

# Water and Heat-Triggered Changes of Bacterial Community Structure and Function in Biological Soil Crusts

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## Research Article

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# Abstract

*Background and Aims* The outstanding ability of biological soil crusts (BSCs) in soil microenvironments regulation is mainly attribute to microorganisms that colonizing in biocrusts. We aimed to investigate the changes of bacterial community structure and function with biocrust succession, as well as their responses to climatic changes across large geographical scales.

*Methods:* Algal BSCs and lichen BSCs were sampled along an aridity gradient on alpine grasslands. Bacterial communities in biocrusts were measured using high-throughput sequencing, and soil underlying biocrusts (0-5 cm) was collected for nutrients determination.

*Results:* Our results indicated that compared with algal BSCs, bacterial community in lichen BSCs was characterized by lower diversity, more complex co-occurrence network and mutually beneficial relationships. The bacterial community assembly was governed mainly by stochastic processes for lichen BSCs, which was different from the almost equally important roles of stochastic and deterministic processes for algal BSCs. Geographical location had a significant effect on bacterial communities in both algal and lichen BSCs, while had a greater effect on lichen BSCs. It is noteworthy that the bacterial diversity of algal BSCs was positively correlated with aridity index, while that of lichens was negatively correlated with aridity index. Moreover, we determined lower soil pH and higher soil phosphorus content underlying lichen BSCs, implying their advantages in soil improvement.

*Conclusions:* Aridity index was one of important driving factors of bacterial community in biocrusts, and its effects were biocrust type dependent. Lichen BSCs had greater effects on soil improvement than that of algal BSCs.

## Introduction

Biological soil crusts (BSCs), comprising 12% of the terrestrial surface area and 70% of the dryland surface soil (Rodriguez-Caballero et al. 2018), are a biological complex of microbiomes, cryptogams (algae, lichens, and mosses), and secretions (Velasco et al. 2017). BSCs are considered as ecosystem engineers, particularly in dry land areas (Bowker et al. 2005), because of their critical role in controlling carbon gain (Kidron et al. 2015) and nitrogen cycling (Elbert et al. 2012; Suet al. 2012), regulating soil hydrology (Strausset et al. 2012), and promoting soil nutrients (Chamizo et al. 2012). Soil quality and microenvironment are significantly improved with the development of crusts; therefore, BSCs have great potential in ecological restoration and desertification control (Zhang et al. 2021). Microorganisms are fundamental components of the soil biocrust and are the main performers of their ecological functions (Housman et al. 2006; Ma et al. 2016). Cyanobacteria and proteobacteria are the dominant bacteria involved in carbon accumulation and nitrogen cycle (Liu et al. 2020). Microbial communities in biocrusts are extremely sensitive to environmental changes (Abed et al. 2019; Nuttapon et al. 2020), as well as natural or anthropogenic disturbances (Steven et al. 2015; Velasco et al. 2019). Moreover, climate change is also an essential driver of microbial communities in biocrusts (Blay et al. 2017). As an important

component of the terrestrial ecosystem, studying microbial community variation with biocrust succession and their responses to climate changes will enhance our understanding of the effects of climate change on structures and functions of terrestrial ecosystems (Delgado-Baquerizo et al. 2016; Wang et al. 2017).

Vital activities and geochemical processes are largely determined by water and heat availability, and the same is true for microbial communities inhabiting the BSCs. Global climate changes, characterized by warming and changes in precipitation patterns, significantly affect microbial community composition and functions in BSCs (Johnson et al. 2012; Ferrenberg et al. 2015; Hagemann et al. 2015; Bowker et al. 2016; Reed et al. 2016). For example, cyanobacteria in BSCs are considered bioindicators of climate change because of their different responses to warming and precipitation decrease (Muñoz-Martín et al. 2019). Various types of BSCs, such as algae, lichen, and moss biocrusts, represent different succession stages of biocrust. Algal biocrusts, the early stage of biocrusts, create conditions favorable for the colonization of moss and lichens by improving soil quality (Song et al. 2014). Microbial community changes with biocrust development (Zhang et al. 2016; Wang et al. 2020). Moreover, various biocrust types respond characteristically to climate change. For example, climate warming reduces the cover, richness, and evenness of lichen biocrusts and increases the abundance of moss biocrusts (De Guevara et al. 2018). Increased precipitation significantly promotes the growth of cyanobacteria and inhibits moss growth (Zelikova et al. 2012). A reduction in precipitation might prevent cyanobacterial biocrusts from reaching more mature successional stages (Fernandes et al. 2018). However, how microbial communities in biocrusts respond to climate change and whether the responses vary with biocrust development remains largely unclear.

Climate gradients are naturally formed along large geographic scales, and are ideal for studying the effects of climate change on terrestrial ecosystems and for various biogeographical studies, which aim to explore the spatial distribution patterns of biodiversity and community assembly processes (deterministic or stochastic processes) (Karimi et al. 2020; Chu et al. 2020; Feng et al. 2019). Community assembly processes are the basis for understanding species co-existence patterns and community composition (Dini-Andreote et al. 2015). Community dynamics are controlled by deterministic factors, such as environmental factors and stochastic processes, including birth, death, and dispersal (Ofițeru et al. 2010). Furthermore, the dominance of deterministic and stochastic processes varies with geographic location and spatial scale (Shi et al. 2018). Microbial community diversity and composition change with biocrust succession. However, little is known about the assembly process of microbial communities in biocrusts at different successional stages.

In the context of global climate change, temperatures at high latitudes and elevations have increased significantly more than in other regions (IPCC 2007; Benito et al. 2011). The Qinghai-Tibetan Plateau, the highest and largest plateau on Earth (Shen et al. 2015) is experiencing rapid warming and precipitation regime changes. Considering its unique geographic location and significant ecological and production functions, the effects of climate change or human activities on biodiversity, vegetation composition, soil characteristics, and ecosystem functions have largely been studied in this area (Sun et al. 2020; Zhang et al. 2019, 2020). In this study, we sampled two types of BSCs with different succession stages from nine

regions on the Qinghai-Tibetan Plateau that varied in altitude, annual temperature, and precipitation. Bacterial community diversity and assembly in the crust layer were measured using high-throughput sequencing, and soil nutrients underlying biocrusts were determined. The nine selected locations were across the north to south gradient along the eastern boundary of the Qinghai Province, covering the main grassland types (alpine steppe and alpine meadow) and showed significant climatic differences. To the best of our knowledge, this research is the most extensive investigation on a geographical scale of the microbial communities in the BSCs in the Qinghai-Tibet Plateau, which has significant application in the utilization of biocrust resources for ecosystem restoration. We hypothesized that 1) microbial community diversity increases with biocrust development, 2) microbial communities in biocrusts with different successional stages responds differently to climatic changes, 3) the community assembly process and co-occurrence network structure changes with biocrust succession, and 4) the ability to improve the underlying soil quality differs between the two biocrust types.

## Materials And Methods

### Study area

Along the eastern edge of Qinghai Province, China, nine regions experiencing different annual mean temperatures and precipitation (AMT and AMP, respectively) were selected for biocrust sampling (Supplemental Fig. S1, Table 1). The AMT and AMP data from 2014 to 2019 were obtained from local meteorological stations. The study areas were characterized by an alpine continental climate with an AMT ranging from -3.12 to 5.09 °C and AMP ranging from 420 to 880 mm. Rain and heat over the same period with the highest temperature and rainfall occurred during the growing season (June–September). The AMT and AMP were typically lower and higher, respectively, at the southern sites (Maqin, Dari, and Jiuzhi) than at the northern sites (Gonghe, Gangcha, and Qilian). The de Martonne aridity index (AI) ( $AI = \text{average precipitation} / (\text{average temperature} + 10)$ ) was used to determine the water and heat conditions of the study sites (Zhou et al. 2020). The main vegetation types in the sampling areas were alpine steppe and alpine meadows, dominated by *Stipa aliena* Keng, *Kobresia pygmaea*, *Elymus nutans*, *Potentilla chinensis*, and *Leontopodium nanum*. BSCs colonized plant interspaces (Supplemental Fig. S1).

Table 1  
Geographical locations and climatic information of sampling sites

ID	Longitude (°E)	Latitude (°N)	Altitude (m)	Annual mean temperature (°C)	Annual mean precipitation (mm)	Aridity index
Gonghe	100.58	36.40	3357	5.09	418.5	27.73
Yushu	97.07	32.85	3805	3.35	565	42.32
Qilian	99.47	38.48	3370	2.42	526.6	42.40
Gangcha	100.57	37.28	3390	0.59	451.2	42.61
Guinan	100.50	36.00	3392	2.74	613.8	48.18
Maqin	100.20	34.45	3700	1.04	606.3	54.92
Maduo	98.13	34.80	4192	-3.12	434.2	63.11
Dari	99.73	33.70	3967	0.23	668.6	65.36
Jiuzhi	100.70	33.05	3563	2.36	887.5	71.80

## Biocrust sampling

Two types of biocrusts with different morphological characteristics and successional stages (algal BSCs with dark color and lichen BSCs with light color) were collected in August 2019 (Fig. 1a). The dark algal BSCs represented the early developmental stages, while the light lichen BSCs represented the late developmental stages. A 30–50 m<sup>2</sup> grassland area was investigated, and regions containing both algal BSCs and lichen BSCs were selected (Muñoz-Martín et al. 2019). The biocrust-layer and the top soil layer (0–5 cm) were separated artificially with a sterilized shovel. Samples were stored at room temperature until they were completely dry for further molecular analysis (Muñoz-Martín et al. 2019). Three crust samples were collected for each biocrust type in each region, resulting in a total of 54 samples (nine regions × two types × three replicates) for 16S rRNA sequencing. Soil samples under the biocrusts were screened with a 2 mm sieve for measuring their physicochemical properties. Soil pH was measured with a pH probe (Orion Star A215, ThermoFisher Scientific, USA), and the water-to-soil ratio was 5:1. The soil total nitrogen (TN) and total carbon (TC) contents were measured using a determinator (Elementar, Hanau, Germany), and the soil total phosphorus (TP) content was measured using HClO<sub>4</sub>-H<sub>2</sub>SO<sub>4</sub> colorimetry.

## DNA extraction, PCR amplification, and sequencing

The sampled BSCs were homogenized with a mill and cooled before DNA extraction. Total DNA was isolated from a 0.5 g biocrust sample using OMEGA: E.Z.N.A.® Soil DNA Kit (Omega Biotek, Norcross, GA, USA) following the manufacturer's instructions. The integrity of the DNA was detected using 1.0% agarose gel electrophoresis, and NanoDrop One (NanoDrop Technologies, Wilmington, USA) was used for purity examination. Specific primers [(5'-GGACTACHVGGGTWTCTAAT-3') and (5'-

ACTCCTACGGGAGGCAGCA-3')] were used to amplify the V3–V4 region of the 16S rRNA. The PCR procedure was performed as described by Wei et al. 2021. PCR products were detected by 1% agarose gel electrophoresis, mixed in equidensity ratios according to the GeneTools Analysis Software (Version 4.03.05.0, SynGene), and then purified using the EZNA Gel Extraction Kit (Omega, USA). Libraries were constructed according to the procedure of the NEBNext Ultra DNA Library Prep Kit for Illumina® (New England Biolabs, USA). The Qubit® 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system were used for quality assessment, after which the libraries were sequenced with the Illumina HiSeq 2500 platform.

## Sequencing data processing

High-quality paired-end (PE) clean reads were obtained by a trimmomatic quality-controlled process (V0.33 <http://www.usadellab.org/cms/?page=trimmomatic>). Subsequently, the reads were merged into one sequence according to the overlapping relationship between PE reads using FLASH software (V1.2.11, <https://ccb.jhu.edu/software/FLASH/>). The allowed minimum overlap length was 10 bp, and the nonconforming sequences with error ratios > 0.2 in the overlapping region of the merged sequence were filtered. The spliced sequences were then filtered using the Trimmomatic software. The effective sequences were clustered into operational taxonomic units (OTUs) using Usearch (V8.0.1517, <http://www.drive5.com/usearch/>), and sequences that shared  $\geq 97\%$  similarity were considered to contain the same OTUs. Chimera sequences and singleton OTUs were removed using the UCHIME de novo algorithm and Usearch ([http://www.drive5.com/usearch/manual/chimera\\_formation.html](http://www.drive5.com/usearch/manual/chimera_formation.html)), separately. Finally, the taxonomic information of the representative OTU sequences was annotated using the GreenGene database (<http://greengenes.secondgenome.com/>) based on the Ribosomal Database Project (RDP) classifier algorithm and the assign\_taxonomy.py script ([http://qiime.org/scripts/assign\\_taxonomy.html](http://qiime.org/scripts/assign_taxonomy.html)) in QIIME.

## Data analysis

Shannon-Wiener index was analyzed using the QIIME (V1.9.1) software (alpha\_diversity.py script) (Lozupone and Knight 2005). Dissimilarities in the bacterial community were shown using principal coordinates analysis (PCoA) plots based on the Bray-Curtis distance. Significant community dissimilarities were tested through permutational multivariate analysis of variance (PERMANOVA) using the “Adonis” function in R (3.6.1). Bacterial community assembly processes were quantified by the  $\beta$ -nearest taxon index ( $\beta$ NTI), which was calculated using the “picante” package in R (Shi et al. 2018), whereby a  $|\beta$ NTI| > 2 suggests that community assembly is mainly controlled by deterministic processes, and  $|\beta$ NTI| < 2 suggests that community assembly is predominately determined by stochastic processes (Stegen et al. 2012). Microbial network analysis was performed by Conet in Cytoscape 3.7.2 and visualized by Gephi 0.9.2. The relationships between bacterial community dissimilarity and the physical separation of sampling sites were analyzed using the “vegan” package in R. The actual geographic distances between the sample sites were calculated based on the latitudes and longitudes of the study sites. Functional prediction of bacteria was performed using Phylogenetic Investigation of Communities

by Reconstruction of Unobserved States (<https://huttenhower.sph.harvard.edu/picrust/>), and communities were compared using the Kyoto Encyclopedia of Genes and Genomes database.

## Results

### **$\alpha$ -diversity of bacterial communities in the biocrusts**

After final quality filtering, the read numbers were rarefied to the minimum size (7,796) to ensure the same sequencing depth, resulting in a total of 1,1298 bacterial OTUs. Of these, 4245 OTUs (37.6% of the total OTUs) were specific for the algal BSCs, 1327 (11.7%) OTUs were specific for the lichen BSC, and 5726 OTUs (50.7%) were shared between both (Supplemental Fig. S2). The rarefaction curves reached a saturation plateau, suggesting that the sequencing depth was sufficient to cover most bacterial species (Supplemental Fig. S3). Our results indicated that sampling locations significantly affected the Shannon index of the bacterial community for both algal BSCs and lichen BSCs. Moreover, the Shannon index of the bacterial communities in the algal BSCs were significantly higher than that in the lichen BSCs (Fig. 1b). Further, the variation degree between the two biocrust types increased as the AI increased (Fig. 1c). To further clarify the variation rules of bacterial diversity in the two biocrusts across large geographical scales, Spearman's correlation analysis was performed between the AI and the Shannon diversity index. Interestingly, we found that the Shannon index was negatively correlated with the AI for the lichen BSCs, while it was positively correlated with the AI for the algal BSCs (Fig. 1d).

### **$\beta$ -diversity, taxonomic composition, and functional genes of the bacterial communities**

Variations in the bacterial community composition were tested using PERMANOVA and visualized through PCoA plots. The results suggested that the bacterial community composition in the algal BSCs significantly differed from that in the lichen BSCs ( $F = 11.9$ ,  $R^2 = 0.186$ ,  $P = 0.001$ ) (Fig. 2a). Furthermore, sampling locations significantly affected the bacterial community composition in the lichen ( $F = 4.77$ ,  $R^2 = 0.679$ ,  $P = 0.001$ ) and algal ( $F = 4.18$ ,  $R^2 = 0.650$ ,  $P = 0.001$ ) BSCs (Supplemental Fig. S4). According to the PCoA plots, sampling sites with higher humidity (Maqin, Maduo, Dari, and Jiuzhi) were grouped together and separated from those with lower humidity for both the algal and lichen BSCs (Fig. 2b).

Differences in the taxonomic composition were also analyzed. At the phylum level, proteobacteria was the dominant phylum, with relative abundance ranging from 26–44% in the algal BSCs and 31–88% in the lichen BSCs along the climatic gradients (Supplemental Fig. S5). The relative abundance of Actinobacteria was higher in areas with low AI (Supplemental Fig. S5). At the genus level, variations in the bacterial taxonomic composition across the different geographical locations were more evidently observed in the lichen BSCs than in the algal BSCs. We also found that Burkholderia was the absolute dominant genus for lichen BSCs in regions that showed high AI (Guinan, Maqin, Dari, and Jiuzhi) (Fig. 2c). In addition, the relative abundance of functional genes associated with ferric uptake, phosphate

transport, and secretion proteins in the lichen BSCs was much higher than those in the algal BSCs, especially in areas with high AI (Fig. 3).

## **Spatial distribution patterns, assemblage processes, and co-occurrence network of the bacterial communities**

The distance-decay relationship, which reflects spatial distribution pattern of biodiversity, has been widely studied in the field of microbial ecology. It describes the reduced similarity in species composition between two communities as the geographic distance increases. Our results indicated that the bacterial communities in both lichen and algal BSCs followed the distance-decay pattern (Fig. 4a). The slope of this linearity curve for the lichen BSCs was higher than the algal BSCs, which indicated a faster turnover rate of the lichen BSCs.  $\beta$ NTI was calculated to determine the relative importance of the deterministic and stochastic assembly processes in the bacterial community assembly. The results indicated that the dominant assemblage processes of the bacterial communities changed with the succession of BSCs. The stochastic processes accounted for 53% for the algal BSCs, whereas it increased to 90% for the lichen BSCs (Fig. 4b). The co-occurrence network of the bacterial community in the lichen BSCs was characterized by higher link number, average degree, clustering coefficient, and network density compared with that in the algal BSCs (Fig. 5). Further, the percentage of positive linkages in the network increased with biocrust succession.

## **Soil characteristics underlying biocrusts**

The physicochemical properties of soils underlying the biocrusts were determined (Fig. 6). Results indicated that soil pH under the lichen BSCs was much lower than that under the algal BSCs at most sampling sites. Soil carbon and nitrogen content showed marginal differences between the two biocrust types, while soil phosphorus content underlying the lichen BSCs was much higher than that underlying the algal BSCs. We also found that the bacterial community in the lichen BSCs was significantly correlated with soil pH, and soil carbon, nitrogen, and phosphorus contents. Further, the bacterial community in the algal BSCs was significantly correlated with soil nitrogen and phosphorus contents (Table 2).

Table 2  
Correlations between bacterial community composition in BSCs and the underlying soil characteristics based on Mantel test

Soil characteristics	Lichen BSCs		Algal BSCs	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
pH (0-5 cm)	<b>0.68</b>	<b>&lt;0.001</b>	0.33	0.09
Total nitrogen (0-5 cm)	<b>-0.51</b>	<b>0.01</b>	-0.36	0.07
Total carbon (0-5 cm)	<b>-0.48</b>	<b>0.01</b>	<b>-0.49</b>	<b>0.01</b>
Total phosphorus (0-5 cm)	<b>-0.44</b>	<b>0.02</b>	<b>-0.55</b>	<b>0.00</b>

## Discussion

### Variations in the bacterial community in the biocrusts with biocrust development

We compared the bacterial communities in the biocrusts with different successional stages through alpha diversity,  $\beta$ -diversity, taxonomic composition, functional genes, network structure, and community assembly processes. Microbial communities of biocrusts are type-dependent, and studies have reported that mature developmental stages with more complex structures show higher alpha diversity than the initial developmental stages (Langhans et al. 2009; Maier et al. 2018; Nuttapon et al. 2020). Another study concluded that the bacterial community diversity initially increased and later decreased with biocrust succession (Chen et al. 2020). Our study proved that the bacterial community in the biocrusts at the later succession stages (lichen BSCs) showed lower diversity compared with biocrusts at the early succession stage (algal BSCs). The biocrusts are usually artificially divided into several distinct developmental stages. However, the succession of BSCs is a continuously changing process, and studies involved in different spans of developmental levels of biocrusts often lead to distinct conclusions.

Microbial community assembly processes can be classified as deterministic and stochastic (Tripathi et al. 2018). Deterministic processes are determined by ecological selection, which is affected by abiotic and biotic factors. Stochastic processes include diffusion and ecological drift (Vellend 2010; Chase and Myers 2011). In the present study, stochastic processes accounted for 53% in the bacterial community assembly for the algal BSCs, while they accounted for 90% for the lichen BSCs, which was in accordance with a study that reported that bacterial communities in biocrusts are mainly driven by stochastic processes with biocrust succession (Li and Hu 2021). However, a three-phase conceptual model proved that stochastic processes primarily dominated community establishment at the initial stages, and the importance of deterministic selection increased with community succession (Dini-Andreote et al. 2015), suggesting an inconsistency between the theoretical model and field test results. Species co-occurrence relationship is an important factor driving microorganism assembly (Maestre et al. 2010). In this study, the proportion of mutualism relationships among microorganisms increased with biocrust succession,

which might be a survival strategy of the microorganisms to adapt to changing microhabitats with biocrust succession.

The distance-decay pattern, which describes the effect of distance on community dissimilarities (Morlon et al. 2008), is critical for understanding the driving factors of community turnover in spatial biodiversity and beta-diversity studies (Ferrier et al. 2010), and has been widely studied in soil microbial biogeography (Feng et al. 2019; Shi et al. 2018). Our results showed that the bacterial communities in both the light and dark BSCs had significant distance-decay patterns, indicating the important role of dispersal limitation in bacterial community assembly, which was consistent with previous studies that involved three biocrust types (alga, lichen, and moss-dominated biocrusts) across northern China (Li and Hu 2021; Su et al. 2020). In addition, the slope of the linear curve for the lichen BSCs was greater than that of the algal BSCs, indicating a faster spatial turnover rate of the bacterial community and greater sensitivity to environmental changes during the late development stages of BSCs.

Network structure of microbe-microbe interactions changed with biocrust succession in the present study. Network density, average degree, and number of linkages increased in the late successional biocrusts, indicating a strong centralization and complication of the late-stage network. However, another study conducted in New South Wales Australia found that the number of linkages increased initially and later decreased along with the early, mid, and late developmental stages (Chilton et al. 2018). Differences in the range of the developmental stages may be responsible for the different results. Previous studies have suggested that the loss of network complexity might be attributed to the decreases in species diversity (Dini-Andreote et al. 2014); however, this was not applicable to this study due to the lower diversity but higher complexity network in late successional biocrusts.

## **Responses of bacterial community in biocrusts with distinct successional stages to aridity**

Local soil characteristics and plant communities are the most important factors influencing the bacterial communities in the biocrusts (Rivera-Aguilar et al. 2009), while climate is the main driving factor at large geographical scales (Zedda et al. 2011). Abed et al. (2019) sampled biocrusts at six different locations and found that bacterial communities in the BSCs were largely determined by soil characteristics and environmental parameters. Moreover, warming strongly reduced the diversity and changed the composition of lichen-dominated BSCs, which eroded the positive impacts of BSCs on ecosystem processes (Escobar et al. 2012). In the present study, we also found significant effects of geographical locations on the bacterial community in the BSCs. Biocrust organisms are fragile and sensitive to climate change (Belnap and Eldridge 2001; Steven et al. 2015). Cold and wet environments could lead to a dramatic decline in the microbial diversity in biocrusts (Blay et al. 2017). However, the direction and magnitude of climate effects differs among the biocrust types. Warming reduces the richness and diversity of lichen-dominated biocrusts, whereas it facilitates moss growth (De Guevara et al. 2018). Previous studies also indicated a significant effect of climate changes on biocrust succession by monitoring changes in biocrust coverage at different developmental stages (Ferrenberg et al. 2015).

Microbial communities of different crusts types respond differently to climate changes. For example, precipitation was positively correlated with the species richness of mosses and negatively correlated with the species richness of cyanobacteria and algae (Li et al. 2017). We found that the AI was negatively and positively correlated with the bacterial diversity for the lichen BSCs and the algal BSCs, respectively. In addition, the variations in the diversity between the two biocrust types were more evident in wetter regions. Decreasing biodiversity has a significantly adverse impact on ecosystem functions (Isbell et al. 2017). Therefore, climate changes in the future might accelerate functional differentiation of different biocrust types.

State-and-Transition Models propose that thresholds exist for different successional stages of ecosystems (Bowker 2010). In desert ecosystems, organic carbon is considered as the threshold of biocrust successions from cyanobacterial BSCs to lichen BSCs, and nitrogen and phosphorus availability determines biocrust succession from lichen to moss (Deng et al. 2020). We predicted that the AI might be an important threshold of bacterial community diversity in the BSCs. The AI of the nine sampling sites ranged from 27 to 71; additionally, the bacterial community diversity increased for the algal BSCs, but decreased sharply for the lichen BSCs when the AI value exceeded 48. Based on this result, we divided the nine sample sites into low aridity index group and high aridity index group. Significant differences in the bacterial community composition were observed between the two groups. Therefore, we believed that this value was a critical transition point for changes in the bacterial microbial community. In addition, the dominant *Burkholderia* genus in regions with high rainfall (Guinan, Maqin, Dari, and Jiuzhi) for the lichen BSCs might restrict the growth of other microorganisms and decrease the microbial community diversity.

## **Effects of the biocrusts on the underlying soil properties**

Biocrusts contribute significantly to soil nutrient accumulations through microbial activities (Mager and Thomas 2011; Kidron et al. 2015). Moreover, BSCs affect nutrient cycling by modifying subsoil microbial community composition (Nevins et al. 2021). Our results revealed that the effects of biocrust on the underlying soil properties were associated with biocrust types and sampling locations. Biocrusts could decrease their underlying soil pH by releasing H<sup>+</sup> or organic acids (Belnap 2011). The soil pH underlying the lichen BSCs was lower than that underlying the algal BSCs, which proved the greater role of biocrusts in the late succession stages on soil improvement than biocrusts in the early succession stages. Organic phosphorus compounds secreted by microorganisms colonizing in biocrusts also increase phosphorus levels in soil underlying biocrusts (Baumann et al. 2018). The higher abundance of functional genes related to phosphorus uptake and transport, and secreted proteins might explain the higher ability of phosphorus accumulation at the late succession stage of biocrust (lichen BSCs). Previous studies reported soil carbon and nitrogen accumulation in the top-layer of soil with crust development (Chamizo et al. 2012); however, we detected marginal differences in the soil carbon and nitrogen contents between the two biocrust types compared with total phosphorus content. Phosphorus transformation in soils is an open-loop process, which is different from carbon and nitrogen cycles. Autotrophs in terrestrial ecosystems can fix carbon and nitrogen in the atmosphere, and heterotrophic microbes release the fixed carbon and nitrogen into the atmosphere as gases by decomposing organic matter. However, phosphorus

barely returns to the atmosphere from terrestrial ecosystems, and only transforms in different forms within terrestrial ecosystems, such as labile phosphorous, relatively stable phosphorous, and organic phosphorous.

## Conclusions

In this study, we explored the variations in the bacterial community composition with biocrust succession stages across large geographical scales. The effect of biocrusts on the underlying soil improvement was also evaluated. We found that bacterial communities in the BSCs were affected significantly by the biocrust types and geographical locations, and geographical locations had greater effects than biocrust types at large geographical scales. With biocrust development, bacterial diversity decreased, co-occurrence network became complicated, and the proportion of coexistence edges increased. Furthermore, we found that stochastic process governed bacterial community assembly for the lichen BSCs. The AI was positively and negatively correlated with the bacterial diversity for the algal BSCs and lichen BSCs, respectively. Moreover, our results indicated that the lichen BSCs had stronger effects on soil acidification and phosphorus accumulation than the algal BSCs. Therefore, crusts types and climatic conditions should be considered when using biological soil crusts for land restoration.

## Declarations

### Statements and Declarations

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### Competing Interests

The authors declare no competing interests.

### Author Contributions

All authors contributed to the study conception and design. Sampling were performed by [Xiaoting Wei], [Fuwen Qin] and [Xinqing Shao]. Data analysis and interpretation were performed by [Xiaoting Wei], [Bing Han] and [Huakun Zhou]. The first draft of the manuscript was written by [Xiaoting Wei]. The revision of the draft was performed by [Miao Liu], and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

### Data Availability

The datasets are available from the corresponding author on reasonable request

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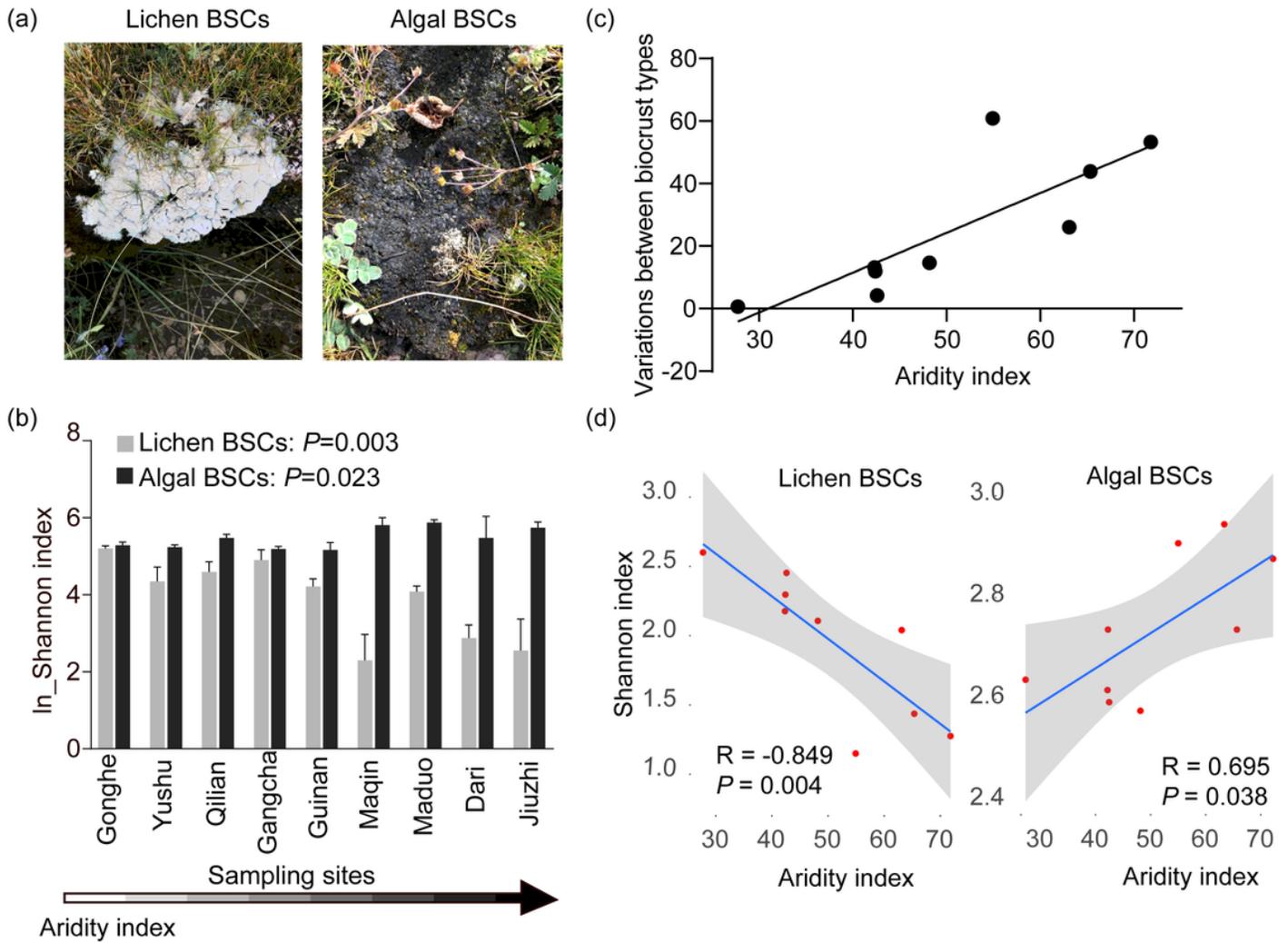
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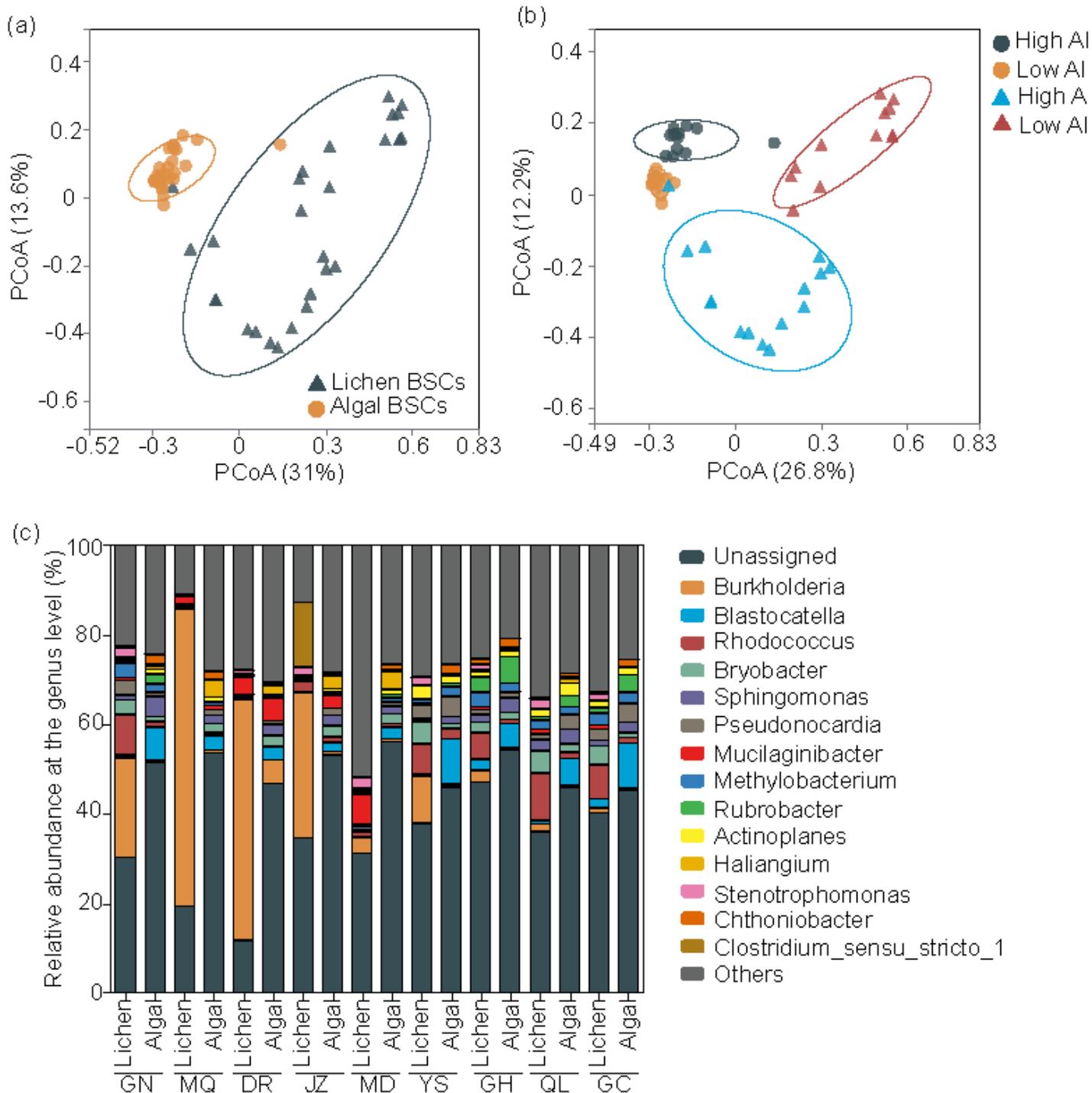
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## Figures



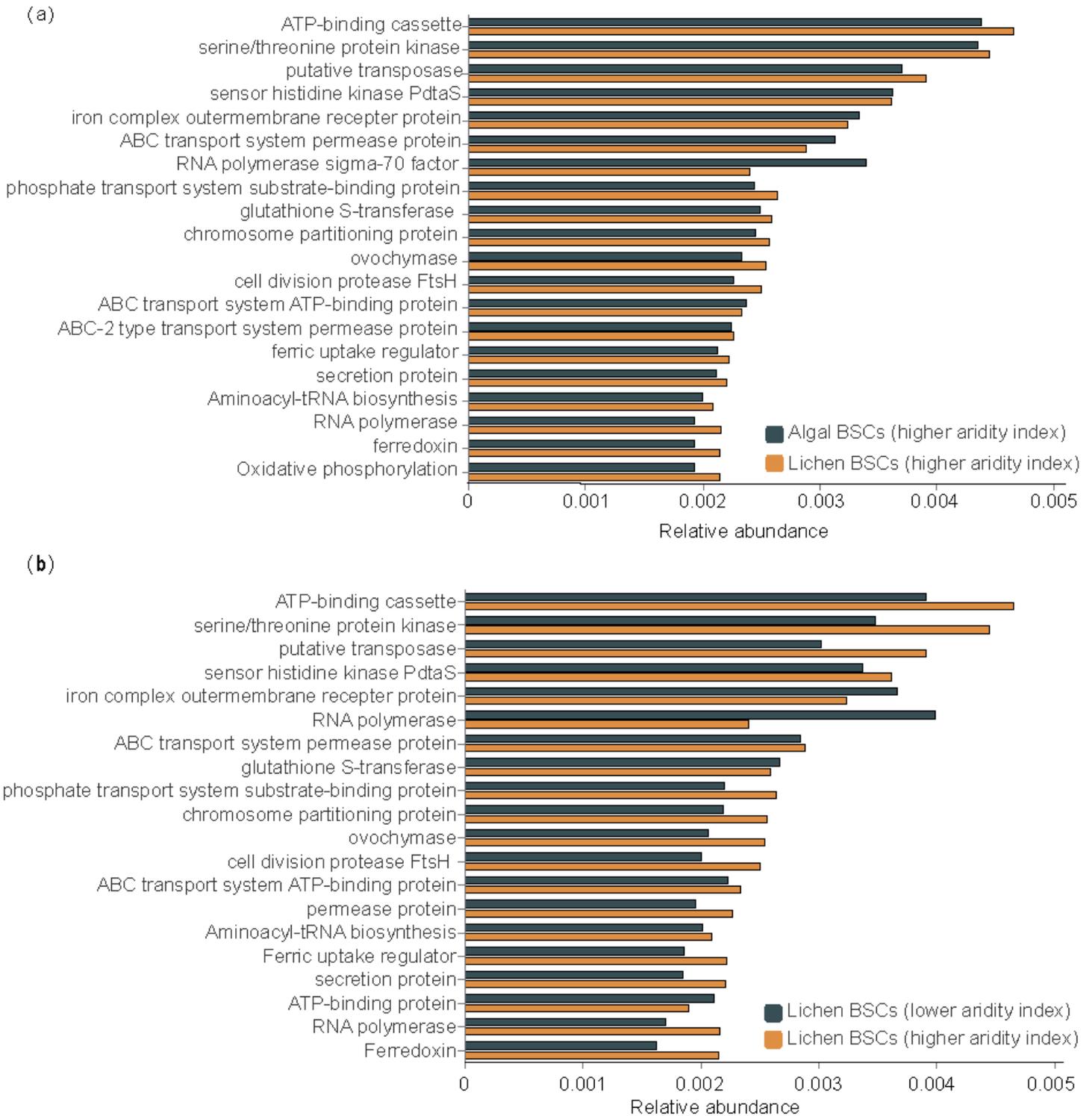
**Figure 1**

Morphological characteristics of biocrusts (A). Bacterial community diversity in biocrusts (B, C). Response of bacterial community diversity to aridity index (D). Variations of bacterial diversity between biocrust types were measured based on the coefficient of variation (CV = standard deviation/mean × 100).



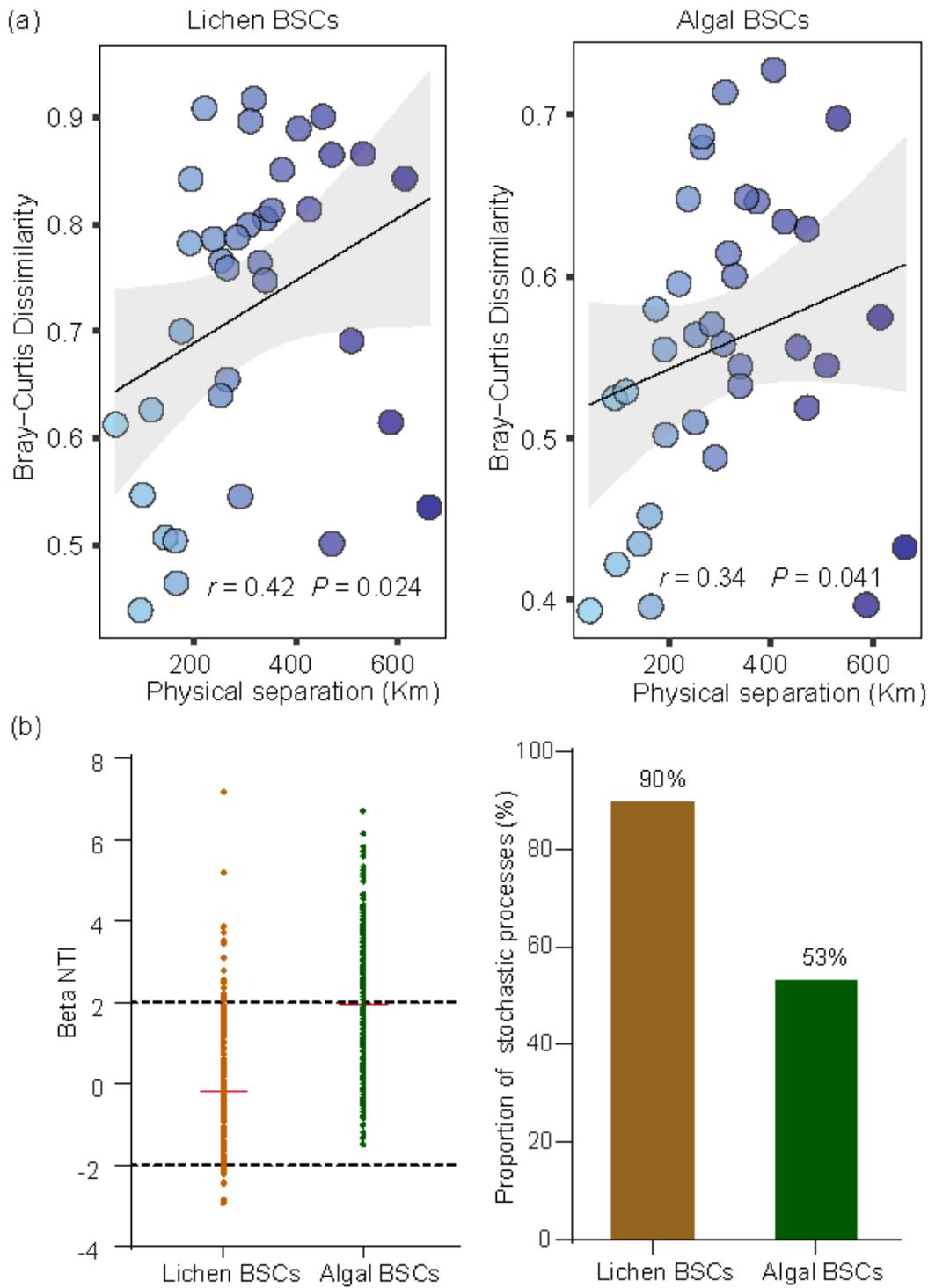
**Figure 2**

PCoA (principal co-ordinates analysis) plots of bacterial communities based on Bray-Curtis distance (A), and the taxonomic composition of bacterial community in biological soil crusts (B). AI-aridity index. High AI-sampling sites with aridity index above 48, and low AI-sampling sites with aridity index lower than 48. GN-Guinan, MQ-Maqin, DR-Dari, JZ-Jiuzhi, MD-Maduo, YS-Yushu, GH-Gonghe, QL-Qilian, GC-Gangcha.



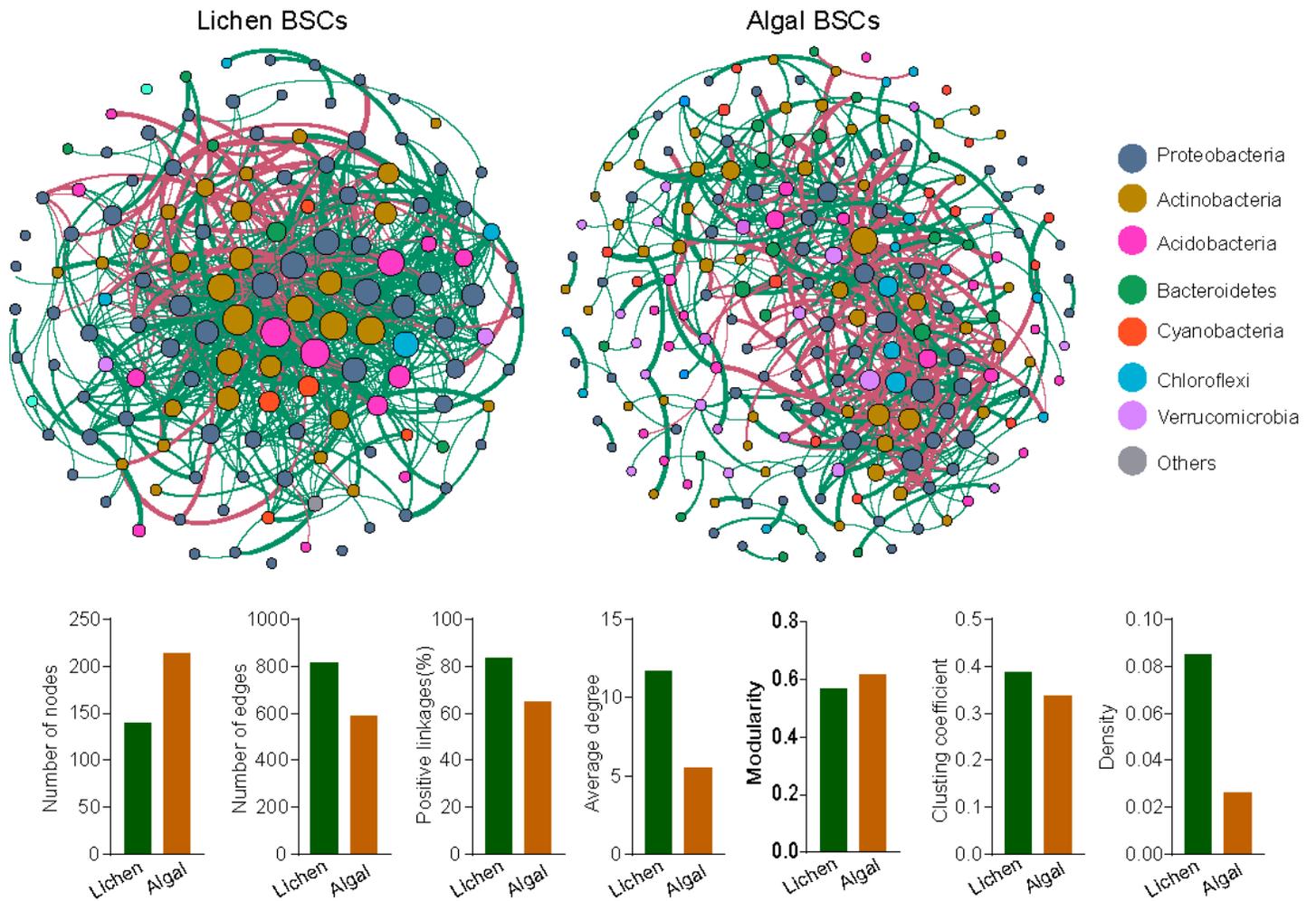
**Figure 3**

Functional genes with significant differences in relative abundance between the two biocrust types at sampling sites with higher aridity index (A), as well as between higher and lower aridity index sites for the lichen BSCs (B).



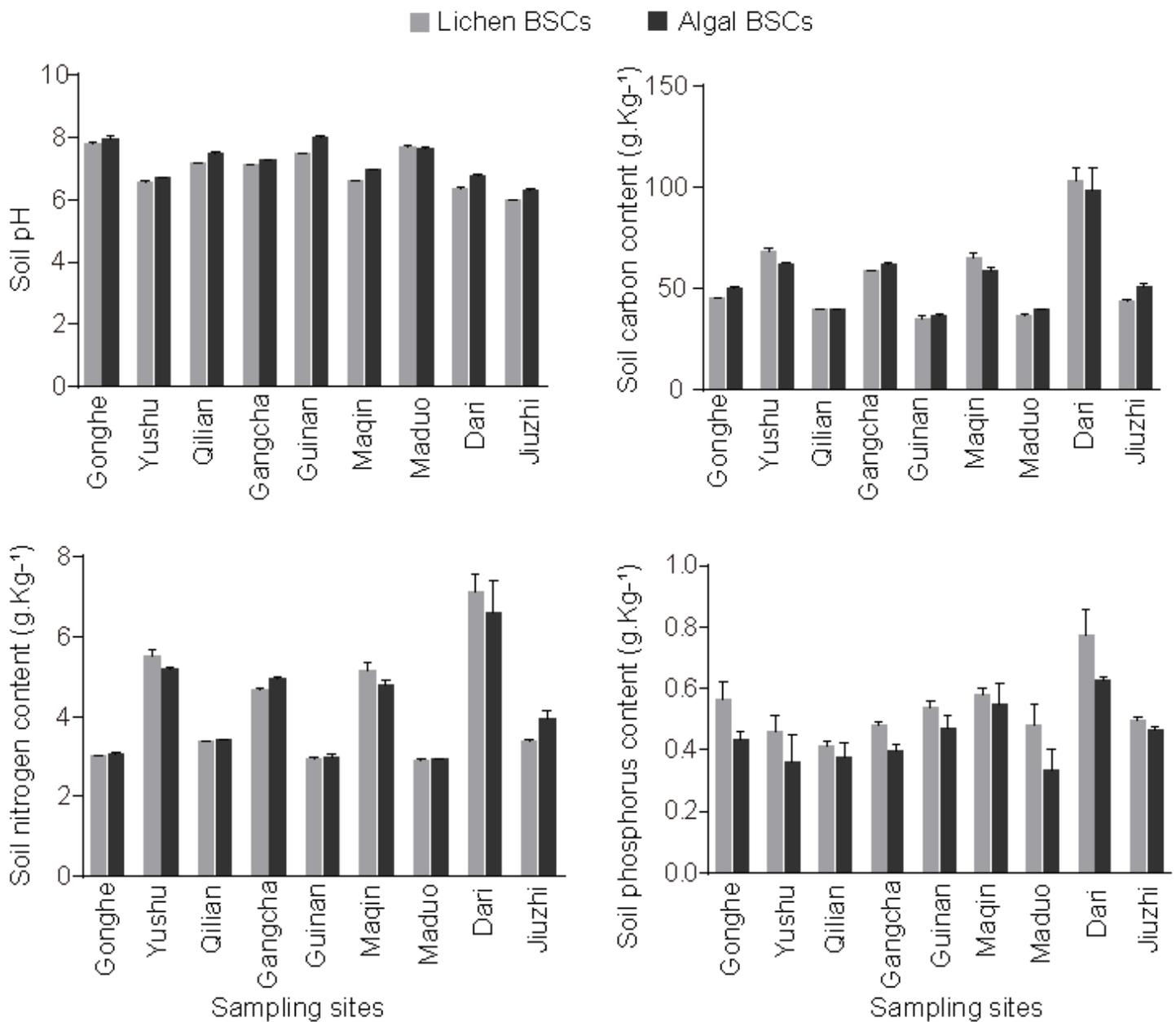
**Figure 4**

The distance-decay relationship and community assembly processes of bacterial community in algal and lichen BSCs.



**Figure 5**

Co-occurrence networks of microbiomes colonizing in biocrusts. The nodes size was proportional to the degree, and the nodes color indicated bacterial phylum taxa. The edges with green color indicated positive correlations, and that with red color indicated negative correlations. The width of edges was proportional to the correlation coefficient.



**Figure 6**

Soil properties underlying algal BSCs and lichen BSCs at each sampling sites (means  $\pm$  SD, n=3).

## Supplementary Files

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