chamizo et al., 2012).
389 Atmospheric nitrogen-fixing organisms increase the total nitrogen content in biocrusts, whereas the
390 excretion of organic compounds by the colonizing organisms contributes to accumulation of SOC (Mager
391 and Thomas, 2011). Soil parameters, such as pH and nutrient availability are considered the most
392 important factors driving the structure of soil bacteria communities (Lauber et al., 2009; Goldfarb et al.,
393 2011; Griffiths et al., 2011; Kuramae et al., 2012; Sánchez-Marañón et al., 2017). Our results also showed
394 significant correlations between several soil bacteria taxa and chemical soil parameters (Table 4). In our
395 study area, the soil bacteria which in general presented positive significant correlations ($p < 0.05$) with
396 SOC, TN and pH (Table 4) were also those with larger abundance of the late-successional biocrust types
397 (Fig. 4).
398 The role of late successional biocrusts dominated by lichens producing higher increases in SOC, TN,
399 aggregate stability and water retention content than the cyanobacteria-dominated biocrusts, has previously
400 been reported (Housman et al., 2006; Chamizo et al., 2012). In the Tabernas Desert, the enzymatic
401 activities involved in C, N and P cycles progressively increased from biocrusts colonized by
402 cyanobacteria to biocrusts dominated by the lichen *D. diacapsis* up to the latest successional stages in
403 biocrusts-dominated by *L. isidiata*. The hydrolytic action of these enzymes could guarantee the presence
404 of readily metabolized low-molecular-weight substrates, which could be used by heterotrophic microbial
405 communities for nutrition and protection against desiccation (Miralles et al., 2012c). Moreover, it has
406 been also shown that in the Tabernas desert, labile C (estimated from the sum of unmineralized C and
407 CO_2-C emitted), carbohydrate-C and polyphenol-C contents were significantly higher in the biocrusts-
408 dominated by *D. diacapsis*, followed by the lichen *L. isidiata* than in biocrusts dominated by
409 cyanobacteria (Miralles et al., 2013). These authors reported other differences in the type of extractable

carbohydrates among those biocrust types, for example, mannitol was present in lichen biocrusts dominated by *D. diacapsis* and *L. isidiata* whereas its presence in cyanobacteria was minimal. These differences in the organic carbon pools could favor the proliferation of different soil bacteria specialized in degrading specific organic compounds (Goldfarb et al., 2011). On the other hand, the effect of the biocrusts on surface wetness and its influence on selecting soil microbial communities should not be ignored either. Soil moisture has a strong effect on microbial communities (Moyano et al., 2013), because microorganisms are dependent on water, and depending on the species, cannot sustain their normal cell activity below a certain water potential (Roe and Conrad, 2013). Some authors (Kidron et al., 2009; 2014) have found high correlations between daylight surface wetness duration and the available water content in the upper centimeter of soil and biocrust chlorophyll content. Lazaro et al. (2008) found that in the Tabernas desert, photosynthetically active radiation and surface temperature were considerably lower in all communities dominated by lichens than in communities dominated by cyanobacteria, while the soil moisture and the duration of dew deposition were higher in biocrusts colonized by lichens. Thus, the combined effects of this set of changes in the physico-chemical and biochemical soil properties by biocrusts, along with the differences in microclimate and soil moisture among the habitats corresponding to the different successional stages, may drive the differentiation of soil microbial communities. Our results showed that SOC, TN and AP content were significatively influenced by the biocrust cover (Supplementary Table 1), increasing in soils colonized by the biocrusts as their percentage of cover increased (Table 1). Therefore, as the biocrust cover increases, it could increase their effect in underlying soil chemical properties, contributing to the selection of bacteria in their underlying layers through of the indirect effect of biocrusts on the soil properties.

Nevertheless, it was also striking that the correlations between the bacterial genera and chemical soil properties were not very high (Table 1), suggesting that in the Tabernas Desert, other factors could also be influencing the structure of microbial communities in soils colonized by biocrusts. Different lichens and cyanobacteria colonies synthetize biochemical metabolites, such as mono and dihydrate calcium oxalates (whewellite and weddellite, respectively), carotenoids, chlorophyll, parietin, emodin, atranorin,

gyrophoric acid, lecanoric acid, fumarprotocetraric acid, rhizocarpic acid, calycin, usnic acid, and so on, with diverse ecophysiological functions (Cockell and Knowland, 1999; Holder et al., 2000, Wynn-Williams and Edwards, 2000; Dickensheets et al., 2000; Edwards, 2007; Miralles et al., 2012b). The synthesis of these biomolecules is species-specific, and *L. isidiata, D. diacapsis, S. lentigera* and cyanobacteria biocrust types are the most important in determining the nature and proportion of those biomolecules in the Tabernas Desert (Miralles et al., 2017). Other authors have shown that some of the secondary metabolites secreted by lichens have antibacterial or antifungal activities (Francolini et al., 2004; Boustie and Grube, 2005; Hodkinson et al., 2012; Kosani´c and Rankovi´c, 2015). The differences in metabolites by biocrust type could contribute to developing selective ecological niches in which some bacterial communities could develop better than others.

4.2. Dominant soil bacterial taxa associated with different biocrust types.

Biocrust type could be a strong predictor of soil bacteria community composition in the Tabernas Desert, as different bacterial taxa could be associated with soils colonized by each biocrust type (Fig. 4). Thus, our results show that the soil bacterial groups, such as *Pirellula, Gemmata, Gemmataceae uncultured, Planctomycetales uncultured ge, Pir4 lineage, Tepidisphaerales unclassified, Pedosphaeraceae ge, Microscillaceae unclassified, TRA3.20 ge,* etc., were much more abundant in or almost exclusive to LI biocrusts (Fig. 4a). Some soil bacteria genera, such as *WD2101 ge, Pirellulaceae uncultured, Pirellulaceae unclassified, 0319.6G20 ge, and Microscillaceae* also showed a clear pattern by successional state of the four biocrust types analyzed, their relative abundance increasing proportionally from the earliest successional stages (IC) to the latest (LI) (Fig. 4a). These results suggest that *L. isidiata* lichen could exert a positive influence on those groups of soil bacteria in the Tabernas Desert.

The soils under the other biocrust types also showed other much more abundant or almost exclusive bacterial groups, suggesting that they could also be favored by the overlying biocrusts. Thus, the SD biocrusts seemed to exert a positive influence on the genera *Truepera, Sphingobacteriaceae unclassified, Devosia, Actinophytocola, and Kribella* (Fig. 4b). The soils colonized by this biocrust shared other groups

of bacteria with relatively similar abundance in soils colonized by *L. isidiata* (as was the case of *Chthoniobacter, Acidobacteria Subgroup 10, Bryobacter, Ohtaekwangia, Opitutus, Burkholderiaceae unclassified, Steroidobacter*, etc. Fig. 4c) or in soils colonized by MC (e.g., *Flavitalea, Rhodocytophaga*). As mentioned above, SD biocrusts occupy an intermediate successional position between *L. isidiata* (LI) and cyanobacteria (MC) (Lázaro et al., 2008). Some authors have found that some lichen-associated bacterial communities are not extensions of those found in surrounding soils, as different lichen species in close spatial proximity harbor dissimilar bacterial communities, suggesting that some bacterial taxa are widespread in different lichen species, probably reflecting their functional role in the lichen symbiosis (Bates et al., 2011).

In MC the most favored genera were *Nostocales uncultured ge, Chroococcidiopsaceae uncultured* and *Coleofasciculaceae unclassified* and also some bacteria, such as *Blastocatella* (Fig. 4d). However, IC exerted a positive influence on the cyanobacterial genus *Leptolyngbya VRUC 135* (Fig. 4e). Other authors also found that filamentous cyanobacteria *Leptolyngbya sp.* are abundant in poorly developed soil crusts and decrease in well-developed lichenized biocrust (Pushkareva et al., 2015). The *Rubrobacter, Solirubrobacter, Geodermatophilus, Longimicrobium, Geodermatophilaceae unclassified,* and *Nitrospira* bacterial groups also showed a clear pattern by successional stage of the four biocrust types (Fig. 4f). Those bacteria decreased in relative abundance from IC to LI, suggesting they are primo-colonizing taxa which are outcompeted when improved soil conditions allow the development of other bacterial groups. In the Tabernas Desert, biocrusts dominated by cyanobacteria are spatially distributed in the areas most exposed to the sun and receiving the highest insolation (Miralles et al., 2012b). Therefore, the stress caused by the higher temperatures, incident solar radiation, and long drought periods seems to be an important factor explaining the occurrence of bacteria resistant to stress, such as the cyanobacteria *Leptolyngbya VRUC 135* and *Rubrobacter* and *Geodermatophilus* bacteria in the incipient colonization of cyanobacteria. These species have been described as thermophilic, being highly resistant to solar radiation (Nakamori et al., 2014; Ferreira et al., 1999; Montero-Calasanz et al., 2014). Their resistance to stress could offer them an advantage, because it would allow them to occupy the most unfavorable places,

where they have little competition with other bacteria that require more humid shady slopes for their development.

Our results have shown that some soil bacteria could be favored by different biocrust types (at their different successional stages), and similarly, the abundance of soil bacteria could be favored by biocrust cover, possibly due to changes in the chemical soil properties and local environment fostered in large part by the biocrust itself and its micro-climatic conditions, such as exposure to solar radiation, in which that biocrust is successful. An additional explanation for the association between bacterial communities and biocrust types could be that bacteria exert a functional role in symbiosis with biocrust organisms. However, the functional roles of bacteria associated with biocrusts remain largely undetermined, so a further study of the genes involved in the metabolism of biogeochemical cycles and their possible connection with biocrusts would be needed.

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Figure captions:

-Figure 1. Total abundance (bacteria g^{-1} soil) of bacteria estimated by qPCR of 16S rRNA in different biocrust types and biocrust covers from 1, representing the maximum percentage of biocrust cover, to 4, the lowest.

-Figure 2. Phylogenetic community composition. Relative abundance of dominant bacteria (>0.1% of total reading) at the phylum level in soils colonized by different biocrust types.

-Figure 3. Principal component analysis. Biplots for the relative abundance of bacterial communities at the lowest classification level (subgroup-to-genus) in soils colonized by four different biocrust types representing a successional gradient (LI- lichen *L. isidiata*; SD- lichens *D. diacapsis* and *S. lentigera*; MC-mature cyanobacteria; IC- incipient colonization of cyanobacteria), and each of them in turn associated with different degrees of biocrust cover (from 1, representing the maximum percentage of biocrust cover, to 4, the lowest; i.e.: LI1 indicates the soil sample colonized by the *L. isidiata* lichen with the maximum percentage of biocrust cover).

-Figure 4. Bacterial taxonomy at the lowest classification level (subgroup-to-genus) associated with soils colonized by four different biocrust types representing a successional gradient (n=12). Fig. 4a: soil bacteria more abundant in soil colonized by *L. issidiata* lichen dominating biocrusts (LI); fig. 4b: soil bacteria more abundant in soil colonized by *D. diacapsis* and *S. lentigera* lichens dominating biocrusts (SD); fig. 4c: soil bacteria more abundant in soil colonized by both LI and SD; fig. 4d: soil bacteria more abundant in soil colonized by mature cyanobacteria (MC); fig. 4f: soil bacteria more abundant in soil colonized by incipient cyanobacteria (IC).

-Supplementary Fig. 1. Rarefaction curves representing the numbers of operational taxonomic units (OTUs) versus the number of valid readings in soils colonized by four different biocrust types representing a successional gradient (LI- lichen *L. isidiata*; SD- lichens *D. diacapsis* and *S. lentigera*; MC-mature cyanobacteria; IC- incipient colonization of cyanobacteria), and each of them associated in turn with different degrees of biocrust cover (from 1, representing the maximum percentage of biocrust cover, to 4, representing the lowest; e.g., LI1 indicates the soil sample colonized by the *L. isidiata* lichen with the maximum percentage of biocrust cover).

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Table 1. Chemical properties of soil samples colonized by different biocrust types and covers (average ± standard derivation).

Biocrust type (A)	Biocrusts covers (a)	SOC (g kg^{-1})	NT (g kg^{-1})	AP (ppm)	EC (mS/cm)	pH
LI	1	20.50 ± 15.27Aa	1.23 ± 0.23Aa	1.78 ± 0.18Aa	484 ± 324Aa	8.02 ± 0.47Aa
LI	2	17.73 ± 12.20Aabc	0.94 ± 0.08Aa	1.58 ± 0.36Aa	502 ± 224Aab	8.21 ± 0.38Aa
LI	3	7.87 ± 0.40Abcd	0.96 ± 0.04Ab	1.49 ± 0.02Aa	579 ± 505Aa	8.23 ± 0.29Aa
LI	4	10.13 ± 4.96Abc	0.89 ± 0.13Ab	1.83 ± 0.11Aa	967 ± 875Aac	8.34 ± 0.34Aa
SD	1	19.47 ± 1.99Aa	1.15 ± 0.15Aa	3.21 ± 0.96Aa	460 ± 166Aa	8.61 ± 0.36Aa
SD	2	12.42 ± 4.13Aabc	1.13 ± 0.13Aa	1.94 ± 0.42Aa	307 ± 18Aab	8.15 ± 0.47Aa
SD	3	6.84 ± 2.11Abcd	0.93 ± 0.03Ab	1.58 ± 0.03Aa	365 ± 59Aa	8.24 ± 0.10Aa
SD	4	8.08 ± 2.55Abc	0.83 ± 0.07Ab	1.25 ± 0.30Aa	880 ± 678Aac	8.13 ± 0.30Aa
MC	1	14.05 ± 3.89ABa	1.13 ± 0.02Aa	1.79 ± 0.14Aa	378 ± 86Aa	8.02 ± 0.02Ba
MC	2	8.03 ± 0.93ABabc	1.04 ± 0.02Aa	1.52 ± 0.14Aa	316 ± 30Aab	7.89 ± 0.08Ba
MC	3	7.07 ± 2.40ABbcd	0.96 ± 0.06Ab	4.03 ± 4.35Aa	350 ± 22Aa	7.94 ± 0.38Ba

MC	4	5.34 ± 1.55ABbc	0.81 ± 0.19Ab	1.47 ± 0.23Aa	352 ± 106Aac	7.84 ± 0.10Ba
IC	1	8.64 ± 2.69Ba	0.95 ± 0.16Aa	1.45 ± 0.27Aa	254 ± 80Aa	8.05 ± 0.08ABa
IC	2	9.12 ± 0.87Babc	1.04 ± 0.10Aa	1.64 ± 0.21Aa	281 ± 38Aab	8.12 ± 0.42ABa
IC	3	6.10 ± 0.51Bbcd	0.81 ± 0.11Ab	1.33 ± 0.10Aa	324 ± 214Aa	8.01 ± 0.53ABa
IC	4	8.66 ± 4.32Bbc	0.80 ± 0.16Ab	2.12 ± 0.29Aa	633 ± 475Aac	8.00 ± 0.16ABa

LI: lichen *Lepraria isidiata*; SD: Lichens *Squamarina lentigera* and *Diploschistes diacapsis;* MC: Mature Cyanobacteria; IC: Incipient Cyanobacteria; SOC: Soil Organic Carbon; NT: Total Nitrogen; AP: Available Phosphorous; EC: Electrical Conductivity. Biocrusts covers are ranged from 1, representing the maximum percentage of biocrust cover, to 4, the lowest. Different capital letters denoting significant differences between biocrust types. Different lowercase letters denoting significant differences between biocrust covers.

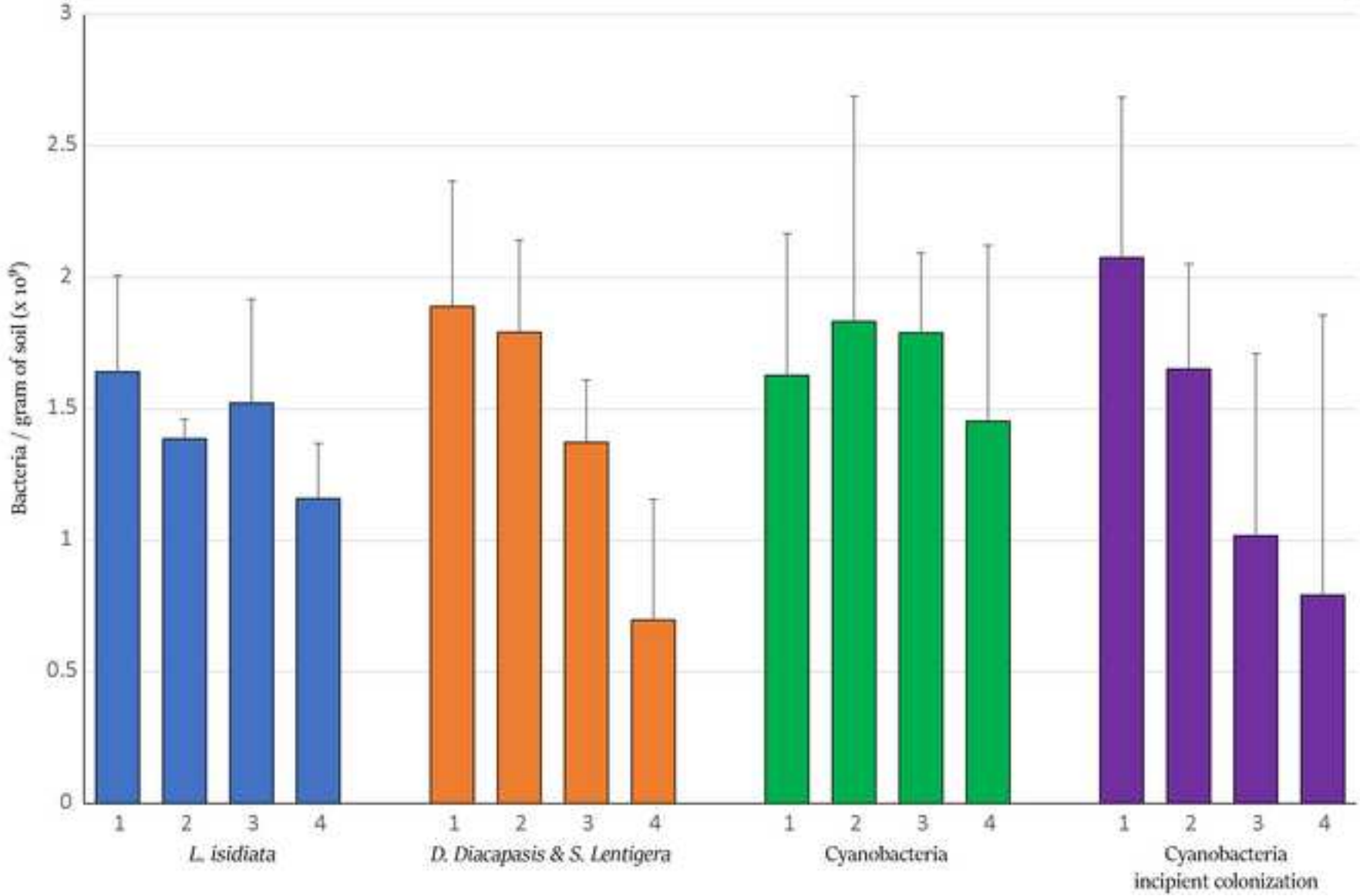
Table 2. Diversity indices for 16S rDNA sequences in soil samples colonized by different biocrust types and covers (average ± standard derivation).

Biocrust types	Biocrusts covers	Coverage	Sobs	Chao	InvSimpson	Shannon	Evenness Pielou (J')
LI	1	0.90 ± 0.02a	3876 ± 534a	7862 ± 1369a	201 ± 30.2a	6.60 ± 0.22a	0.80 ± 0.01a
LI	2	0.89 ± 0.00a	3972 ± 79a	8534 ± 502a	221 ± 24.6a	6.68 ± 0.03a	0.81 ± 0.00a
LI	3	0.89 ± 0.01a	4069 ± 372a	8928 ± 792a	239 ± 30.0a	6.73 ± 0.16a	0.81 ± 0.01a
LI	4	0.91 ± 0.01a	3725 ± 244a	7264 ± 1027a	188 ± 26.3a	6.55 ± 0.07a	0.80 ± 0.01a
SD	1	0.90 ± 0.01abc	3716 ± 220b	8395 ± 968ab	202 ± 37.9b	6.58 ± 0.03b	0.80 ± 0.00b
SD	2	0.91 ± 0.00abc	3473 ± 99b	7040 ± 326ab	152 ± 47.7b	6.41 ± 0.14b	0.79 ± 0.02b
SD	3	0.90 ± 0.00abc	3733 ± 24b	8140 ± 572ab	172 ± 26.0b	6.54 ± 0.07b	0.79 ± 0.01b
SD	4	0.91 ± 0.02abc	3379 ± 468b	7540 ± 1456ab	134 ± 98.8b	6.14 ± 0.42b	0.76 ± 0.04b
MC	1	0.92 ± 0.01bcd	3079 ± 469c	7257 ± 1171b	109 ± 61.4c	5.94 ± 0.47c	0.74 ± 0.05c
MC	2	0.91 ± 0.01bcd	3193 ± 201c	7447 ± 1174b	81 ± 39.7c	5.92 ± 0.28c	0.73 ± 0.03c
MC	3	0.92 ± 0.01bcd	3142 ± 213c	6852 ± 478b	81 ± 27.9c	5.97 ± 0.16c	0.74 ± 0.02c
MC	4	0.92 ± 0.01bcd	2907 ± 164c	6623 ± 597b	50 ± 7.1c	5.74 ± 0.03c	0.72 ± 0.00c
IC	1	0.92 ± 0.00bc	2894 ± 133c	6513 ± 473b	73 ± 30.4c	5.81 ± 0.23c	0.73 ± 0.02c
IC	2	0.91 ± 0.01bc	3304 ± 403c	7302 ± 1014b	104 ± 78.2c	6.05 ± 0.47c	0.75 ± 0.05c
IC	3	0.91 ± 0.02bc	3318 ± 430c	7626 ± 1938b	130 ± 39.2c	6.18 ± 0.23c	0.76 ± 0.02c
IC	4	0.92 ± 0.02bc	2900 ± 657c	6175 ± 1421b	131 ± 47.0c	6.06 ± 0.26c	0.76 ± 0.02c

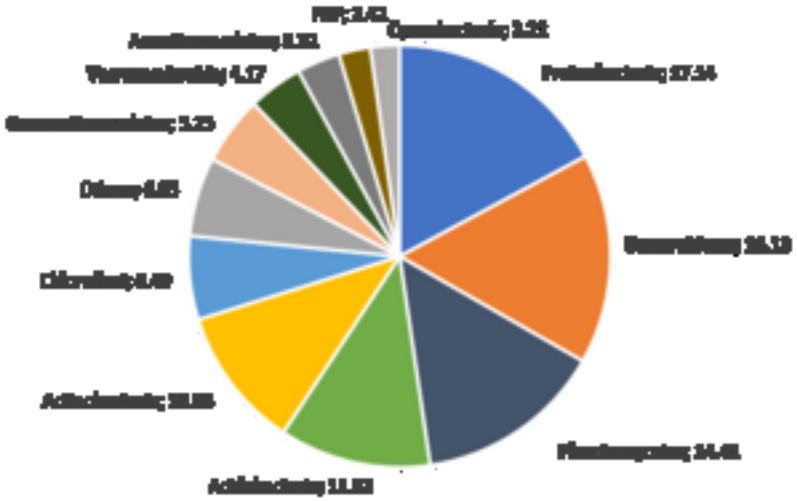
LI: lichen *Lepraria isidiata*; SD: Lichens *Squamarina lentigera* and *Diploschistes diacapsis;* MC: Madure Cyanobacteria; IC: Incipient Cyanobacteria. Biocrusts covers are ranked from 1, representing the maximum percentage of biocrust cover, to 4, the lowest. Lower letters denote significant differences among soils colonized by different biocrust types. No significant differences were found among biocrusts covers.

Table 3. Significant differences in soil bacterial communities found by PERMANOVA analysis, by biocrust type, biocrust cover and interaction of both.

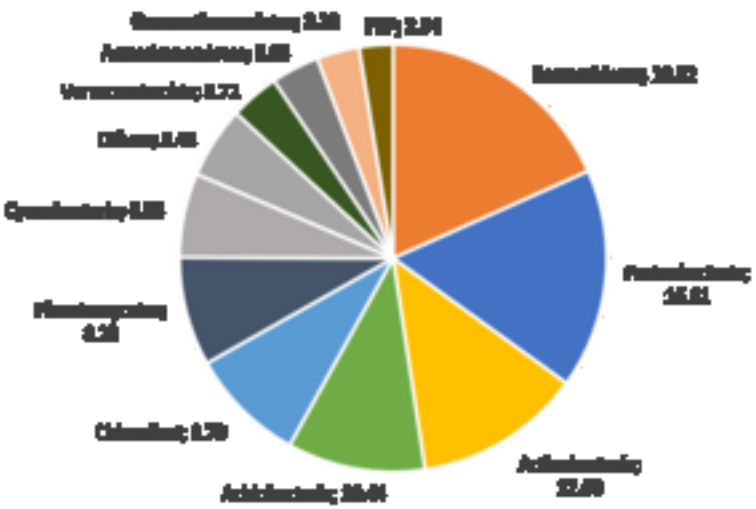
	df	SS	MS	Pseudo-F	P(perm)	perms
Biocrusts types	3	13501	4500.2	8.645	0.0001	9901
Biocrusts covers	3	1606	535.3	1.028	0.4127	9873
Biocrusts types x Biocrusts covers	9	3778	419.7	0.806	0.9036	9826
Res	32	16658				
Total	47	35542				


Bacteria / gram of soil (x 10⁹)
3
2.5
2
1.5
1
0.5
0
1 2 3 4
L. isidiata
1 2 3 4
D. Diacapasis & S. Lentigera
1 2 3 4
Cyanobacteria
1 2 3 4
Cyanobacteria
incipient colonization

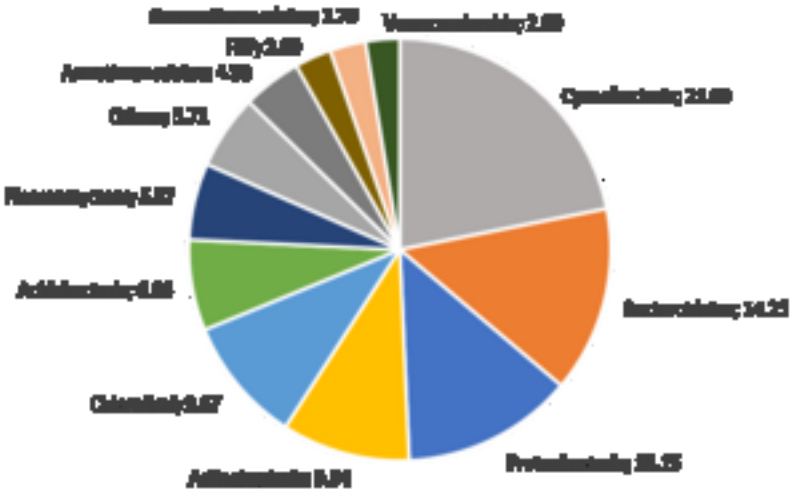
Phyla in biocrusts dominated by *Lepraria* lobificans lichen (%)



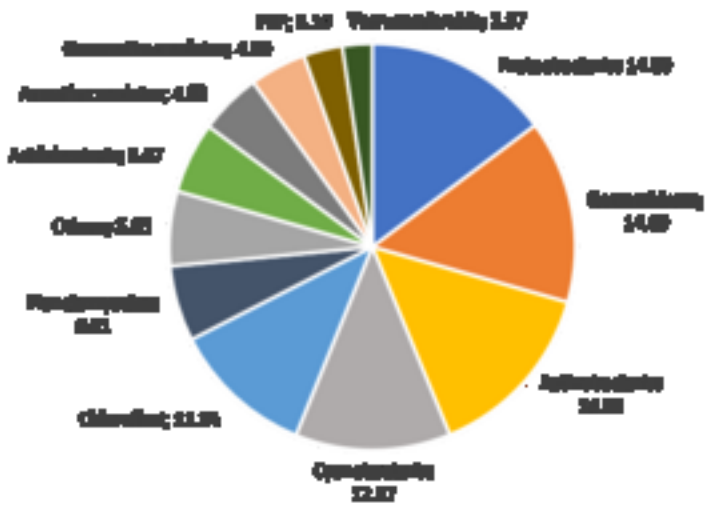
Phyla in biocrusts dominated by D. diacapsis and S. fenligera lichens (%)

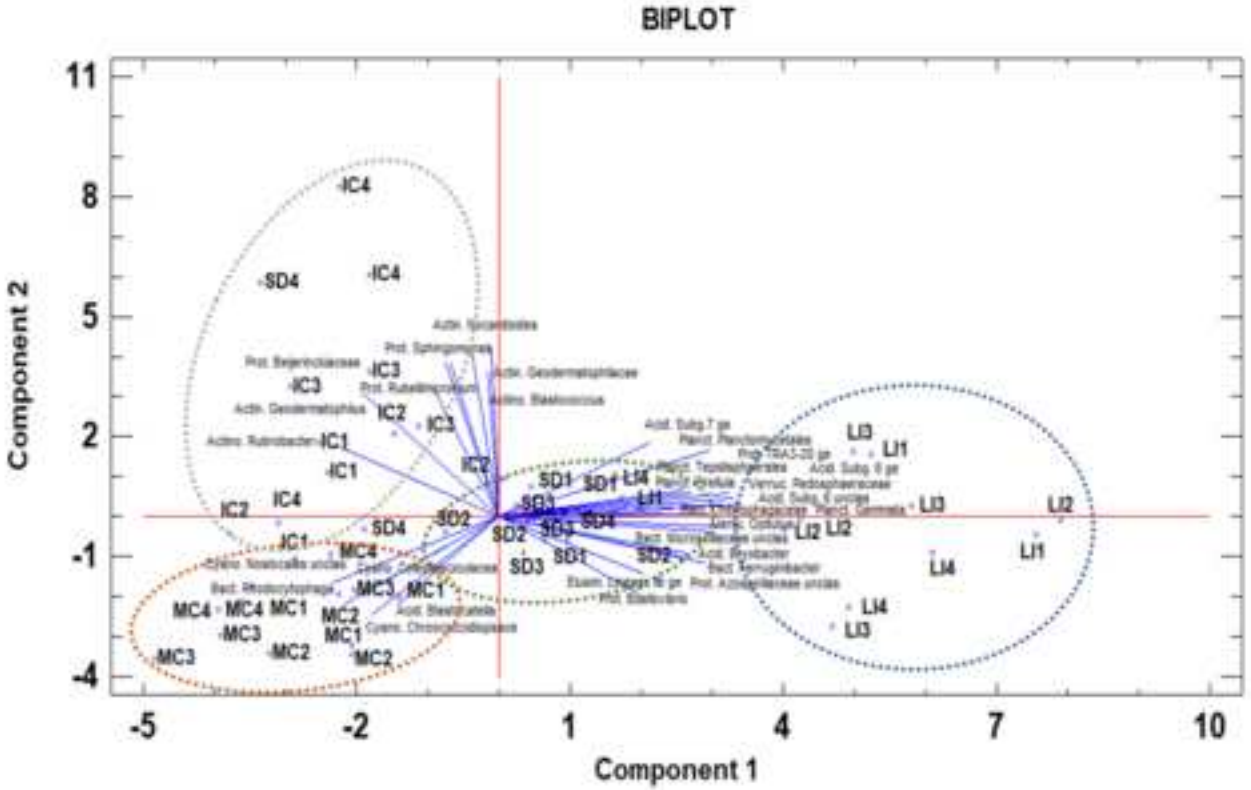


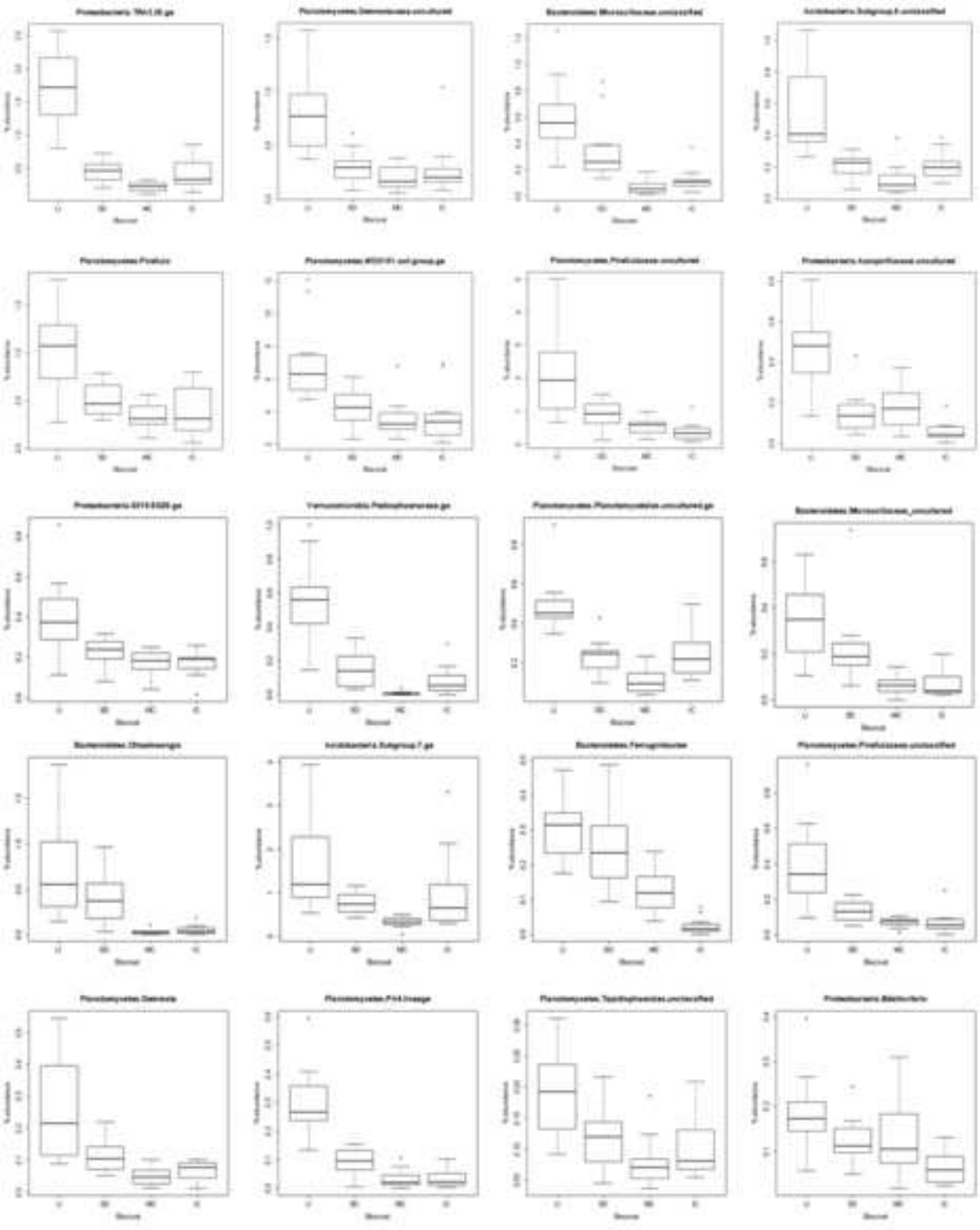
Phyla in biocrust dominated by Cyanobacteria (%)

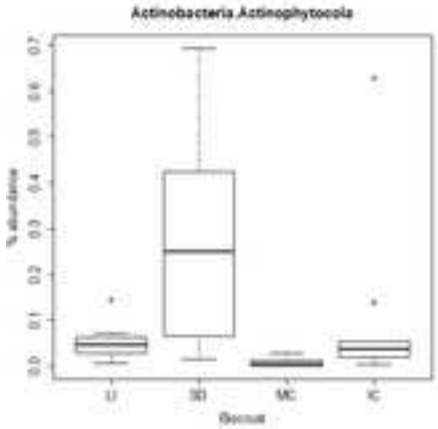


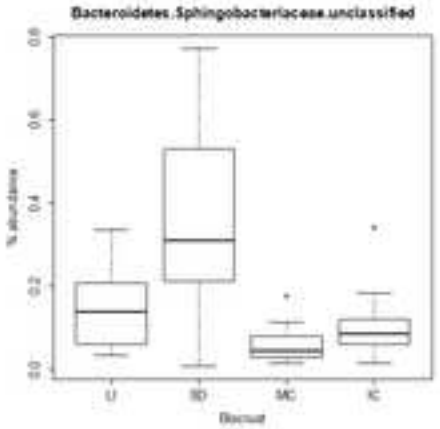
Phyla in biocrusts dominated by incipient colonization of Cyanobacteria (%)

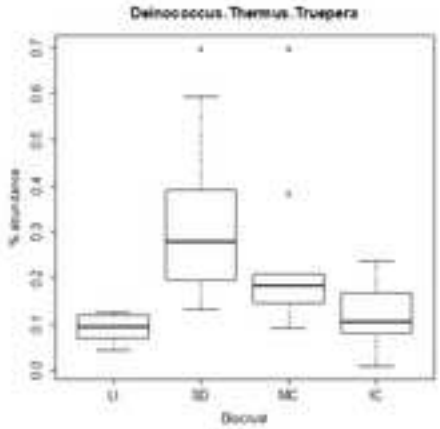


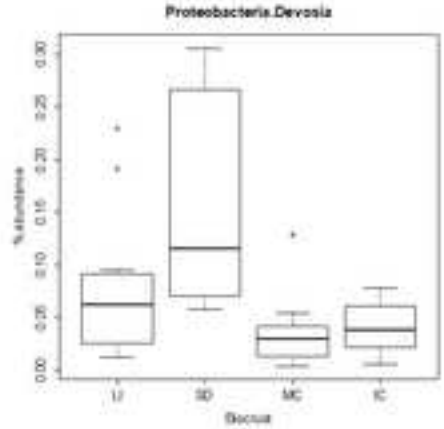

BIPLOT
Component 2
Component 1
11
8
5
2
-1
-4
-5
-2
1
4
7
10
IC4
IC4
SD4
IC3
IC3
IC2
IC3
IC1
IC1
IC2
IC4
IC2
SD4
IC1
MC4
MC4
MC4
MC1
MC3
MC2
MC3
MC1
MC3
MC2
MC2
SD2
SD2
SD3
SD1
SD1
SD4
SD2
SD3
LI4
LI1
LI3
LI1
LI2
LI2
LI3
LI2
LI4
LI1
LI4
LI3

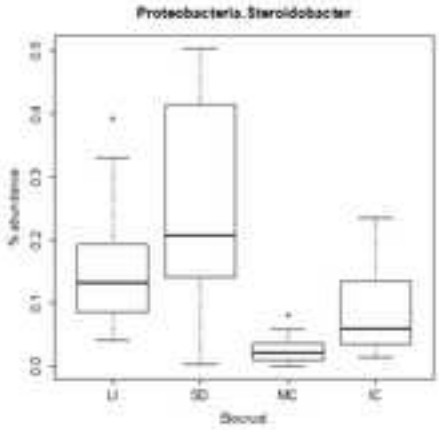


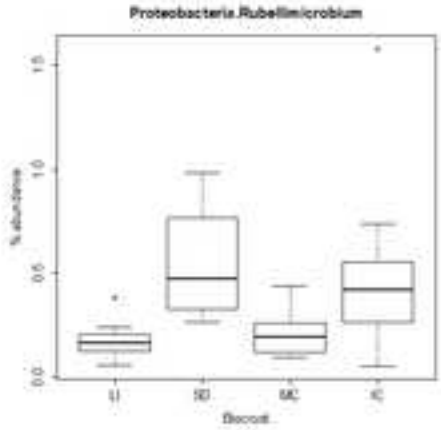

Actinobacteria.Actinophytocola
% abundance
U
SD
MC
IC
Biocrust

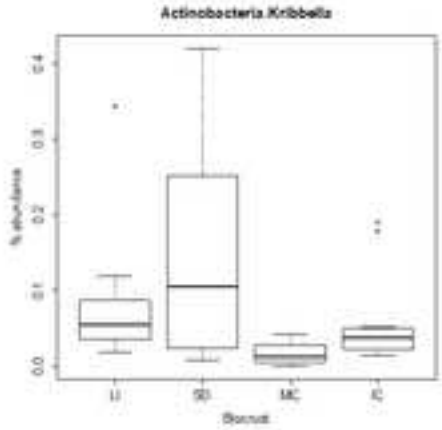

Bacteroidetes.Sphingobacteriaceae.unclassified
% abundance
U
SD
MC
IC
Biocrust

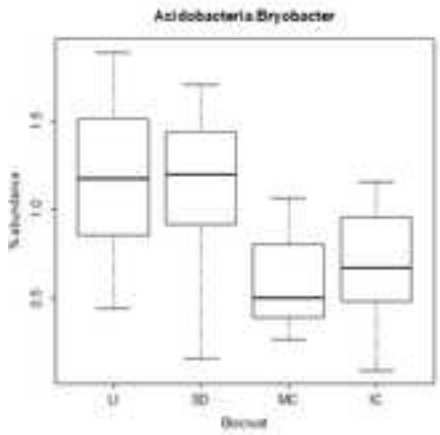

Deinococcus.Thermus.Truepera
% abundance
U
SD
MC
IC
Biocrust

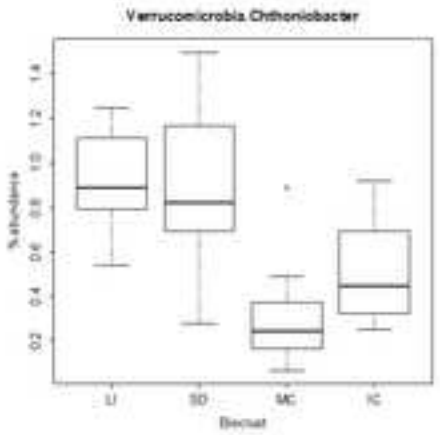

Proteobacteria.Devosia
% abundance
U
SD
MC
IC
Biocrust

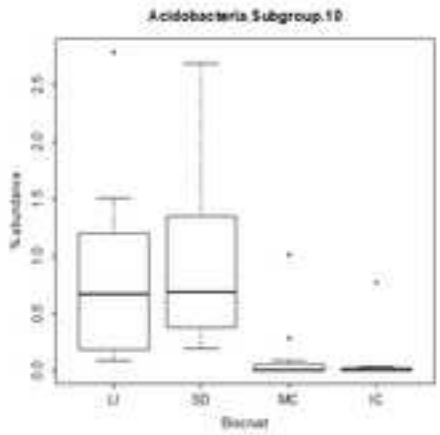

Proteobacteria.Steroidobacter
% abundance
U
SD
MC
IC
Biocrust

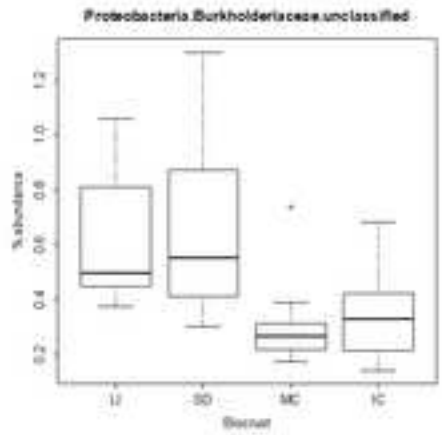

Proteobacteria.Rubellimicrobium
% abundance
U
SD
MC
IC
Biocrust

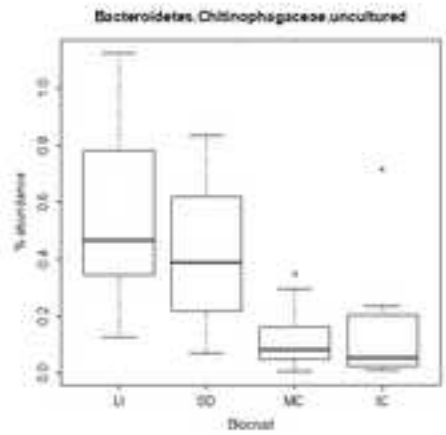

Actinobacteria.Kribbella
% abundance
U
SD
MC
IC
Biocrust

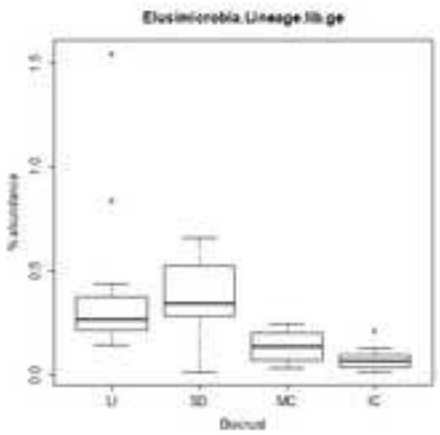

Acidobacteria.Bryobacter
% abundance
LI
SD
MC
IC
Biocrust

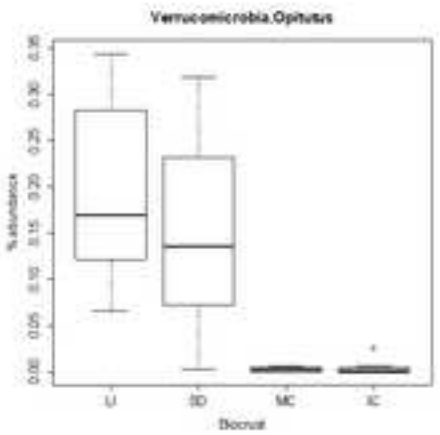

Verrucomicrobia.Chthoniobacter
% abundance
LI
SD
MC
IC
Biocrust

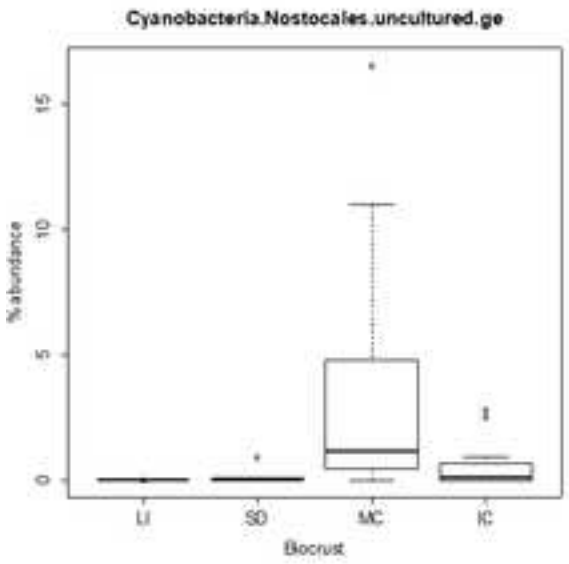

Acidobacteria.Subgroup.10
% abundance
LI
SD
MC
IC
Biocrust

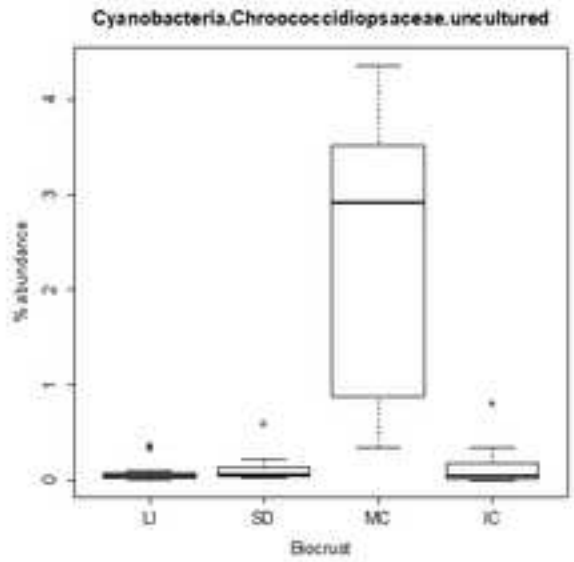

Proteobacteria.Burkholderiaceae.unclassified
% abundance
LI
SD
MC
IC
Biocrust

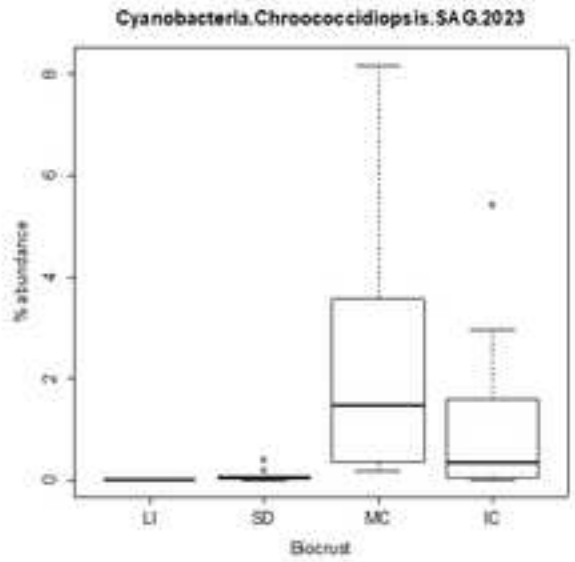

Bacteroidetes.Chitinophagaceae.uncultured
% abundance
LI
SD
MC
IC
Biocrust

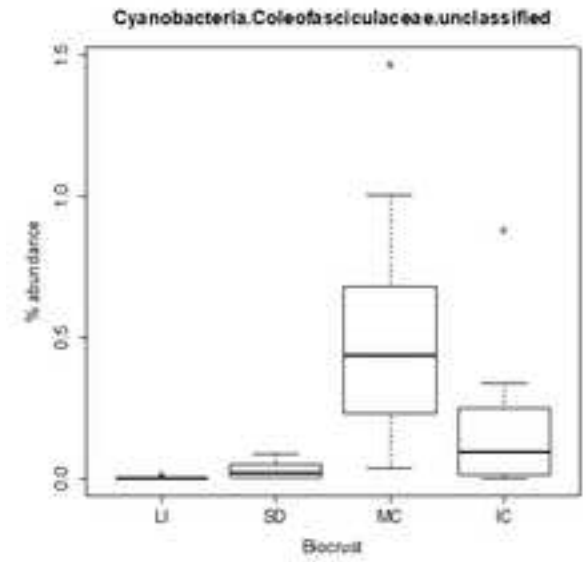

Elusimicrobia.Lineage.IIb.ge
% abundance
LI
SD
MC
IC
Biocrust

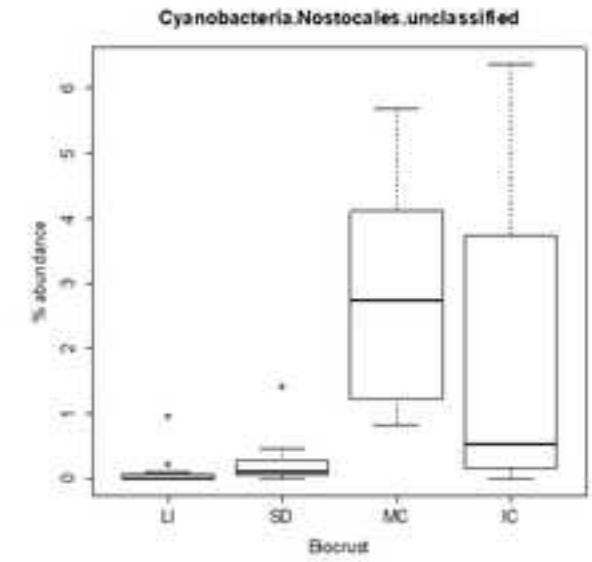

Verrucomicrobia.Opitutus
% abundance
LI
SD
MC
IC
Biocrust

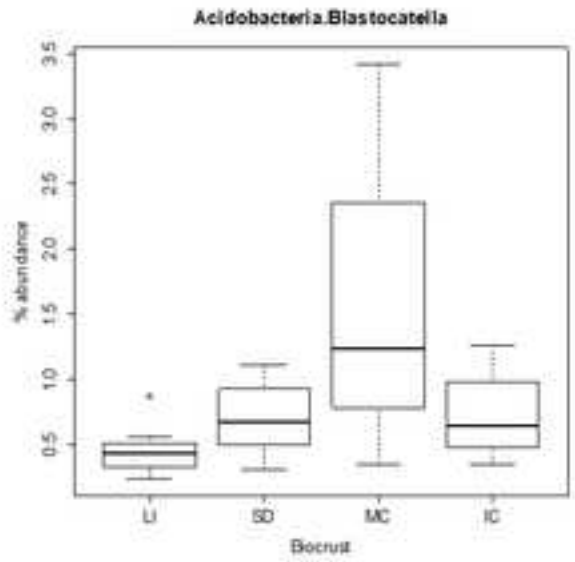

Cyanobacteria.Nostocales.uncultured.ge
% abundance
0
5
10
15
LI
SD
MC
IC
Biocrust


Cyanobacteria.Chroococcidiopsaceae.uncultured
% abundance
0
1
2
3
4
LI
SD
MC
IC
Biocrust


Cyanobacteria.Chroococcidiopsis.SAG.2023
% abundance
0
2
4
6
8
LI
SD
MC
IC
Biocrust


Cyanobacteria.Coleofasciculaceae.unclassified
% abundance
0.0
0.5
1.0
1.5
LI
SD
MC
IC
Biocrust


Cyanobacteria.Nostocales.unclassified
% abundance
0
1
2
3
4
5
6
LI
SD
MC
IC
Biocrust


Acidobacteria.Blastocatella
% abundance
0.5
1.0
1.5
2.0
2.5
3.0
3.5
LI
SD
MC
IC
Biocrust