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Soil nutrients, enzyme activities, and microbial communities differ among biocrust types and soil layers in a degraded karst ecosystem

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ABSTRACT

Being different from the well-known and extensively researched counterparts in arid and semi-arid regions, biological soil crusts (BSCs) in degraded karst ecosystems with subtropical humid climate have been paid little attention. In this study, we investigated the differences of nutrient content, enzyme activity, and microbial communities between different types of BSCs (cyanobacterial crusts, moss crusts, moss-cyanobacteria mixed crusts, and bare soils), and between the BSCs layer and underlying soil. The results showed that the soil nutrient (total nitrogen [TN], total phosphorus [TP], and NH_4-N) contents and enzyme activity (urease) in the late-successional stage (moss crusts) were higher than those in early-successional stages (cyanobacterial crusts, moss-cyanobacteria mixed crusts, and bare soils). The species richness of the bacterial community increased with the succession of BSCs, but that of the fungal community did not change. The diversity and composition of both bacterial and fungal communities were not significantly different among biocrust types. The nutrient content (TN, NH_4-N), enzyme activity (urease, nitrate reductase, sucrase), and the richness of bacteria and fungi were significantly higher in BSCs layer than in subsurface soils. For either bacteria or fungi, there was a significant difference in community species composition but not in diversity between BSCs layer and subsurface soils. Besides, the results of network topological properties showed that the network structures of both bacteria and fungi in the BSCs layer were more complex than that in the subsurface, and positive links dominated both networks. These results indicate the importance of the BSCs layer especially moss crusts for nutrient accumulation and microbial genetic resources, and provide an essential basis for further understanding the ecological functions of BSCs in degraded karst ecosystems with subtropical humid climate.

1. Introduction

Karst is a natural landform that forms a double-layered spatial structure at the surface and the subsurface after the dissolution of soluble rocks by acidic water (Gombert, 2002). Karst ecosystems are fragile since long parent rock formation time, shallow and barren soil layer, high rate of bedrock exposure (Xie et al., 2015). Although having good hydrothermal conditions, South China Karst in the humid subtropical climate zone is the most comprehensive, complex, and longest evolving ecologically fragile area on earth regarding karst landscape (Jiang et al., 2014). Excessive or unreasonable human activities have triggered loss of vegetation, severe soil erosion and large-scale rock desertification, leading to the degradation of the ecosystems in South China Karst (Su et al., 2002). In degraded karst ecosystems, the harsh habitats are highly selective for plants. Due to the unique morphological structure and physiological characteristics, biological soil crusts (BSCs) occupy specific ecological niche and are widely distributed.

As a complex community of cyanobacteria, lichens, mosses, fungi, and algae on the soil surface (Belnap, 2003), BSCs are crucial engineers regulating ecosystem functions (Eldridge et al., 2020). These functions include regulating carbon and nitrogen cycles (Büdel et al., 2018; Torres-Cruz et al., 2018), conservation of soil and water (Gao et al., 2020), impact on soil microbial communities and functions (Liu et al., 2017; Su et al., 2020), regulation of soil hydrology (Belnap et al., 2013),

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etc. BSCs have become a new and vital research hotspot in recent decades because of their irreplaceable roles of the restoration and reconstruction in many degraded ecosystems in arid and semi-arid areas (Belnap, 2003; Bowker et al., 2018; Zhao et al., 2020).

Compared to bare soils, BSCs are better at fixing C, N, and P (Delgado-Baquerizo et al., 2015), increasing water infiltration (Chamizo et al., 2012), trapping nutrient-rich fine particles in the air (Belnap, 2003), and driving soil hydrolytic enzyme activity (Miralles et al., 2012a). Many studies found that soil properties are closely related to BSCs types and usually reach optimum at the late-successional stage in arid and semi-arid areas (Lázaro et al., 2008). Later successional stages of BSCs predominated by mosses produce higher carbohydrate and polyphenol contents than early successional BSCs (e.g., cyanobacterial crusts and lichen crusts) (Miralles et al., 2013; Liu et al., 2014) and show high N-cycle enzyme activity that hydrolyzes low-molecular-weight substrates and increases nitrogen availability in the soil (Miralles et al., 2012b). Consequently, the provision of nutrients by different types of BSCs shapes significantly different soil microbial communities at microscale (Chilton et al., 2018; Zhang et al., 2018). However, a few studies drew the different conclusion that the presence or not of BSCs cover accumulate significantly different soil nutrients and microbial communities, while the accumulation differences have nothing to do with BSCs type (Bao et al., 2019; Pombubpa et al., 2020; Nevins et al., 2021).

BSCs colonize the soil surface and are typically 1–15 mm thick (Colesie et al., 2014), suggesting that the effects of BSCs on soil physicochemical and biological properties may be limited to the topsoil (Miralles et al., 2012a; Miralles et al., 2012b; Pointing and Belnap, 2012). In general, the soil physicochemical properties (e.g., water content, total nitrogen [TN], and organic matter), enzymatic activity (e.g., dehydrogenase, urease, and phosphatase), and microbial biomass of BSCs layer were significantly higher than those of the subsurface layer in arid and semi-arid zones (Rao and Burns, 1990; Miralles et al., 2012a; Kakeh et al., 2018). Moreover, the species composition of microbial communities in the BSCs layer and subsurface layer are significantly different (Steven et al., 2013; Pombubpa et al., 2020).

Degraded karst ecosystems in humid subtropical climate zone is inherently endowed with better hydrothermal and soil conditions compared to arid and semi-arid regions, so that BSCs here are likely to be quite different from those in arid and semi-arid regions. So far, among the limited number of BSCs researches in degraded karst areas, most are about moss crusts. It was reported that moss crusts had a positive effect on soil nutrients and buffered the negative effects of the degradation in karst ecosystems (Cheng et al., 2020a). Compared with subsurface soil, moss crust layer effectively accumulates more soil nutrients (Cheng et al., 2020b). There are quite a lot of aspects regarding BSCs in degraded karst ecosystems to be explored. This study aimed to discover the influences of BSCs on soil nutrients, enzyme activities, and microbial communities in degraded karst landscapes. We hypothesized that, in degraded karst ecosystems in subtropical humid climate zone, 1) with the succession of BSCs, soil nutrients increase, enzymatic activities and microbial diversity change, and 2) compared with the subsurface soils, the BSCs layer accumulates significantly more soil nutrients and has higher enzymatic activities, microbial richness and diversity, as well as alters species composition of microbial communities. To test our hypothesis, we collected different BSCs and bare soils and corresponding soil under the BSCs in degraded karst ecosystems to quantify the differences in soil nutrients, enzyme activities, and microbial communities. This study will improve our understanding of the ecological function of BSCs in subtropical humid karst regions and provide a valuable theoretical basis for the effective management and conservation of degraded karst ecosystems.

2. Materials and methods

2.1. Description of study area

The study area, Huajiang karst gorge (25° 37′ 40″ ~ 25° 42′ 30″ N, 105° 35′ 00″ ~ 105° 43′ 20″ E), is located in Guanling County, Guizhou Province, belonging to the Beipan River Basin of the Pearl River System, with an altitude range of 600–1200 m. The study area has a subtropical monsoon climate, with an average annual temperature of 18.4 °C and average yearly precipitation of 1100 mm. The rainy season is from May to October, accounting for 83% of the annual rainfall. The soil type is calcareous soil developed from limestone. The study area exhibits a large area of exposed surface rocks (70%), shallow and infertile soils, low vegetation cover, and a desertification-like landscape (Wei et al., 2010; Li et al., 2017). BSCs are dominated by mosses or cyanobacteria seized the opportunity to fill the ecological niche of this degraded ecosystem (Fig. 1).

2.2. Soil sampling

Crusts and soils were sampled on October 24, 2020, after 9 days without rain. Four 20 m × 20 m plots were randomly selected in the study area, with all three types of BSCs (cyanobacterial crusts, moss-cyanobacteria mixed crusts [hereafter: mixed crusts] and moss crusts) developing in each plot, and each plot was at least 100 m apart (Table S1). Each crust type was randomly sampled five duplications in each plot, and the closest distance between the sampling points was about 10 m. Crust layer was sampled at depth of 0–1 cm, subsurface layer at 1–3 cm, and the bare soil at 0–1 cm. Five samples for each crust type in the same plot were well mixed, and totally 28 mixed samples were obtained. Each soil sample was divided into three parts, respectively for microbial DNA extraction, enzyme activity measurements, and nutrient analyses.

2.3. Analyses of soil physicochemical characteristics and enzyme activities

Soil organic carbon (SOC) was analyzed by the dichromate oxidation approach (Nelson and Sommers, 1983). Total nitrogen (TN) was determined using the Kjeldahl technique (Bremner, 1960). Total phosphorus (TP) was analyzed by the molybdenum blue method (Pan et al., 2003). Nitrate (NO_3-N) was analyzed by phenol sulfonic acid colorimetry (Nicholas and Nason, 1957). Ammonium (NH_4-N) was measured by the indophenol blue colorimetry (Dorich and Nelson, 1983). Available phosphorus (AP) was extracted using $HCLO_4$-H_2SO_4 and measured by the ascorbic acid/molybdate reagent blue color method. The composition of soil particles was analyzed by a laser particle size analyzer (Mastersize 2000, Malvern Instruments Ltd., UK). The soil particle-size can be divided into three grades: clay (0–2 μm), silt (2–50 μm), and sand (50–2000 μm). Soil water content (SWC) was determined by drying 10 g of soil at 105 ℃ for 24 h. Soil pH was measured in a 2.5:1 water/soil suspension using a pH meter (FE20, Mettler Toledo, Shanghai, China). The thickness of the BSCs layer was measured using a digital Vernier caliper, and crusts were separated from the soil with water through a 2 mm sieve and dried at 65 ℃ for 24 h for the measurement of BSCs biomass. According to the manufacturer's protocols, soil sucrose (SC), urease (UE), nitrate reductase (NR), and alkaline phosphatase (ALP) were measured using soil enzyme assay kits (SinoBestBio, China) (Cheng et al., 2021).

2.4. DNA extraction, PCR amplification, and sequencing

Soil microbial DNA was extracted from 0.5 g freeze-dried soil of each sample using the Fast DNA® Spin Kit for Soil (MP Biomedicals, OH, USA) following the manufacturer's directions. The final DNA concentration and purification were determined using a NanoDrop 2000

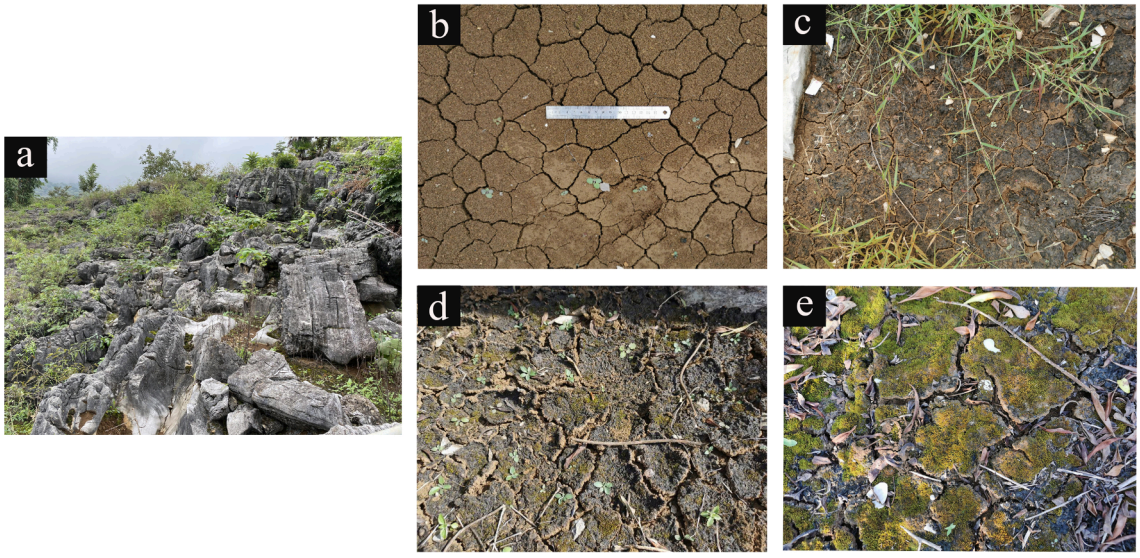

Fig. 1. Degraded karst landscapes (a) in huajiang karst gorge, and different types of biocrusts: bare soils (b), cyanobacterial crusts (c), moss-cyanobacteria mixed crusts (d), moss crusts (e).

UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA), and extracted DNA quality was checked by 1% agarose gel electrophoresis. To consider the composition and structure of soil bacterial and fungal communities, we amplified the V3–V4 place of the bacterial 16S rRNA gene with primers 338F/806R (Huang et al., 2019) and the ITS1 place of the fungal ITS rRNA gene with primers ITS1F/ITS2R (Yao et al., 2017), respectively, on Illumina MiSeq platform (Illumina, San Diego, USA) of the Major Bio-Pharm Technology Co. Ltd. (Shanghai, China). The OTU abundance information of each sample was normalized according to the sequence reading standard corresponding to the piece with the smallest sequence (27,363 reads for 16S rRNA gene and 67,866 reads for ITS rRNA gene). For further details on PCR amplification, Illumina MiSeq sequencing, and pyrosequencing statistics processing, refer to the description in Supplementary Materials.

2.5. Network construction and analyses

The co-occurrence network is constructed and analyzed through the molecular ecological network pipeline (http://ieg2.ou.edu/MENA/mainly.cgi). More information about pipeline theories and properties can be found in the reference (Deng et al., 2012). The threshold value of 0.92 was used to construct soil bacterial and fungal networks in order to compare the networks under the same conditions. Two networks of soil bacterial and fungal communities from BSCs layer samples and subsurface samples were built, respectively, with the default settings except the majority's setting (only keeping genera with 8 in total 12 samples). The networks were visualized in Gephi (version 0.9.2; https://gephi.org/). The node size was proportional to the node degree, and the node color represented the microbial phylum. The edge between each pair of nodes represented a positive (in red) or a negative (in green) Pearson correlation. Network topological properties, including average degree, average clustering coefficient, average path distance, connectivity, and modularity, were calculated based on Deng et al. (2012).

Mantel test analysis explored the association between environmental factors and microbial communities by the software QIIME. To further investigate the effects of environmental variables on bacterial and fungal networks, we used two-factor network analysis; Spearman correlation coefficients ($P < 0.05$ and $|r| > 0.5$) between soil microbial communities and environmental factors were calculated using Networkx software. The networks were constructed in Gephi.

2.6. Statistical analyses

Soil physicochemical characteristics and enzyme activities between BSCs types and bare soils were compared using a one-way ANOVA test followed by a Tukey post-hoc test (SPSS 22.0, Chicago, IL, USA). Non-parametric Wilcoxon signed-rank test was used to compare soil physicochemical characteristics and enzyme activities between BSCs layer and subsurface soil (SPSS 22.0, Chicago, IL, USA). Chao1, Shannon, and Sobs indexes were calculated by software Mothur (Schloss et al., 2009). Non-metric multidimensional scaling (NMDS) plots and analysis of similarity (ANOSIM) test with 999 permutations based on Bray Curtis distance metric in R with the Vegan package were used to visualize and assess the effects of BSCs types, soil depths on soil microbial community composition (Zhou et al., 2017). Linear discriminant analysis effect size (LEfSe) was used to investigate potential biomarkers (across five taxonomic levels, from phylum to genus for soil bacterial and fungal communities) within microbiomes specifically enriched in different types of BSCs and bare soils, and soil layers based on $P < 0.05$ and LDA score > 2.0 (Segata et al., 2011).

2.7. Accession numbers

Sequence reads of bacteria and fungi generated in this study were deposited in the SRA in the NCBI database underneath the accession numbers PRJNA716424 and PRJNA716438, respectively.

3. Results

3.1. Physicochemical characteristics and enzyme activities

The dominant species of three types of BSCs (cyanobacterial crusts, mixed crusts and moss crusts) are respectively *Microcoleus vaginatus*, *Lyngbya* sp. - *Trichostomum brachydontium*, and *Trichostomum brachydontium* (Table S1). Thickness and biomass of BSCs increase significantly along with the successional series.

Compared with the other two types of BSCs and bare soil, moss crusts improved soil physical and chemical properties and enzyme activity (Table 1). TN in moss crusts was 55.5%, 83.0%, and 102.8% greater than mixed crusts, cyanobacterial crusts, and bare soils, respectively. A similar trend was observed for TP, SOC, and NH_4-N: The contents of TP and NH_4-N in moss crusts were the highest compared with other soil cover types; SOC in moss crusts was one times higher than that in

Table 1
Physicochemical characterization and enzyme activities in different types of biocrusts.

	Bare soils (control)	Cyanobacterial crusts	Mixed crusts	Moss crusts
TN ($g \cdot kg^{-1}$)	2.50 ± 0.29a	2.77 ± 0.48a	3.26 ± 0.55a	5.07 ± 0.75b
TP ($g \cdot kg^{-1}$)	1.00 ± 0.25a	1.04 ± 0.11a	1.06 ± 0.11a	1.83 ± 0.19b
SOC ($g \cdot kg^{-1}$)	57.44 ± 11.52b	29.38 ± 4.25a	43.01 ± 7.25ab	58.77 ± 5.36b
NH_4-N ($mg \cdot kg^{-1}$)	0.21 ± 0.08a	0.73 ± 0.06a	1.00 ± 0.17ab	1.83 ± 0.78b
NO_3-N ($mg \cdot kg^{-1}$)	1.10 ± 0.43a	0.78 ± 0.22a	0.89 ± 0.32a	1.40 ± 0.57a
AP ($mg \cdot kg^{-1}$)	51.69 ± 4.88a	31.88 ± 5.20a	35.27 ± 8.40a	42.38 ± 13.59a
pH	7.83 ± 0.37a	7.82 ± 0.35a	7.92 ± 0.09a	7.82 ± 0.19a
SWC (%)	11.76 ± 3.03a	5.50 ± 2.55a	6.30 ± 2.22a	7.38 ± 2.69a
Clay (%)	6.64 ± 0.68a	8.66 ± 0.74a	8.22 ± 0.81a	11.41 ± 1.40b
Silt (%)	80.42 ± 0.66a	85.09 ± 1.15b	84.61 ± 0.73b	84.67 ± 1.18b
Sand (%)	12.94 ± 0.85c	6.25 ± 1.17b	7.17 ± 0.89b	3.92 ± 0.49a
UE ($U \cdot g^{-1}$)	319.10 ± 63.09a	411.32 ± 129.96a	595.84 ± 146.01ab	748.68 ± 191.04b
SC ($U \cdot g^{-1}$)	43.66 ± 1.89a	49.43 ± 0.64b	47.75 ± 1.16b	50.29 ± 0.65b
NR ($U \cdot g^{-1}$)	3.76 ± 0.48a	16.66 ± 1.02b	4.83 ± 1.08a	12.06 ± 4.23b
ALP ($U \cdot g^{-1}$)	10.55 ± 0.27a	10.02 ± 0.51a	10.68 ± 0.44a	10.08 ± 0.40a

*Values represent means ± standard errors (n = 4). Different lowercase letters indicate significant differences between different types of biocrust and bare soil ($P < 0.05$).
*Abbreviations: TN, total nitrogen; TP, total phosphorus; SOC, soil organic carbon; NH_4-N, ammonium, NO_3-N, nitrate; SWC, soil water content; AP, available phosphorus; UE, urease; SC, sucrase; NR, nitrate reductase; ALP, alkaline phosphatase.

cyanobacterial crusts. Sand content in bare soils was 0.8, 1.1, and 2.3 times greater than mixed crusts, cyanobacterial crusts, and moss crusts, respectively. Clay content in moss crusts was significantly higher than other soil cover types. The urease content in moss crusts was 0.8 and 1.3 times higher than that in cyanobacterial crusts and bare soils, respectively. The content of sucrase in bare soils was significantly lower than other types of crusts. The cyanobacterial crusts had the highest nitrate reductase content compared with different types of BSCs and bare soils. There was no difference in AP, NO_3-N, pH, Alkaline phosphatase, and water content between the three types of BSCs and bare soils.

Most soil quality indicators and enzyme activities in the BSCs layer were higher than in the subsurface of BSCs (Table 2). In detail, BSCs significantly increased the contents of TN, NH_4-N, and Sand by 7%, 55%, and 30%, respectively, compared to the subsurface of BSCs. Similarly, urease, sucrase, and nitrate reductase activity in the BSCs layer was 138%, 9%, and 159% higher than that in the subsurface of BSCs, respectively. Conversely, the quantity of SWC in the subsurface of BSCs was 117% higher than that in the BSCs layer. Other soil factors had no significant effect on soil layers.

3.2. Species diversity and composition of soil microbial community

The richness indexes of the bacterial community in moss crusts and mixed crusts were significantly higher than those in bare soils (Fig. 2a, e). Still, there was no difference in diversity index between different soil cover types (Fig. 2c). There was no difference in fungal richness and diversity indices between different crusts types and bare soils (Fig. 2b, d, f).

Table 2
Physicochemical characterization and enzyme activities between the biocrusts layer and subsurface.

	Biocrusts layer	Subsurface
TN ($g \cdot kg^{-1}$)	3.70 ± 1.16b	3.43 ± 0.96a
TP ($g \cdot kg^{-1}$)	1.27 ± 0.36a	1.19 ± 0.25a
SOC ($g \cdot kg^{-1}$)	43.72 ± 13.31a	49.52 ± 20.53a
NH_4-N ($mg \cdot kg^{-1}$)	1.19 ± 0.69b	0.77 ± 0.59a
NO_3-N ($mg \cdot kg^{-1}$)	1.03 ± 0.48a	1.53 ± 0.85a
AP ($mg \cdot kg^{-1}$)	36.51 ± 10.64a	44.53 ± 10.30a
pH	7.85 ± 0.24a	7.98 ± 0.17a
SWC (%)	0.06 ± 0.03a	0.13 ± 0.02b
Clay (%)	9.43 ± 1.74a	8.80 ± 1.49a
Silt (%)	84.79 ± 1.06a	84.89 ± 1.67a
Sand (%)	5.78 ± 1.63a	6.31 ± 1.89a
UE ($U \cdot g^{-1}$)	585.28 ± 209.59b	246.10 ± 118.19a
SC ($U \cdot g^{-1}$)	49.16 ± 1.35b	45.02 ± 2.67a
NR ($U \cdot g^{-1}$)	11.18 ± 5.52b	4.31 ± 1.91a
ALP ($U \cdot g^{-1}$)	10.26 ± 0.54a	10.40 ± 0.44a

*Values represent means ± standard errors (n = 12). Different lowercase letters indicate significant differences between different soil layers ($P < 0.05$).

For all the three types of BSCs, bacterial community richness index of BSCs layer was significantly higher than subsurface soil (Fig. 3a, e). The fungal community richness index showed the same trend (Fig. 3b, f). However, there was no difference in bacterial or fungal community diversity in the BSCs layer compared with the subsurface soil (Fig. 3c, d).

The bacterial and fungal community structure was not significantly affected by soil cover type (Fig. 4). Compared with the subsurface soils, the species composition of both bacterial and fungal communities in the BSCs layer was quite different (Fig. 5).

3.3. Microbial communities with statistically significant differences

The LEfSe analysis revealed that 49 bacterial biomarkers and 13 fungal biomarkers were sensitive to soil cover type (Fig. S1). *Clostridia* (Firmicutes) and *Cyanobacteriia* (Cyanobacteria) had the most significant effect scores among bacteria (LDA scores = 2.7) (Fig. S1), and *Sordariomycetes* (Ascomycota) (LDA score = 5.0) had the largest effect scores among fungi in bare soils (Fig. S1). *Actinobacteria* (Actinobacteriota) had the most significant effect scores among bacteria (LDA scores = 2.1) (Fig. S1), and *Leotiomycetes* (Ascomycota) (LDA score = 4.2) had the largest effect scores among fungi in cyanobacterial crusts (Fig. S1). *Anaerolineae* (Chloroflexi) had the largest effect scores among bacteria (LDA scores = 2.5) (Fig. S1), and there was no significant fungal biomarker detected in mixed crusts (Fig. S1). *Anaerolineae* (Chloroflexi) had the most significant effect scores among bacteria (LDA scores = 3.8) (Fig. S1), and *Eurotiomycetes* (Ascomycota) (LDA score = 3.5) had the largest effect scores among fungi in moss crusts (Fig. S1).

The LEfSe analysis revealed 238 bacterial biomarkers and 51 fungal biomarkers sensitive to soil layers (Fig. S2). For bacteria, there were 141 biomarkers in the BSCs layer and 97 biomarkers in the subsurface soils. 78 biomarkers were associated with Proteobacteria, and 30 biomarkers with Bacteroidota in the BSCs layer; 23 biomarkers were associated with Acidobacteria, 20 with Firmicutes and 12 with Actinobacteria in the subsurface soils. For fungi, there were 31 biomarkers in the BSCs layer and 20 in the subsurface soils. 16 biomarkers were related to Ascomycota, and 9 to Basidiomycota in the BSCs layer; 12 biomarkers associated with Ascomycota were found in the subsurface soils.

3.4. Network properties in soil layers and the relationship between network structure and environmental factors

Network analysis revealed that the BSCs significantly altered the topological network of soil bacteria and fungi (Fig. 6 and Table 3). The bacterial and fungal networks of the BSCs layer were much larger (e.g., more nodes and edges) and more complex (e.g., higher average degree,

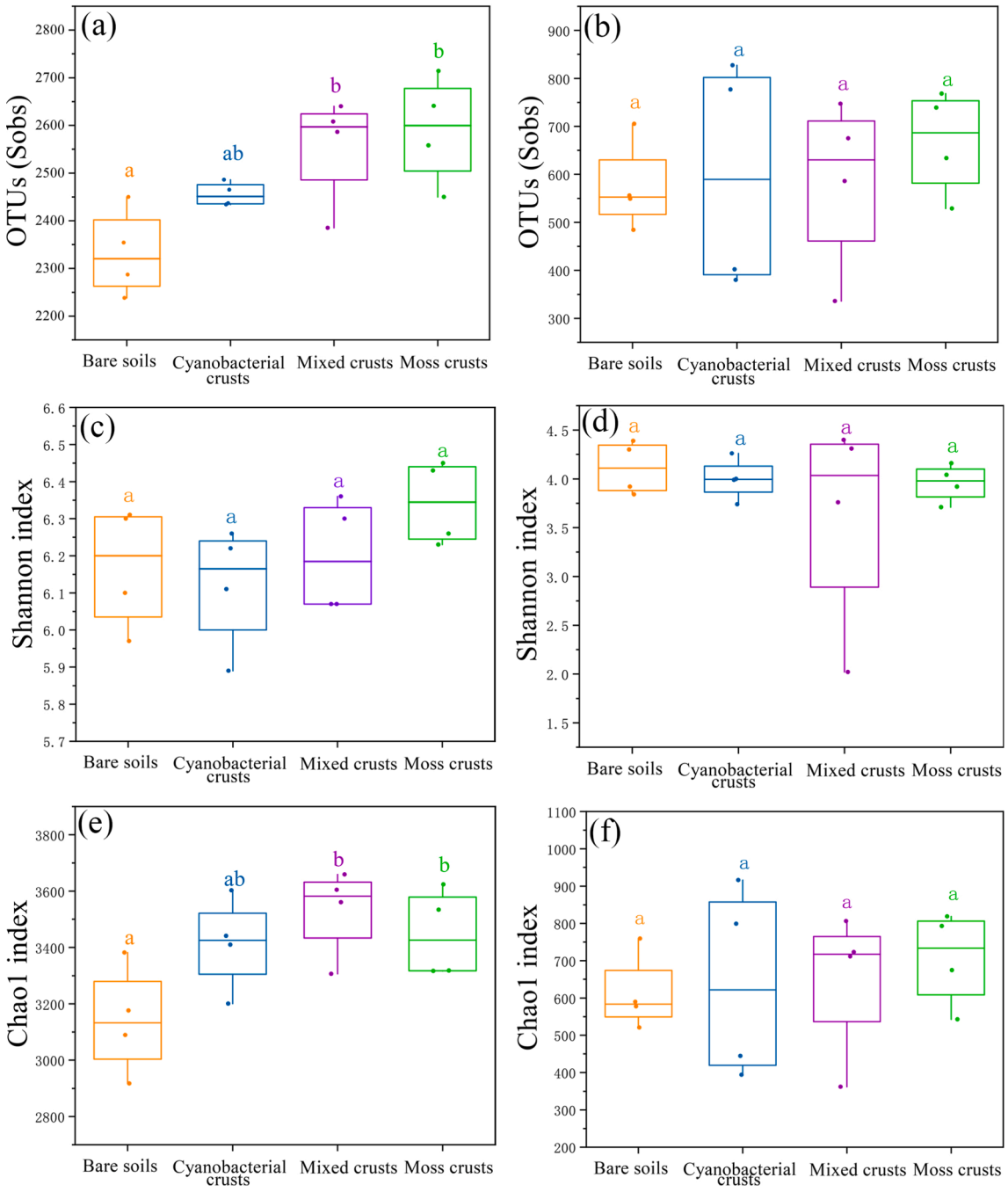

Fig. 2. Sobs, Shannon, and Chao1 indices of soil bacterial (a, c, e) and fungal communities (b, d, f) in different crust types (n = 4). Welch's *t*-test significant differences ($P < 0.05$) are indicated by different letters.

average clustering coefficient, and connectivity) than those of the subsurface soil (Table 3). In addition, the positive edges of bacteria and fungi were much higher than the negative edges in both the BSCs layer and subsurface soil (Table 3). Proteobacteria and Ascomycota were the most significant contributors to nodes and correlations across BSCs and subsurface soils for bacteria and fungi, respectively (Fig. 6).

Mantel test analysis revealed that bacterial community in all the samples was closely related to TN ($P = 0.002$), TP ($P = 0.047$) and urease ($P = 0.005$); fungal community was closely related to TN ($P = 0.040$) and urease ($P = 0.015$) (Table S2). For network structure in all the samples, urease, TN, SWC, NH_4-N, Clay, alkaline phosphatase, and nitrate reductase significantly influenced both bacterial and fungal networks. Urease recorded the highest node connectivity in the bacterial (node degree = 11; Fig. 7a) and fungal networks (node degree = 7; Fig. 7b).

4. Discussion

4.1. Effects of biocrust types and soil layers on soil properties

The nutrient content and enzyme activity of moss crusts differed from other cover types, and there were no significant differences in other types, which partially deviated from our first hypothesis. Late-successional BSCs (e.g., moss crusts) are more photosynthetically efficient than early-successional ones (e.g., cyanobacterial crusts) (Lan et al., 2012a; Lan et al., 2012b; Lan et al., 2019). Researchers have found

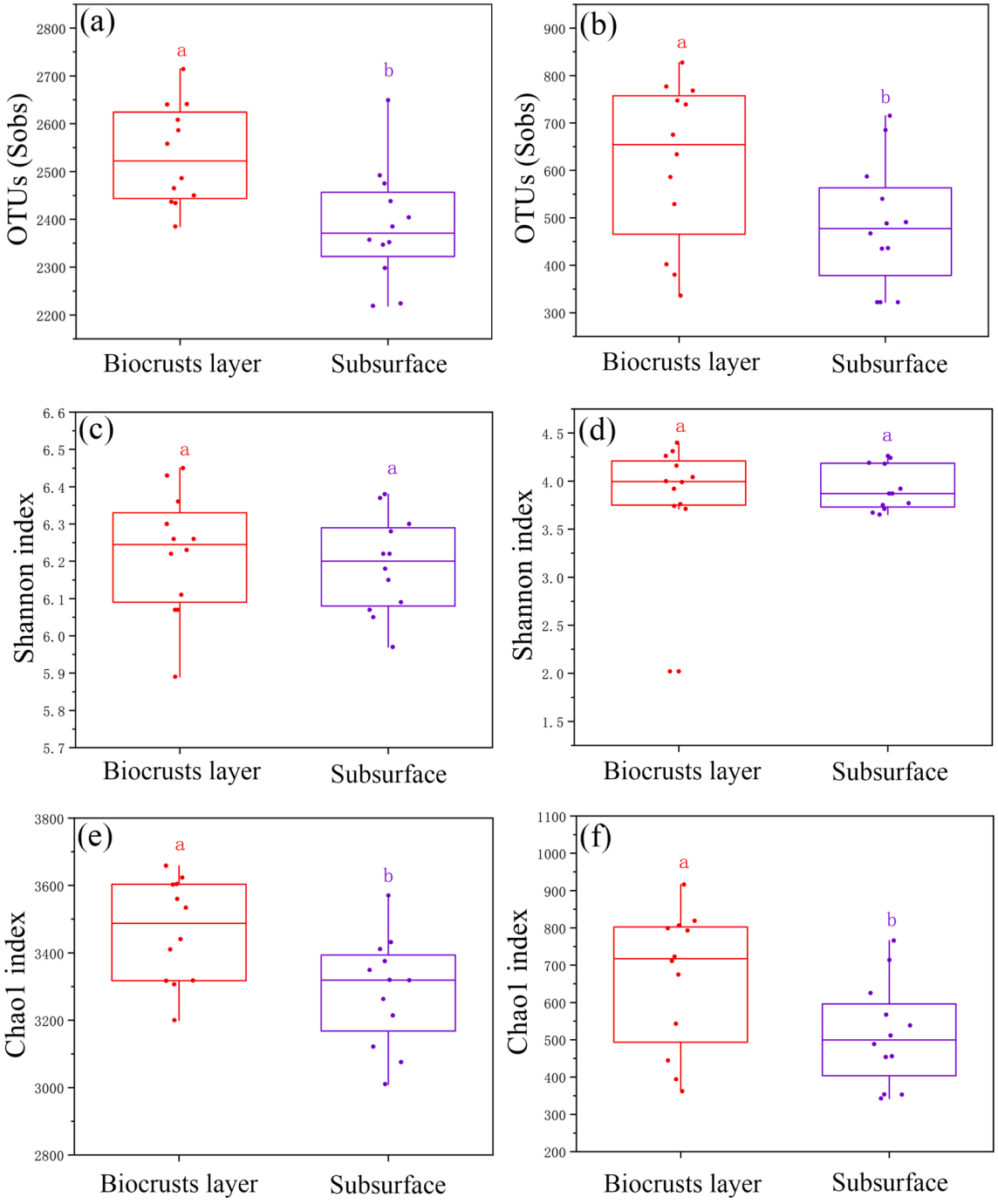

Fig. 3. Sobs, Shannon, and Chao1 indices of soil bacterial (a, c, e) and fungal communities (b, d, f) in different soil layers (n = 12). Welch's *t*-test significant differences ($P < 0.05$) are indicated by different letters.

that Late-successional BSCs significantly increase the organic matter content, nitrogenase activity, and polysaccharides than those in early-successional crusts in arid and semi-arid ecosystems (Housman et al., 2006; Mager and Thomas, 2011; Yu et al., 2012). The increased organic matter and polysaccharides provide abundant carbon sources for microorganisms and invertase, which can increase microbial biomass and enzyme activity (Katsalirou et al., 2010; Zhou et al., 2012; Zhang et al., 2015). The present study showed that soil nutrients (TN, TP, NH_4-N) and enzyme activities (urease, sucrose) were significantly higher in moss crusts than other cover types (Table 1). It was suggested that moss crusts have a substantial nutrient retention effect in degraded karst ecosystems in subtropical humid climate zone. In the present study, the BSCs accumulated fine particles (clay and silt), especially moss crusts, may because of relatively excellent dust capture and fixation capacity of moss crusts (Williams et al., 2012; Williams et al., 2013). The fine particles effectively promote the formation of complex agglomerate structure, better soil structure and increase soil water holding capacity (Felde et al., 2014; Xiao and Veste, 2017). Therefore, moss crusts can improve soil structure of the few centimeters of most top soil in degraded karst ecosystems.

Urease and nitrate reductase (related to the N cycle), and sucrose (related to the C cycle) were significantly higher in BSCs layer than in the subsurface (Table 2). The above changes in enzyme activity corresponded with our results that the contents of TN and NH_4-N were

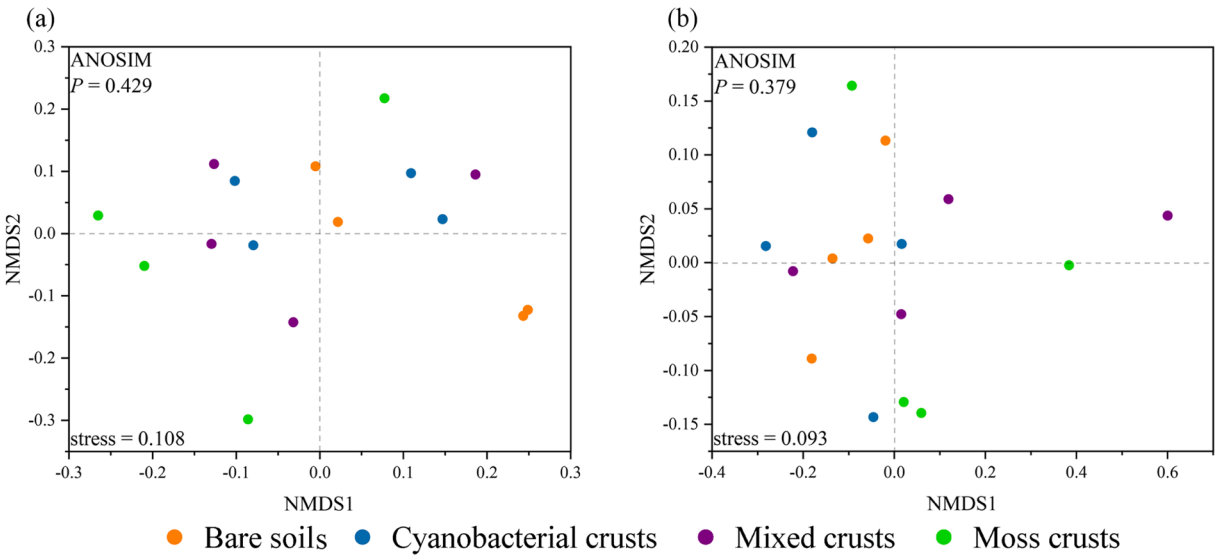

Fig. 4. Non-metric multidimensional scaling (NMDS) ordinations and analysis of similarity (ANOSIM) test showing the difference of soil bacterial (a) and fungal (b) community structure in different crust types and bare soils (n = 4).

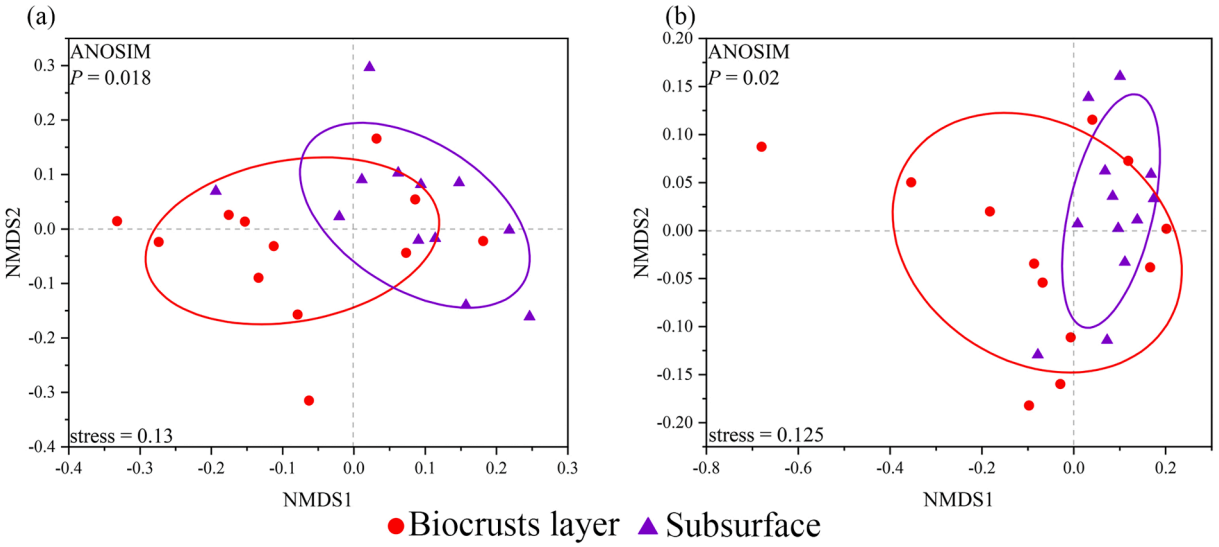

Fig. 5. Non-metric multidimensional scaling (NMDS) ordinations and analysis of similarity (ANOSIM) test showing the difference of soil bacterial (a) and fungal (b) community structure in different soil layers (n = 12).

significantly higher in the BSCs layer than in the subsurface soils. Soil fertility improvement promotes the microbial activity in BSCs (Xiao and Veste, 2017), resulting in a significant increase in extracellular enzyme activity associated with N cycle. There were no significant change of TP, AP, and alkaline phosphatase in BSCs layer compared with the subsurface soils. It indicates that BSCs in degraded karst areas may not affect the availability of inorganic phosphorus.

4.2. Effects of biocrust types and soil layers on species diversity and composition of microbial community

The α-diversity index of bacteria and fungi did not increase with the succession of BSCs. Their community structure had no significant difference between different cover types. This doesn't fit our first hypothesis. Soil bacterial and fungal communities were significantly associated with TN and urease (Table S2). Nitrogen is an essential nutrient for microbial growth, and changes in its availability can affect microbial activity (Liu et al., 2018). Urease converts organic nitrogen in the soil into inorganic nitrogen used by plants and microorganisms (Fiona et al., 2013). In the present study, the TN, NH_4-N content, and urease activity of BSCs in the late-successional stage were higher than those in the early stage BSCs (Table 1). Comparatively limited nutrients at early successional BSCs stages constrain microbial abundance (Maier et al., 2018). Therefore, the bacterial communities in mixed crusts and moss crusts had the highest species richness in the study area (Fig. 2a, e). Crust type did not affect fungal species richness or diversity (Fig. 2c) in this study. The reason for this may be that bacteria utilize nitrogen to a greater extent than fungi (Reay et al., 2019). The species composition of bacterial or fungal community also did not differ between different types of crusts. In addition to the influence of BSCs, soil microbial communities are significantly influenced by various factors such as geographic location, soil properties, topography, microclimate, and the surrounding environment (Wang et al., 2020; Pombubpa et al., 2020). Therefore, a more detailed understanding of the microbial community of BSCs requires a combination of multiple factors to be considered.

The microbial richness of bacteria and fungi in the BSCs layer were

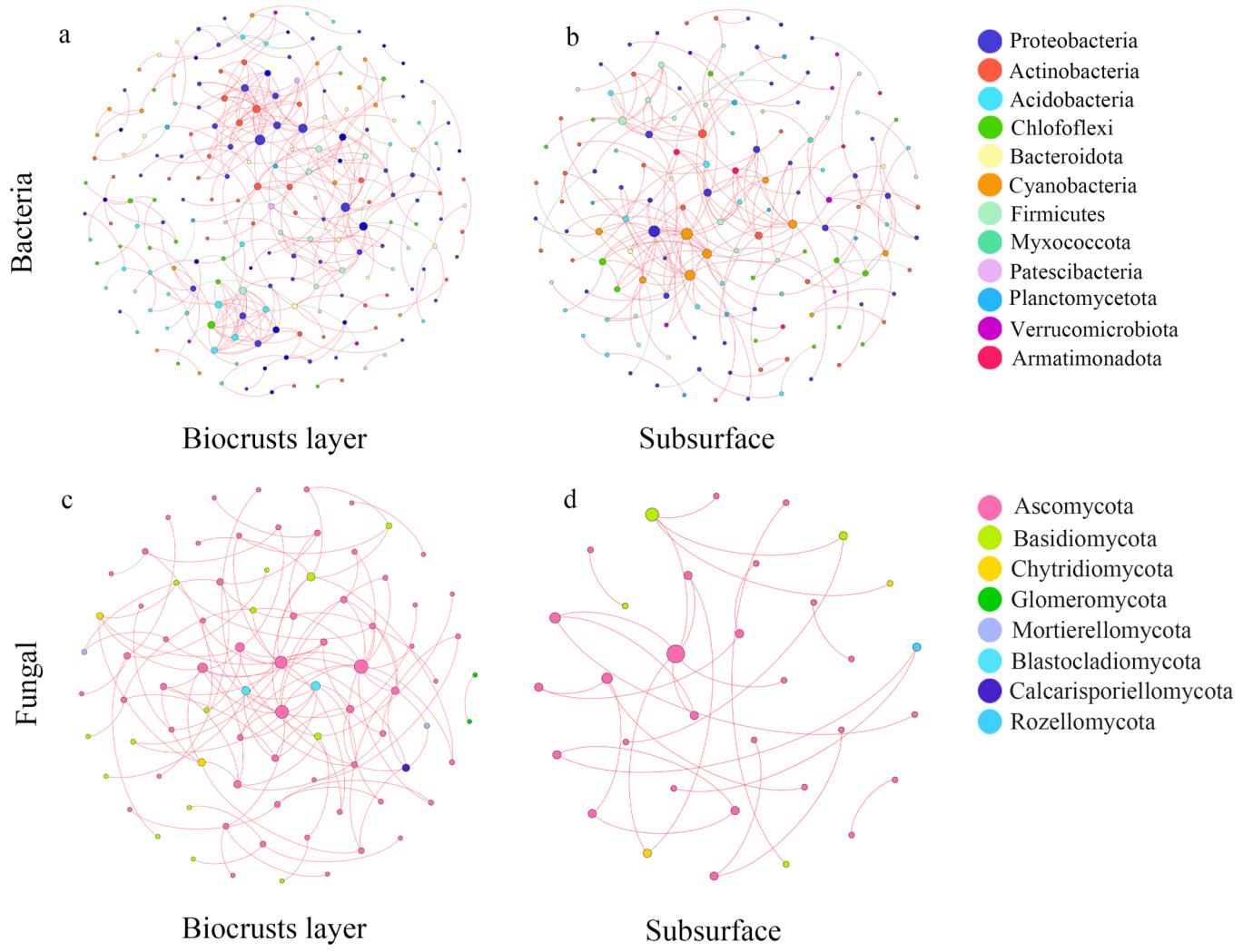

Fig. 6. Co-occurrence networks of soil bacterial and fungal communities in different soil layers. The nodes are colored by the phylum level. The size of each node is proportional to the node degree. The link between each pair of nodes represents positive (red) and negative (green) correlations. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Network topological properties between the biocrusts layer and subsurface.

Network properties	Bacterial networks		Fungal networks	
	Biocrusts layer	Subsurface	Biocrusts layer	Subsurface
Total nodes	206	147	82	33
Total edges	327	199	118	28
Positive edges	306	191	117	28
Negative edges	21	8	1	0
Similarity threshold	0.92	0.92	0.92	0.92
Average degree	3.175	2.707	2.878	1.697
Average clustering coefficient	0.214	0.188	0.153	0.105
Average path distance	6.015	4.591	4.541	2.695
Connectivity	0.563	0.513	0.712	0.223
Modularity	0.779	0.757	0.651	0.732

significantly higher than those in the subsurface. Species composition of microbial community was different in different soil layers. This fits our second hypothesis. As shown in the results of this study, the species richness of either bacteria (Fig. 3a, e) or fungi (Fig. 3b, f) was significantly higher in the BSCs layer than in the subsurface, and the species composition of bacterial (Fig. 5a) and fungal community (Fig. 5b) differed significantly between soil layers. Based on the results of the NMDS analysis in Fig. 4 and Fig. 5, it was shown that the most significant differences in bacterial and fungal community structure in the degraded karst ecosystems were at the vertical profile (between BSCs layer and subsurface).

The differences in soil properties may be the main reason for the differences in microbial communities. Cryptogams (i.e., mosses, lichens, cyanobacteria, and green algae) in the BSCs accumulate soil nutrients and fertility by trapping deposited nitrogen and dust or N_2 fixation (Housman et al., 2006; Chamizo et al., 2012). The increase in soil nutrients provides material and energy for microorganisms and increases the microbial population (Barger et al., 2016). In the present study, several species in the phylum Proteobacteria in the BSCs layer were higher than in the underlying soils (Fig. S2). Most nitrogen-fixing and ammonia-oxidizing bacteria belong to Proteobacteria (Li et al., 2020), implying that Proteobacteria plays an essential role in the nitrogen cycle of BSCs layer. Soil urease, nitrate reductase, and sucrase are crucial to soil C and N cycling, respectively (Ge et al., 2010). The results of this study showed that the activities of urease, sucrase, and nitrate reductase in the BSCs layer were significantly higher than those in the subsurface soils (Table 2), which is caused by the differences in nutrient and physical characteristics in different soil layers (Zhang et al., 2009). Therefore, it indicates that BSCs can accelerate soil carbon and nitrogen

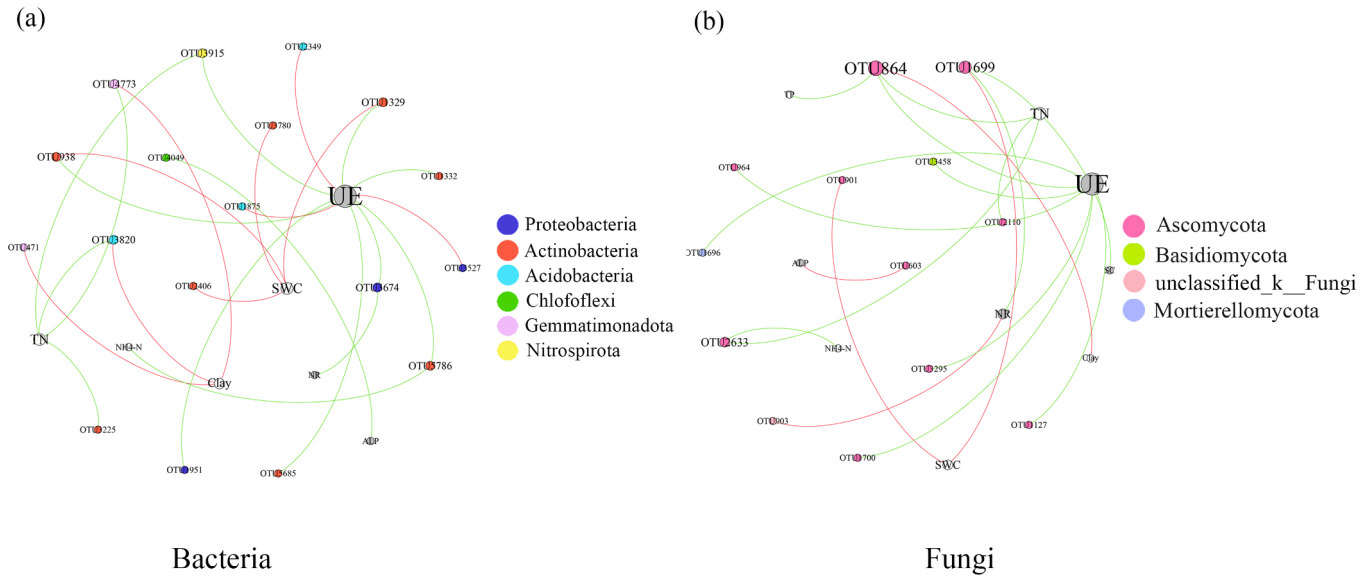

Fig. 7. Networks between environmental variables and their directly connected (Spearman's $|r| > 0.5$, P - value < 0.05) nodes from all the samples. (a) Bacterial network. (b) Fungal network. Node size is proportional to node connectivity. Node color represents various phylogenetic phyla. Red lines indicate positive interactions, and green lines indicate negative interactions. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

turnover compared to the underlying soil.

4.3. Microbial co-occurrence network in the biocrusts layer and subsurface

Networks of soil microbial communities are critical for revealing connections among network members and providing insights into microbial responses to environmental degradation (Banerjee et al., 2019). The network of bacterial communities in the BSCs layer and subsurface in this study respectively had a more complex structure (higher connectivity and clustering coefficients) than the fungal communities' network. The reason for this may be that bacteria usually grow faster than fungi (Rousk and Bååth, 2011). The presence of nutrients will stimulate the growth of bacteria, thereby increasing competition for nutrients. In this study, the highest proportions of bacterial and fungal networks in the BSCs layer and subsurface were respectively in Proteobacteria and Ascomycota, indicating that these two phyla play an essential role in the network structure. That might because a variety of genera in the phylum Proteobacteria can fix carbon (Li et al.. 2020), while the phylum Ascomycota is associated with accelerated soil C and N cycling and soil organic C content (Liu et al., 2020). Positive and negative links are fundamental properties in networks, indicating positive and negative interactions respectively. Positive interactions may reflect cooperation between species or overlapping ecological niches, and negative interactions may reflect competition between species or separation of ecological niches (Deng et al., 2016; Ghoul and Mitri, 2016). In this study, positive linkages were much higher than negative linkages for bacteria and fungi networks in either BSCs or subsurface, potentially suggesting that the relationships between microbial communities are based more on cooperation than competition (Newman, 2006). Furthermore, positive linkages may expand or create ecological niche spaces for interacting species (Freilich et al., 2018). The total nodes, total number of edges, and positive links of bacteria and fungi in the BSCs layer are also higher than in the subsurface, indicating that the microbial ecological network in the BSCs layer is more complex than the subsurface soils. The stability of the microbial community in the BSCs layer is stronger than that in the subsoil, which is conducive to the stability of the ecosystem (van der Heijden et al., 2010). The presence of BSCs has a positive effect on improving soil health in degraded karst ecosystems with subtropical humid climate.

5. Conclusions

Our results showed that BSCs at the late-successional stage (moss crusts) could significantly increase soil nutrient content and enzyme activity. Compared to the subsurface soil layer, BSCs layers had significantly higher soil nutrient content and enzyme activity. The diversity and network structure of microbial communities were mainly influenced by soil variables, especially TN and urease, indicating BSCs could promote the nitrogen cycle process, improve soil nutrients, and thus increase the number and diversity of microorganisms in degraded karst ecosystems.

With the succession of the BSCs, the richness of bacteria was increased, the fungal community was not affected, while no change in species diversity or composition of bacterial or fungal community was found. The bacterial and fungal richness of the BSCs layer were higher than subsurface soils, and species composition was significantly different between two layers. Besides, the network structure of bacteria and fungi in the BSCs layers was more complex than that in the underlying soil. In conclusion, the BSCs layer especially moss crusts are important for soil nutrient accumulation and microbial genetic resources. This study improves our understanding of the ecological function of BSCs in subtropical humid karst regions and provide a valuable theoretical basis for the effective management and conservation of degraded karst ecosystems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.catena.2022.106057.

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