

The microbiome in lichen and moss biocrust differentially contribute to carbon and nitrogen cycles in arid ecosystems

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Research Article

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Abstract

Biological soil crusts (biocrusts) are the main cover in arid and semi-arid regions worldwide. Microbes are an essential component in biocrusts and mediate many critical biochemical processes. However, little is known about the taxonomic composition and the functions of microbiomes in lichen and moss biocrusts. The shotgun metagenomic sequencing was used to compare two biocrust types (lichen-dominated and moss-dominated) in this study to reveal the microbial genes and metabolic pathways involved in carbon and nitrogen cycling. The results showed that the most abundant phylum in the two types of biocrust microbiomes was Actinobacteria, but their composition differed for the other abundant phyla (Proteobacteria, Cyanobacteria, Bacteroidetes, and Acidobacteria). The relative abundance of carbohydrate-active enzymes and enzymes associated with carbon and nitrogen metabolism significantly differed in both biocrust types. There is a clear dominance of respiration pathways over carbon fixation pathways in the microbial communities of both biocrust types. The genes encoding carbon monoxide dehydrogenase were more abundant than those encoding ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCo) involved in the C fixation. N₂ fixation had low metabolic pathways diversity in biocrust microbiomes, while nitrogen reduction dominated. The assimilatory nitrate reduction genes showed higher relative abundance in lichen biocrusts, while the dissimilatory nitrate reduction genes showed higher relative abundance in moss biocrusts. Dissolved organic carbon and soil organic carbon were considered as critical environmental factors affecting biocrust type. Our study suggests that biocrust type has significant effects on microbial biodiversity and biogeochemical cycling, providing new insights into biocrust microbiome responses to climate change at the gene level.

Introduction

Drylands cover approximately 40% of the terrestrial surface on the Earth [1], and up to 40% of the soil surface in drylands is covered with biocrusts that play critical roles in a range of ecological processes [2–4]. Biocrusts provide a habitat for soil organisms to become a complex mosaic layer and accelerate the development and succession of soil ecosystems [5–8]. Bryophytes colonizing the soil surface in the interspaces between plants reduce the threat of degradation and desertification in drylands due to the growing human population and global change [2, 7].

Many studies have confirmed that biocrusts are an essential participant in the carbon and nitrogen cycle and a vital source of soil organic carbon and nitrogen in dryland ecosystems [9, 10]. Globally, higher carbon and nitrogen fixation by biocrusts in different ecosystems. It is estimated that the global annual net C uptake by biocrusts is up to 3.6 Pg a⁻¹, with 1 Pg a⁻¹ fixed by biocrusts in arid and semi-arid regions [11]. Biocrusts may contribute approximately 27–53% of the biological terrestrial N fixation globally [12]. Nonetheless, they are still the main contributor to carbon and nitrogen emissions [8, 13, 14]. Different organisms, such as cyanobacteria, lichen, and moss, support different biocrust types, which often result in different successional stages [5, 6, 15, 16]. The capacity of different biocrusts to fix and emit nutrients varies greatly and depends on the succession stage. Generally, early succession has higher carbon

fixation efficiency [3, 17, 18], and later succession has higher nitrogen fixation, carbon, and nitrogen emission efficiency of biocrusts [8, 19–23].

High-throughput technologies have shown that microbes are the most abundant organisms in biocrusts [24], performing numerous functions in dryland ecosystems [25, 26]. Studies have shown that the composition and structure of microbial communities differ between biocrust types and successional stages, resulting in different carbon and nitrogen function [27–29], which was more likely to be the result of the functional characteristics contained in the microbiome. It has been suggested that some microbial processes may be influenced by the abundance and diversity of relevant functional genes, which can be regarded as parameters for assessing the potential function of microbial communities [30]. The metagenome profiles of the carbon- and nitrogen-related metabolic pathways differed between bacterial and moss biocrusts in the Tengger Desert, China [28], and the bacteria and fungi differentially contributed to carbon and nitrogen cycles during biocrust succession based on GeoChip 5.0 analysis [31]. However, most studies have focused on the same study site, so there is little understanding of microbial carbon and nitrogen function in other dryland ecosystems. Biocrust type, microbial composition, and ecological function vary significantly between sites due to climate and spatial heterogeneity. Therefore, it is necessary to explore soil microbiomes involved in biogeochemical cycles in biocrusts of diverse dryland ecosystems to provide a theoretical basis for regulating source–sink relationships [32].

This study aimed to determine differences in microbiome genetic diversity involved in C and N metabolism between two distinct biocrust types widely distributed in the southeastern Mu Us Sandland using shotgun metagenomic sequencing. We hypothesized that the two biocrust types differ in nutrient transformation processes due to different microbiome compositions and structures. Our research questions were: (1) Does the soil microbiome reflect the different biocrust stages? (2) Which microbial groups or metabolic potentials present significant differences in two biocrust types? (3) Which key chemical factors drive the community structure of the biocrust microbiome? This study offers new insights into microbial regulation mechanisms for biogeochemical cycling in two biocrust types.

Materials And Methods

Study site

The research was conducted in October 2019 in the Yuyang District, Yulin City, in the northern part of Shaanxi Province, China (109°36' to 109°36' E, 38°32' to 38°32' N) (Fig. 1A). The site is located in the southeast of the Mu Us Sandland, where the mean annual temperature (MAT) is 8.1°C, and mean annual precipitation (MAP) is 287 mm, of which 62% precipitation between July and September [33]. The soil texture is sandy in the 0–1 m profile, with an average bulk density of 1.5 g cm⁻³ [34]. Biocrusts commonly cover open areas between shrubs (Fig. 1B). They are dominated by lichen crust with an uneven surface, dark in color, and about 9 mm thick following the rainy season (Fig. 1C) and moss crusts with a rough surface, about 12 mm thick, dark in color when dry and green when wet (Fig. 1D).

Biocrust sampling

Six representative plots (20 m × 20 m) were established for lichen and moss biocrusts (Table S1). The distance between plots with the same biocrust type was > 1,000 m. Three representative subplots (5 m × 5 m, at least 10 m apart) were selected within each plot. The plant litter on the biocrusts was removed before sample collection. Five biocrusts samples were randomly collected from each subplot using a sterile cutting ring (internal diameter 9.0 cm, depth 1 cm) and combined. Three composite replicates of two biocrust types were collected from the six plots. The samples were sieved (2.0 mm mesh) to remove visible roots and stones and divided into three parts for further analysis. The first part (approximately 250 g) was stored at 4°C for analyzing soil properties. The second part (approximately 250 g) was transported on ice to the laboratory immediately after collection and freeze-dried at - 80°C for DNA extraction.

Soil pH was determined after creating a 1: 2.5 (10 g / 25 mL) soil-to-distilled water slurry. Electrolytic conductivity (EC) was measured with a ratio of 1: 5 (w/v) using an EC meter (Leici, Shanghai, China). Soil water content (SWC) was gravimetrically determined after oven drying at 105°C for 24 h. Soil organic carbon (SOC) was determined using the dichromate oxidation method [35]. Total nitrogen (TN) was determined using a Kjeldahl Apparatus Nitrogen Analyzer (FOSS2200). Dissolved organic carbon (DOC) was extracted with ultrapure water (water: soil 2: 1) from fresh soil and centrifuged at 3,000 rpm for 5 min, then determined using a TOC analyzer (Elementar, Germany). Nitrate (NO_3^-) and ammonium (NH_4^+) concentrations were analyzed using 20g soil extracted with 50 mL of 2 M KCl, after shaking for 30 min, the solution was filtered and determined using a continuous flow analyzer (Skalar, Breda, The Netherlands). Soil mineral nitrogen was calculated as the sum of NO_3^- and NH_4^+ . Soil microbial biomass carbon (MBC) and nitrogen (MBN) were measured using the chloroform fumigation-extraction method. MBC infiltrates was determined by the potassium dichromate method [36], and MBN was estimated by the Kjeldahl method [37]. MBC and MBN were computed by determining the differences between the fumigated and unfumigated samples with conversion factors of 0.38 for MBC [36] and 0.54 for MBN [37].

Metagenomic analysis

Six biocrust samples (two treatments · three replicates) were subjected to metagenomic sequencing. Soil DNA was extracted from 0.5 g biocrust using the Fast DNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, United States). We used a TBS-380 mini-fluorometer (Turner BioSystems, Sunnyvale, CA, United States) and NanoDrop 2000 spectrometer (Thermo Fisher Scientific, Waltham, MA, United States), respectively, to quantify the concentration and purity of the DNA. We used 1% agarose gels examined the DNA quality. 1 µg of qualified DNA per sample was fragmented by sonication to generate approximately 300 bp fragments using a Covaris S220 (Covaris, Inc., MS, United States). The fragments were end-repaired, poly(A)-tailed, ligated to full-length adaptors, purified, and amplified using PCR to generate sequencing libraries using a NEBNext®Ultra™ DNA Library Prep Kit for Illumina (NEB, USA), according to manufacturer's instructions. Libraries were analyzed for size distribution using an Agilent 2100

Bioanalyzer and quantified using real-time PCR [38]. Sequencing was conducted at the Allwegene Technology Co., Ltd. (Beijing, China).

The raw metagenomic data were screened to remove short length (< 50 bp) and low quality reads (quality value < 20) to generate clean reads. The clean data were assembled using SOAPdenovo [39]. Scaffolds were split into 'N' sequences to generate contigs. Contigs > 300 bp in length were used for further analysis. The open reading frames (ORFs) in the contigs were predicted using MetaGeneMark [40]. The abundance of unique contigs in every metagenome was calculated after mapping the corresponding clean data to the unique contigs using SoapAligner. For taxonomic analysis, the unique contigs were compared against the MicroNT datasets of the National Center for Biotechnology Information (NCBI) with microbial reference genomes that included bacteria, fungi, archaea, and viruses [38]. The resulting alignment hits (e-values > 1×10^{-5}) were filtered out. The lowest common ancestor algorithm for the MEGAN software was used to identify every contigs taxon [41]. Predicted ORFs were clustered in CD179 HIT (parameters: 95% identity, 90% coverage) [42]. The relative gene abundance was defined as the ratio of the sum of the sequencing depth of every base in the predicted gene to the gene length. Metabolism cycling analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, non-supervised orthologous groups (eggNOG) database, and orthologous groups of proteins (COG) database. BLASTP (Version 2.2.28 +) [43] was used for amino acid alignment against the KEGG database. The metagenomic data have been submitted to the NCBI with the biosample accession number SUB10878155.

The summary of the sequencing data is presented in Supplementary Table S2. A total of 248,348,478 high-quality sequences were obtained from the six samples. After sequence assembly and gene prediction, we obtained 3,075,206 contigs. On this basis, a non-redundant gene catalog of 3,798,346 was constructed to describe the overall information of all genes retrieved from the biocrusts.

Statistical analyses

T-test analysis of the soil physicochemical parameters, microbial composition, and metabolic pathways in two types of biocrusts was performed using SPSS 23.0. Principal coordinate analysis (PCoA) was used to visualize the relative differences in community taxonomy and the functional composition (eggNOG and KEGG pathway) of the two biocrust types. The permutational multivariate analysis of variance (PERMANOVA) was further used to reveal the correlation between taxonomic and functional compositions in different biocrust types. Random Forest analysis was performed using MicrobiomeAnalyst at <https://www.microbiomeanalyst.ca/> [44, 45]. Redundancy analysis (RDA) was used to test the effects of soil biogeochemical characteristics on microbial communities using the vegan package in R (4.0.2 version). Heatmaps were visualized by using TBtools [46].

Results

Soil physicochemical properties of biocrusts and their relationship with the microbiome

Most soil properties varied significantly between the two biocrust types. Moss biocrusts had lower pH, MBN, NO_3^- , and EC than lichen biocrusts, while the reverse was true for the remainder, such as SOC, DOC, NH_4^+ , and MBC, aside from soil moisture content and TN (ANOVA, $P < 0.05$) (Table 1).

Table 1
Soil physicochemical properties of two types biocrusts.

Soil physicochemical properties	Lichen biocrusts	Moss biocrusts
SWC (%)	11.60 (1.71) ^a	14.59 (2.22) ^a
pH	8.12 (0.09) ^a	7.35 (0.15) ^b
EC ($\mu\text{s}\cdot\text{cm}^{-1}$)	171.7 (27.38) ^a	90.5 (3.63) ^b
SOC ($\text{g}\cdot\text{kg}^{-1}$)	12.84 (1.89) ^b	19.95 (1.76) ^a
DOC ($\text{mg}\cdot\text{kg}^{-1}$)	56.12 (5.07) ^b	72.2 (4.51) ^a
TN ($\text{g}\cdot\text{kg}^{-1}$)	1.06 (0.24) ^a	1.45 (0.16) ^a
NO_3^- ($\text{mg}\cdot\text{kg}^{-1}$)	52.26 (10.28) ^a	17.45 (5.97) ^b
NH_4^+ ($\text{mg}\cdot\text{kg}^{-1}$)	4.36 (0.75) ^b	23.38 (6.26) ^a
MBC ($\text{mg}\cdot\text{kg}^{-1}$)	773.42 (95.98) ^b	1099.67 (130.86) ^a
MBN ($\text{mg}\cdot\text{kg}^{-1}$)	84.55 (17.10) ^a	40.95 (2.89) ^b
Notes: Data are mean values (n = 3). The numbers in brackets are the standard deviations. Different lower case letters within a row indicate significant differences between biocrust type at $\alpha = 0.05$ level using Duncan's method.		

The Random Forest analysis indicated differences in the abundance pattern of DOC representatives and, to a lesser extent, SOC and others (Fig. S1A). We selected the top five environmental factors according to importance ranking and microbial communities for RDA analysis. The RDA showed that the first two axes explained 88.54% of the cumulative variation in the microbial communities in the two biocrust types (as constrained by the measured soil environmental variables), with the first axis explaining 79.30% and the corresponding second axis explaining 9.24%. Among the soil factors, SOC (0.8975) and DOC (0.8665) were strongly related to microbial community structure with the highest correlated variables in axis 1 (Fig. S2, Table S3).

Microbiome comparison between lichen and moss biocrusts

The β -diversity index differed between the two biocrust types, but no statistically significant pattern occurred at the species level ([PERMANOVA] F-value: 7.704, R-squared: 0.65824, p-value = 0.1) (Fig. 2). No significant differences occurred between lichen biocrusts and moss biocrusts at the eggNOG pathway level ([PERMANOVA] F-value: 11.137, R-squared: 0.73575, p-value = 0.1) or KEGG pathway level ([PERMANOVA] F-value: 12.371, R-squared: 0.75567, p-value = 0.1), but the applied DESeq2 statistical test, revealed that moss biocrust samples were functionally more similar than lichen biocrust samples.

Actinobacteria, Bacteroidetes, and Acidobacteria were more abundant (relative abundance > 1%) in moss biocrusts than lichen biocrusts. At the same time, Proteobacteria and Cyanobacteria were less abundant in the moss than lichen biocrusts (Table S4) (t-test, $P > 0.05$). Actinoplanes, Streptomyces, Pyrinomonas, Pseudonocardia, Bradyrhizobium, and Micromonospora were significantly more abundant in moss biocrusts than lichen biocrusts, and Microvirga, Conexibacter, and Microcoleus were considerably less abundant in moss biocrusts than lichen biocrusts (t-test, $P > 0.05$). Geminocystis and Truepera behaved differently between biocrust types, according to the Random Forest analysis (Fig. S1B).

Carbon and nitrogen-related pathways in lichen and moss biocrusts

Focused on microbial metabolic pathways between the two biocrust types, the annotated genes (relative abundance > 1%) of function-related contribute to significant differences. Seven KEGG pathways (carbon metabolism, amino sugar and nucleotide sugar metabolism, fatty acid metabolism, pyrimidine metabolism, mismatch repair, alanine, aspartate, and glutamate metabolism, and nucleotide excision repair) were overrepresented in the moss biocrusts compared to lichen biocrusts. The lichen biocrusts had overrepresented pathways for ABC transporters, quorum sensing, porphyrin and chlorophyll metabolism, arginine and proline metabolism, glutathione metabolism compared to moss biocrusts (t-test, $P > 0.05$) (Fig. 3).

Most of the carbohydrate-active enzyme (CAZyme) contents differed between the biocrust types. Moss biocrusts had a higher relative abundance of glycoside hydrolases, polysaccharide lyases, and carbohydrate-binding modules than lichen biocrusts. In comparison, lichen biocrusts had a higher relative abundance of glycosyltransferases and carbohydrate esterases than moss biocrusts (t-test, $P > 0.05$) (Table S5). The relative abundance of enzymes associated with carbon and nitrogen metabolism in microbiomes differed between lichen and moss biocrusts. For example, moss biocrusts had significantly more carbohydrate hydrolases than lichen biocrusts, while lichen biocrusts had significantly more aromatic hydrolases than moss biocrusts. Moss biocrusts had higher nitrogen dissimilation, while lichen biocrusts had higher nitrogen assimilation than moss biocrusts (t-test, $P > 0.05$) (Fig. 4).

Of the 14 carbon and nitrogen metabolism pathways (Table S6), glycolysis was the most important pathway in both biocrusts, followed by the pentose phosphate pathway. Benzoate degradation, tryptophan metabolism, and lysine degradation were more abundant in moss biocrusts than lichen biocrusts. In contrast, lysine biosynthesis and vancomycin resistance were more abundant in lichen biocrusts than moss biocrusts. Moss biocrusts had more *coxS* genes encoding carbon monoxide dehydrogenases than lichen biocrusts (Table S7).

Of the genes involved in the nitrogen cycle, the gene *hzo* participating in anammox was not found (Fig. 5). Lichen biocrusts had a higher abundance of *nirK*, *napA*, *narB*, and *nirA* than moss biocrusts, while moss biocrusts had a higher abundance of *nasA*, *nasB*, and *nirB* than lichen biocrusts (t-test, $P > 0.05$) (Table S8). However, the total abundance of gene involved in the nitrogen cycling process, such as assimilatory NO_3^- reduction and nitrogen fixation, did not significantly differ between biocrust type (t-test, $P > 0.05$) (Table S9).

Discussion

Overall, a comparative metagenomic analysis on the microbiomes of lichen and moss biocrusts in the Mu Us Sandland is presented in our study, revealing distinct differences in taxonomic and functional diversity of microbiomes in C and N cycles between two types of biocrust and supplying useful information for understanding biochemical processes and nutrient cycling in the desert.

Effect of biocrust type and (bio-)chemical parameters on the microbiome

Our results suggest that biocrust type greatly affects the microbiome's species composition, gene, and metabolic diversity (Fig. 2). Well-developed biocrusts have an abundance of Actinobacteria, Bacteroidetes, and Acidobacteria [47, 48], consistent with the alkaline character of desert soils [49–51], especially in the Tabernas desert [29]. According to Vetrovsky et al. [52] and Qiao et al. [53], Actinobacteria can degrade complex compounds, such as polysaccharides and phenolic compounds, and Bacteroidetes can degrade xylan to improve the nutritional status of biocrusts. Acidobacteria may be a vital contributor to degrade the polymorphs in plant residues [54]. Critical for biocrust formation, Proteobacteria could secrete extracellular polysaccharides for bonding sand particles to prevent soil wind erosion and contribute the most to N_2 -fixation in the early stages of biocrust formation. [55, 56]. Kidron et al. [57] indicated that Cyanobacteria rapidly colonized the soil surface due to their rapid growth, migration, and strong adaptability to extreme environments. However, being self-sufficient for carbon and nitrogen assimilation [58–60]. Moss biocrusts had more abundant Actinobacteria, Bacteroidetes, and Acidobacteria than lichen biocrusts, while lichen biocrusts had more abundant Proteobacteria and Cyanobacteria than moss biocrusts (Table S4). The results indicated that biocrusts at the early succession stage are rich in carbon and nitrogen-fixing micro-organisms that aim to accumulate topsoil

nutrients, while biocrusts at the late succession stage are dominated by micro-organisms that mainly degrade complex compounds to provide effective nutrients for vascular plant establishment [27, 31, 61].

The genera *Actinoplanes*, *Streptomyces*, *Pyrinomonas*, *Micromonospora*, *Microvirga*, *Conexibacter*, and *Microcoleus* are found in dry and extreme arid habitats [28] and were presented in the two biocrust types analyzed in this study (Table S4). In particular, the two biocrust types differed significantly in the abundance of *Microcoleus*, a dominant photosynthetic organism in Cyanobacteria biocrusts [62]. According to the Random Forest analysis, *Geminocystis* and *Truepera* were the biomarkers distinguishing biocrust type (Fig. S1B). *Geminocystis*, found in lakes, belongs to Cyanophyta and fixes carbon and nitrogen [63]. *Truepera*, a bulbous bacterium that resists harsh environments [64], belongs to *Deinococcus-Thermus* and may efficiently digest soil organic matter [65].

As changes in the biomass and soil physiochemical environment markedly altered the composition and structure of microbial communities, we predicted that the different physiochemical environments of biocrusts would result in selective microbial growth, which would be seen as a shift in microbial community structure and accompanying gene content. Consistent with Yang et al. [66] and Fierer and Jackson [67], our research found that soil biogeochemical properties were closely related to microbial communities. The analyses revealed that DOC and SOC were the main environmental drivers of microbial community β -diversity in both biocrust types (Fig. S1A and S2), which is consistent with previous studies [49, 68–71]. In addition, DOC and SOC had strong positive correlations with the microbial community structure in both biocrust types. In this study, moss biocrusts had significantly higher organic carbon, soluble carbon, and MBC than lichen biocrusts (Table 1), supporting our previous research revealing the carbon sink's role in soil carbon input in moss biocrusts [27].

Biocrust microbiome and its influence on carbon and nitrogen fixation and transformation

Our second significant finding was biocrust types differed in the relative abundance of CAZymes and enzymes associated with carbon and nitrogen metabolism (Table S5, Fig. 4). Moss biocrusts had significantly more glycoside hydrolases (e.g., glycoside hydrolases, carbohydrate-binding module) and polysaccharide lyases (e.g., polysaccharide lyases) than lichen biocrusts, indicating that moss biocrust microbiomes secrete more biodegradable carbohydrate enzymes. In contrast, lichen biocrust had more glycosyltransferases and carbohydrate esterases, suggesting that the glycosyltransferases in microbiomes catalyzed the attachment of activated sugars to different receptor molecules, such as proteins, nucleic acids, oligosaccharides, and lipids, secreting carbohydrate esterases to produce glycosidic bonds [72]. The microbiomes in both biocrust types used different substrates to decompose organic carbon. Moss biocrusts usually used carbohydrates as a carbon source, while lichen biocrusts used aromatic compounds. The microbiomes in moss biocrusts reduced NO_3^- and nitrite to NO or NH_3 , explaining the lower NO_3^- and higher NH_4^+ than lichen biocrusts. The microbiomes in lichen biocrusts fixed N_2 and synthesized amino acids, further promoting microbial growth and reproduction, explaining the higher MBN content than moss biocrusts.

The metagenome profile of the carbon- and nitrogen-related metabolic pathways also differed between lichen and moss biocrusts. In addition, the high abundance of genes associated with amino acid biosynthesis (e.g., lysine and arginine) in both biocrust types (Table S6) indicates their importance for microbiome adaptation to desert environments and life processes in general [28]. Biocrusts are a major source of SOC in drylands [17, 73, 74]. Interestingly, our results show a clear dominance of respiration over carbon fixation in microbial communities associated with both biocrust types (Table S6), in contrast to other findings in the Tengger Desert, where mostly carbon fixation metabolic pathways were found in bacterial and moss biocrusts [28]. In our results, the most important pathways in both biocrusts were glycolysis and the citrate cycle, which are essential for microbial respiration [29]. These differences could be due to changes in the soil microbial populations and thus metabolism due to the enormous spatial heterogeneity in arid desert soils.

The metagenomic data revealed that the *rbcL* and *rbcS* genes, encoding the rate-limiting enzyme-RuBisCo, were deficient, with no significant difference between lichen and moss biocrusts (Table S7). The key *coxL*, *coxS*, and *coxM* genes in the carbon cycle, encoding carbon monoxide dehydrogenases—a bidirectional enzyme allowing organisms to both make use of CO as a source of energy (CO oxidation) and CO₂ as a source of carbon (CO₂ fixation) [75]—differed slightly between biocrust types. Interestingly, these genes were 9-fold (lichen crust) to 18-fold (moss crust) more abundant than RuBisCo genes (Table S7). Thus, the high abundance of CO dehydrogenases in biocrusts suggests that these genes contribute to CO₂ fixation through the Calvin cycle or chemosynthetic carbon fixation in some deserts, such as the Antarctic desert and Tengger Desert [28, 76, 77].

Nitrogen availability is the most important factor limiting the primary production of biocrusts in arid environments except for water [78, 79] and largely depends on the microbiomes driving N cycling [80]. Studies have shown that nitrogen inputs in desert environments strongly depend on the nitrogen-fixing microbiomes in biocrusts [26, 81, 82]. The Low diversity and abundance of genes in N₂ fixation pathways in our study (Table S9) is a distinguishing feature of biocrust microbiomes [28]. More than 80% of the nitrogen in the soil is transformed into available nitrogen in the form of ammonium and nitrate nitrogen by the mineralization mediated by microbiomes for being absorbed and utilized by plants [66]. We found that the conversion of urea to NH₄⁺ (ammonification) by the *ureC* gene and N₂ to NH₄⁺ (N fixation) by the *nifH*, *nifD*, and *nifK* genes have high levels of potential functionality in providing nitrogen for biocrusts in the nitrogen cycle. Consistent with previous literature, we found a high level of denitrification in biocrusts (Fig. 5, Table S9), revealing that denitrification may enable N₂O and NO production and prevent NO₃⁻ production [25]. Both assimilatory and dissimilatory NO₃⁻ reduction were identified in these samples (Table S9). The microbiomes of lichen and moss biocrusts were rich in assimilatory and dissimilatory NO₃⁻ reduction genes, respectively (Table S9). NO₃⁻ was reduced to nitrite by *nasA*, *nasB*, *narB*, and *NR* genes for assimilatory NO₃⁻ reduction or *napA* and *napB* for dissimilatory NO₃⁻ reduction, and further reduced to NH₄⁺ by *nirA* for assimilatory NO₃⁻ reduction or *nirB*, *nirD*, *nrfA*, and *nrfH* for dissimilatory NO₃⁻ reduction (Fig. 5). The conversion of NO₃⁻ to NH₄⁺ (assimilatory or dissimilatory NO₃⁻ reduction) could

increase soil NH_4^+ derived from NO_3^- during desert revegetation over time. It may be used for the amino acid synthesis of the microbiome in moss crusts and provide an available nitrogen source for moss crusts growth. However, because the *hzo* gene was not detected during biocrust succession in this study, the conversion of NH_4^+ to N_2 (anammox) was likely not to be active in the N cycle of biocrusts in arid and semi-arid regions [31, 81].

Combined ammonification, NO_3^- reduction (assimilative or dissimilatory), and denitrification accounted for 93.96% and 91.49% of the gene abundance for nitrogen-metabolism-related pathways in lichen and moss biocrusts, respectively (Fig. 5, Table S9). Therefore, the transformation of NO_3^- to NH_4^+ may explain the high amino acid biosynthesis, keeping the nitrogen bio-bound and avoiding nitrogen losses due to leaching and denitrification. The reproduction and colonization of biocrusts regulate plant-available nitrogen content and promote nitrogen mineralization and availability in soil, which is an important biological factor affecting soil nitrogen cycling.

Conclusion

This study details the microbiome composition and C and N cycles-related metabolism in two types of biocrusts in the southeastern Mu Us Sandland. Our findings suggest that the biocrust type significantly affects species, gene, and functional diversity. The study offers insight into the molecular basis of biogeochemical processes in biocrusts. Our results showed there is a clear dominance of loss over fixation in microbial communities' carbon and nitrogen cycles associated with both biocrust types at the level of gene expression. The metabolic pathways of NO_3^- reduction were affected by biocrust type. However, although significant differences in the relative abundance of genes for C and N cycling processes in different biocrusts types may potentially lead to accelerating nutrient cycling process, more systematic, in-depth studies are needed to clarify the rates and extent of various stimulated nutrient cycling processes, and their impacts on the over-all biocrusts nutrient dynamics in this ecosystem.

Declarations

Supplementary data

Supplementary data to this article can be found in supplemental material.

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Author contributions

Chongfeng Bu conceived the ideas and designed the methodology, Chang Tian, Jingwen Pang, Yahong Li and Qi Guo collected the data, Chang Tian and Jingwen Pang analyzed the data and wrote the manuscript, Shufang Wu and Kadambot H.M. Siddique contributed critically to the drafts. All authors gave final approval for publication.

Data availability

All data is publically available on the SRA database under BioProject PRJNA793166.

Competing Interests

The authors declare no competing interest.

Ethics Approval Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

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Figures



Figure 1

(A) Location and (B) landscape of the (C) lichen biocrust and (D) moss biocrust collection site.

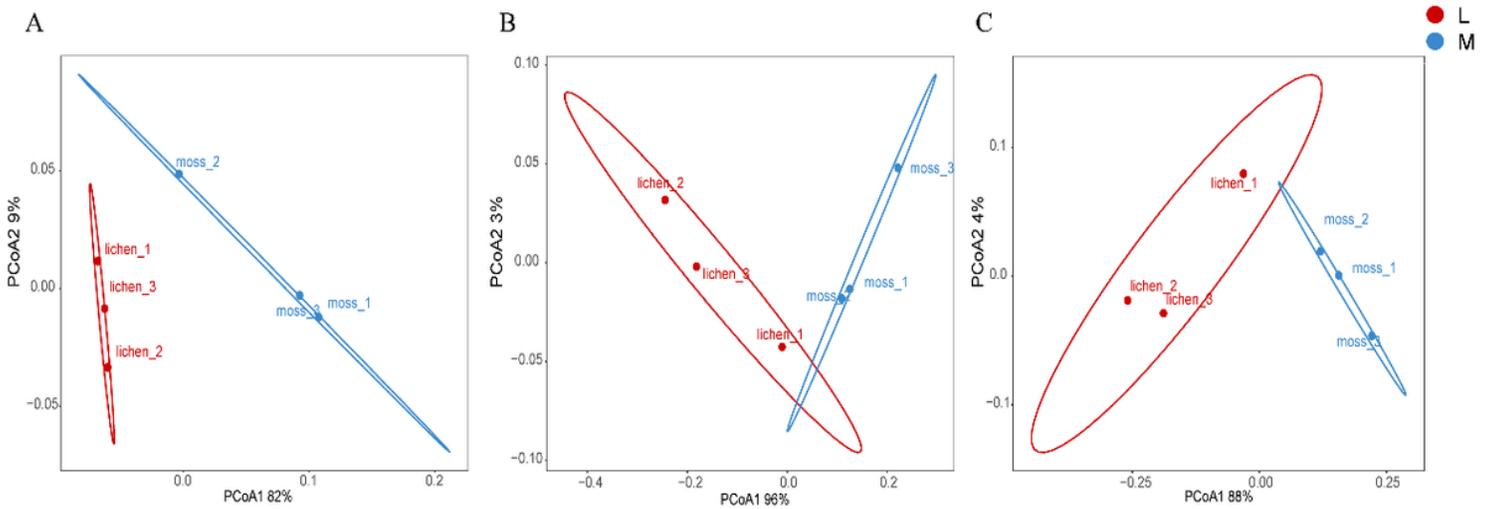


Figure 2

Effect of biocrust type on the taxonomic and functional composition of microbiomes. Red is lichen biocrust and blue is moss biocrust. (A) Species level, (B) KEGG pathway level, and (C) eggNOG pathway level.

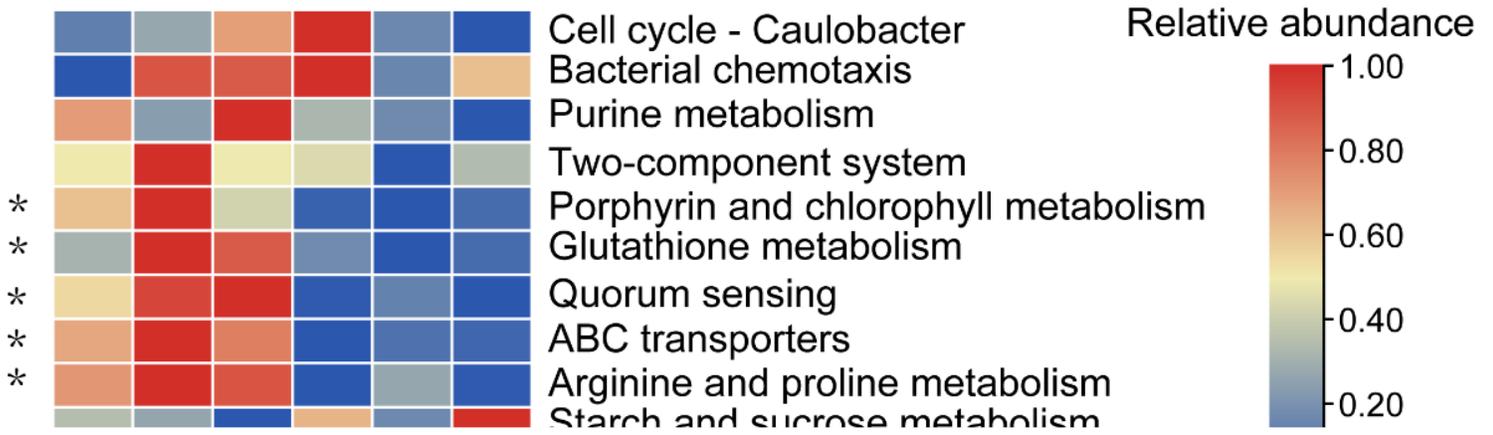


Figure 3

Functional comparison between lichen and moss biocrusts at the KEGG pathway level-3 (relative abundance >1%).

Figure 4

Relative abundance of enzymes associated with carbon and nitrogen metabolism in lichen and moss biocrusts at the COG pathway level.

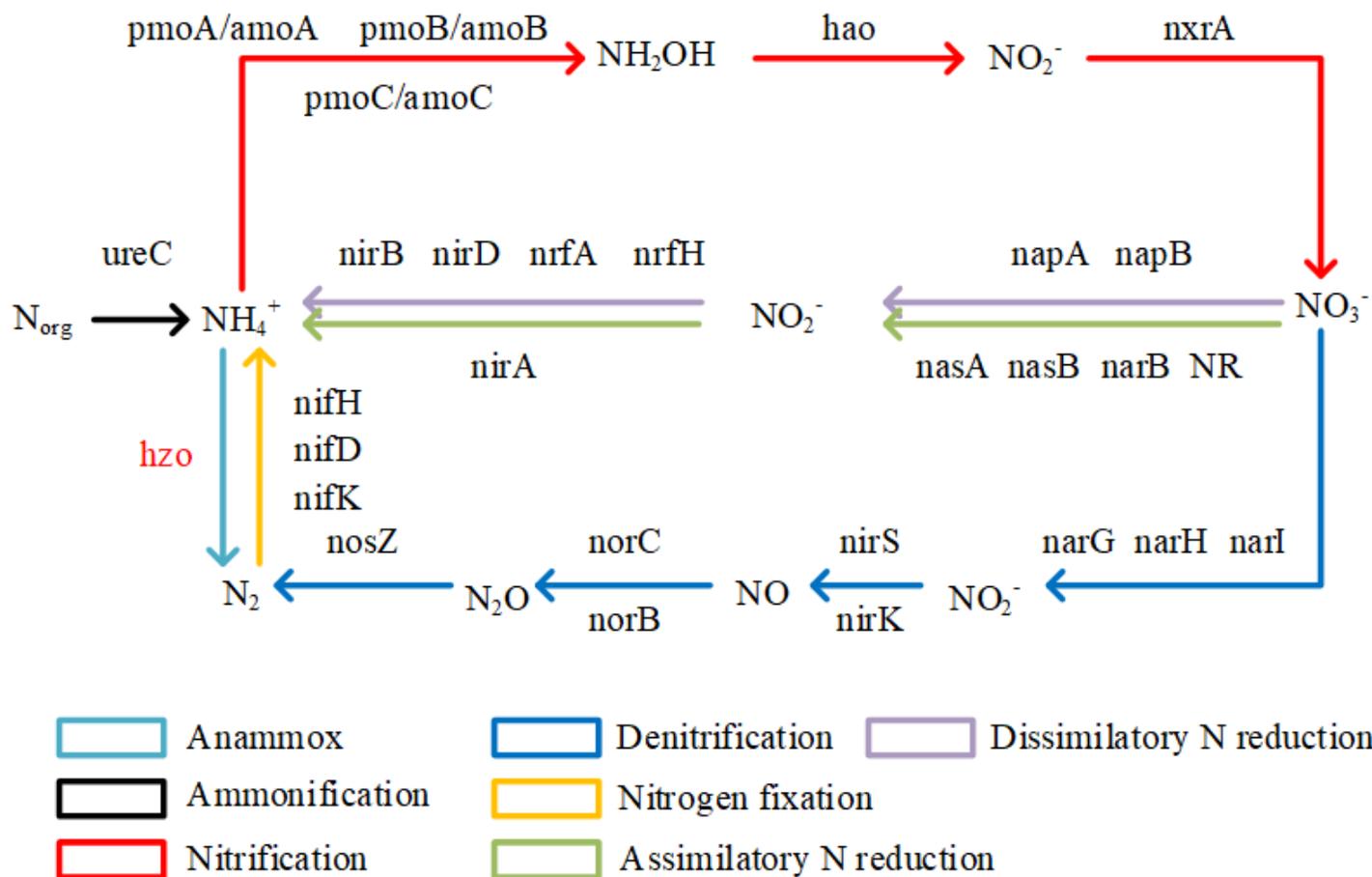


Figure 5

Normalized average signal intensity of bacterial genes involved in the nitrogen cycle of lichen and moss biocrusts. Red-colored genes were not detected by shotgun metagenomic sequencing

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