Microbial community structure and function potential of biological soil crusts in the Mu Us Desert, northern China

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Abstract

Aims This study assessed microbial community structure and functional potential in different biological soil crusts (BSCs) involved in key biogeochemical processes (C and N cycling) in desert ecosystems.

Methods Changes in microbial composition and function gene abundance in three BSCs (algal, lichen, and moss crusts) were assessed using shotgun metagenome sequencing to explore whether different BSCs exhibited different microbial community structures, and C and N metabolic potential.

Results The microbial community and functional structure differed significantly among the three BSCs; differences were largely driven by soil organic carbon and total nitrogen. The different relative abundance of genes involved in both C and N cycling promoted different microbial metabolic potential in BSCs. The C and N input process genes in lichen crusts were higher than algal crusts and moss crusts; The C degradation genes (both labile and recalcitrant C) and N loss genes (nitrification and denitrification) were higher in moss crusts.

Conclusions Microbial community structure and microbial function structure changed in different BSCs types. In addition, lichen crusts possessed higher C and N fixation potential and moss crusts owed higher C metabolic and N loss potential, which could result in positive soil nutrient feedback and improve BSCs development.

Key words Biological soil crusts, Microbial community structure, Microbial function structure, Carbon cycling, nitrogen cycling, Shotgun metagenome sequencing

Introduction

Drylands cover approximately 40% of the terrestrial surface of the Earth (Reynolds et al. 2007), and most area of drylands is covered with biological soil crusts (BSCs) that play critical roles in a range of biochemical cycling processes (Eldridge and Greene 1994; Li 2012; Belnap et al. 2016). For instance, BSCs increase soil C, N, and other nutrients, and accelerate the development and succession of soil ecosystems (Lan et al. 2012, 2013; Maestre et al. 2012; Weber et al. 2015). Microbes mediate many critical biochemical processes such as nutrient cycling, organic matter decomposition, and plant production (Delgado-Baquerizo et al. 2016, 2018). However, there are gaps in role of microbes in different types of BSCs in the biochemical cycles. Consequently, understanding microbial community structure and gene function changes in different types of BSCs provide insight into BSC function, especially in desert ecosystems.

Microbes are important component in BSCs which are called 'the living skin of the earth' (Bowker et al. 2018). BSCs provide a habitat for soil organisms to become a complex mosaic layer (Liu et al. 2017a, b; Steven et al. 2013) that largely influence microbial abundance and activity (Bates et al. 2010; Delgado-Baquerizo et al. 2016). Previous studies on the microbial composition and diversity in BSCs mainly focused on the complex mosaic layer of BSC layers (0–1 cm) and their underlying soil (1-5) (Bowker et al. 2018; Liu et al. 2017a, b; Zhang et al. 2016). Nevertheless, studies also more concenteated on Cyanobacteria-dominated BSCs in arid and semiarid regions (Abed et al. 2010; Steven et al. 2013; Yeager et al. 2004). Microbial biomass and composition not only differ between the BSCs layer and different soil depths, but also changed in different BSCs succession stage (Steven et al. 2013, 2014),

which suggest that different types of BSCs layer play different role in soil restoration owing to the change of relative abundance of cyanobacteria that is essential to C and N cycling in desert regions (Liu et al., 2018). One study revealed that in biogeochemical cycling (C, N, S, and P cycles), the functional gene structures of microbial communities in differently aged BSCs were highly homologous, which provided limited information for understanding the microbial function in different BSC types (Liu et al. 2018). Therefore, it is vital importanrt to probe microbial community structure in different types of BSCs layers (0-1 cm), and microbial function changes in different types of BSC layers. Trough studying the functional gene structures and metabolic potentials of different types of BSC layers could better be a better way to understand their different role of nutrient cycling.

BSCs are particularly important in arid and semi-arid regions in which soil organic C and N are limited resources, and viewed as an important C sink for their considerable photosynthetic potential (Grote et al. 2010; Thomas et al. 2008). It is estimated that the global annual net C uptake by BSCs is up to 3.6 Pg a^{-1}, with 1 Pg a^{-1} fixed by BSCs in arid and semi-arid regions (Elbert et al. 2009). In addition, BSCs are not only the most important source of N in desert ecosystems (Zaady et al. 2010), but also participate in N cycling through autotrophic N fixation (Belnap 2011). BSCs may contribute approximately 27–53% of the biological terrestrial N fixation on a global scale (Ciais et al. 2014; Elbert et al. 2012), which is extremely important in supplying soil nutrients in drylands. However, there were rely information in BSCs C and N function under microbes participation.

Although High-throughput sequencing technologies have shown that microbes are ,performing numerous functions in dryland ecosystems (Abed et al. 2013; Belnap 2001, 2011; Delgado-Baquerizo et al. 2013; Elliott et al. 2014; Liu et al. 2017a, b; Maestre et al. 2012; Steven et al. 2014). Probing functional genes and the functional composition of microbial communities using shotgun metagenome sequencing, instead of making predictions using taxonomic information could be a more efficient and effective method to investigate BSC function (Barberán et al. 2012; Yang et al. 2014). Therefore, investigate the change in microbial functional genes of different BSCs types is imperative for us to understand their microbial metabolic functions.

In this study, we applied shotgun metagenome sequencing methods to gain insight into the microbial community and function structure, and the relative abundance of C and N cycle related genes in three dominant BSC types (algal, lichen, and moss crusts) in the Mu Us Desert, Northern China. The specific aims of this study were to investigate how the microbial community and function structure difference in different BSC types, how the genes involved in the C and N cycling processes change, and whether different microbial metabolic potentials are displayed in the different BSC types of desert ecosystems. Our study could provide an overall picture of microbial community and functional structures, and the potential critical roles of microbes in C and N cycling in different BSC types, which may provide a better understanding of the biochemical processes and nutrient cycling in desert ecosystems.

Materials and methods

Research site, soil sampling, and chemical analysis

The research was conducted at the Yanchi Research Station (37°04′-38°10′ N and 106°30′–107°47′ E, 1,550 m above sea level), Ningxia Province, northern China. The site is located on the southwestern fringe of the Mu Us Desert, where the mean annual temperature is 8.1°C, and the mean annual rainfall is 287 mm, 62% of which falls between July and September (Jia et al. 2014). The soil texture of the study area is sandy in the 0- to 1-m profile, with an average bulk density of 1.5 g cm^{-3} (Lai et al. 2016). The research site is dominated by sparse shrub species including *Artemisia ordosica*, *Hedysarum mongolicum*, *Hedysarum scoparium*, *Salix psammophila*, and *Agropyron cristatum* (Lai et al. 2016). BSCs commonly cover gaps between the shrubs in the research site, and are dominated by algal crusts, lichen crusts, and moss crusts (Fig. 1).

BSC samples were collected in July 2017 during the plant biomass production peak. Three plots (100 m × 100

m each) were established for each type of BSC (Table 1). The algal crust plot was approximately 500 m from the lichen and moss crust plots, and the lichen crust plot was 100 m away from the moss crust plot. Three 20 m × 20 m sampling subplots were randomly selected in each plot, separated by 20 m. The plant litter on the BSCs was removed before sample collection. Twelve BSC samples were randomly obtained from each subplot using a sterile cutting ring (internal diameter 9 cm, depth 1 cm). The samples from each subplot were mixed to create one composite sample. Each composite sample was combined, homogenized, and sieved through a 2-mm mesh to remove gravel and litter. A total of nine composite samples were collected from three plots. Then, each composite sample was divided into three subsamples: the first part (approximately 200 g) was air-dried for physicochemical analysis, the second (approximately 200 g) was stored at 4°C for a maximum of 30 days for microbial biomass analysis (Černohlávková et al. 2009), and the third (approximately 100 g) was stored at -78.5°C on dry ice for metagenomic analysis.

The soil water content (SWC) was gravimetrically determined after oven drying at 105°C for 24 h. The total soil organic carbon (SOC) was determined using the dichromate oxidation method (Walkley and Black 1934). The total nitrogen (TN) content measured by 0.5 g sample, 10 mL of sulfuric acid, 5 g of FOSS catalyst mixture, and digestion at 420 C° for 2 h to determined using a Kjeldahl Apparatus Nitrogen Analyzer (FOSS2200). The total phosphorus (TP) was measured by molybdenum anti-colorimetric method which decomposed with perchloric acid and sulfuric acid developed in ultraviolet spectrophotometer (Shimadzu UV-2550). The soil pH was measured after creating a 1 : 2.5 (10 g / 25 mL) soil-to-distilled water slurry.

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were measured using the chloroform fumigation-extraction method. MBC in filtrates was determined by the potassium dichromate method (Vance et al. 1987), and MBN was estimated by the Kjeldahl method (Brookes et al. 1985). MBC and MBN were computed by determining the differences between the fumigated and unfumigated samples with conversion factors of 0.38 for MBC (Vance et al. 1987) and 0.54 for MBN (Brookes et al. 1985).

Shotgun metagenome sequencing, processing, and data analysis

All nine samples were subjected to shotgun metagenome sequencing using the Roche 454 pyrosequencing approach. For each sample, the total microbial community DNA was isolated from 1.0 g of soil using the MoBio Power Soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. Firstly, DNA degradation degree and potential contamination were monitored on 1% agarose gels; Secondly, DNA purity was checked using the NanoPhotometer® spectrophotometer (Implen Inc. Westlake Village, CA, USA); and thirdly, DNA concentration was measured using a Qubit® dsDNA Assay Kit in a Qubit® 2.0 Fluorometer (Life Technologies, CA, USA). After DNA testing, a total of 1 μg of qualified DNA per sample was fragmented by sonication to generate approximately 350 bp fragments. The fragments were end-repaired, poly(A)–tailed, ligated to full-length adaptors, purified, and amplified using PCR to generate sequencing libraries using an NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA), following the manufacturer's instructions. Libraries were analysed for size distribution using an Agilent 2100 Bioanalyzer and quantified using real-time PCR (Wang and Sun 2018). Sequencing was conducted at the Novogene Bioinformatics Institute (Beijing, China).

The raw metagenomic data were screened to remove adaptor fragments and low-quality reads, and to generate clean reads (Table S1; Fig. S1). The specific processing steps are as follows: a) remove the reads which contain low quality bases (default quality threshold value ≤38) above a certain portion (default length of 40 bp); b) remove the reads in which the N base has reached a certain percentage (default length of 10 bp); c) remove reads which shared the overlap above a certain portion with Adapter (default length of 15 bp). Then, the clean data were assembled to creat Scaffolds by using SOAPdenovo and the parameters are -presets meta-large (--min-count 2 --k-min 27 --k-max 87 --k-step 10) (Luo et al. 2012). The assembly with the longest N50 in every metagenome was selected for

subsequent analysis. Scaffolds were split into 'N' sequences to generate scaftigs (i.e., continuous sequences within scaffolds). Scaftigs longer than 500 bp were used for further analysis. The Scaftigs assembled from both single and mixed are all predicted the ORF by MetaGeneMark (V2.10, http://topaz.gatech.edu/GeneMark/) software, and filtered the length information shorter than 100 nt from the predicted result with default parameters (Zhu et al., 2010). For ORF predicted, CD-HIT software (identity threshold of 95%) (V4.5.8, http://www.bioinformatics.org/cd-hit) is adopted to redundancy and obtain the unique initial gene catalogue (the genes here refers to the nucleotide sequences coded by unique and continuous genes), the parameters option are -c 0.95, -G 0, -aS 0.9, -g 1, -d 0. The Clean Data of each sample is mapped to initial gene catalogue using SoapAligner (soap 2.21) and get the number of reads to which genes mapped in each sample with the parameter setting are -m 200, -x 400, identity ⩾ 95%. Filter the gene which the number of reads ⩽2 in each sample and obtain the gene catalogue (Unigenes) eventually used for subsequently analysis. The average of Unigenes in alag crusts, liche crusts and moss cruts were 1116698, 1961147 and 775262 respectively (Table S1).

DIAMOND software (Buchfink et al., 2015; V0.7.9, https://github.com/bbuchfink/

diamond/) is used to blast the Unigenes to the sequences of Bacteria, Fungi, Archaea and Viruses which are all extracted from the NR database (Version: 20161115,https://www.ncbi.nlm.nih.gov/) of NCBI with the parameter setting are blastp, -e 1e-5 (Wang and Sun 2018). The resulting alignment hits with e-values larger than 1×10^{-5} were filtered out. The lowest common ancestor algorithm for the MEGAN software was used to identify every scaftig taxon (Huson et al. 2011). Predicted open reading frames were clustered in CD-HIT with default parameters (-c 0.95, -G 0, -aS 0.9, -g 1, -d 0) (Li et al., 2006). 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; Kanehisa et al. 2004). To evaluate all the function potential, we estimate gene categories in KEGG level 1. To estimate the C fixation potential, we specifically focused on genes for C4-dicarboxylic acid, Calvin, reductive tricarboxylic acid (rTCA), 3-hydroxypropionate, and reductive acetyl CoA cycles, based on a report by Ren *et al*. (2018) in KEGG level 3. To estimate the C degradation potential, we specifically focused on genes responsible for the catabolic processes of various C-complexes with varying decomposability, ranging from labile C (e.g., monosaccharides and polysaccharides) to recalcitrant C (e.g., cell wall constituents and phenols), based on a report by Zhang *et al*. (2017) in KEGG level 3. To estimate the N cycle potential, we specifically focused on genes responsible for the nitrification, denitrification, dissimilatory nitrate reduction, assimilatory nitrate reduction, anammox, N fixation, and N mineralization, based on a report by Tu *et al*. (2017) in KEGG level 3. BLASTP (e-value $\leq 1 \times 10^{-5}$) was used for amino acid alignment against the KEGG database (KEGG level 3). The metagenomic data have been submitted to the NCBI with the biosample accession number SUB5359866.

Statistical analysis

Multiple comparisons of the soil physicochemical parameters were performed using Tukey's honestly significant difference (HSD) test in the different BSC types. Multiple comparisons the relative abundance of bacterial, archaea, and fungal phyla, as well as C and N cycle related genes were performed using Kruskall-Wallis test paired with a post-hoc Dunn's test. Non-metric multidimensional scaling (NMDS) was used to evaluate the overall differences in bacterial, archaea, and fungal community structures based on the Bray–Curtis similarity index. This measure was assessed using data for the number of genes at the phylum level. The Mantel test was used to determine the major environmental attributes shaping microbial community structures and microbial functional structures. The principal coordinate analysis (PCoA) was used to visualize the relative differences in phylum community taxonomy, and the functional composition of all genes among different BSC types (Anderson 2003). The permutational multivariate analysis of variance (PERMANOVA) was further used to reveal the correlation

between taxonomic and functional compositions in different BSC types (Anderson 2005). Linear regression analyses were also used to identify the relationships between the relative change in phylum or functional gene abundance caused by different BSC types. All statistical analyses were performed using R version 3.3.2 (R Core Team 2016).

Results

Soil properties

SOC, SWC, TN, MBN, and MBC content were markedly higher in moss crusts than in algal and lichen crusts (Fig. 2; $P < 0.05$). In addtion, the SOC in lichen crusts was greatly higher than algal crusts ($P < 0.05$). There were no significance difference between algal and lichen crusts in SWC, TN, MBC and MBN ($P > 0.05$). TP were significantly lower in algal crusts compered to lichen crusts and moss crusts ($P < 0.05$); pH were significantly lower in moss crusts compered to algal crusts and lichen crusts ($P < 0.05$).

Taxonomic and functional structure

Taxonomic analysis of the classified reads showed that bacteria were the dominant component (91.29% relative abundance average) with a low proportion of archaea (1.24%), fungi (0.14%), viruses (0.03%), and others (unclassified sequences; 7.42%) in the three BSC types. The relative abundance of bacteria in moss crusts was significantly lower than that in algal and lichen crusts (Table 2; $P < 0.05$), whereas that of fungi and archaea was relatively higher in moss crusts.

Across the three types of BSCs, the dominant microbial phyla were Proteobacteria (24.83%, average in relative abundance), Actinobacteria (21.75%), Cyanobacteria (14.68%), Acidobacteria (9.17%), Bacteroidetes (4.40%), Gemmatimonadetes (2.42%), Chloroflexi (1.46%), and Planctomycetes (1.24%) (relative abundance > 1%, Table 3). The dominant bacterial phyla compositions in the three BSC types were significantly different (Table 3; $P < 0.05$). The relative abundance of Proteobacteria and Cyanobacteria in moss crusts were significantly lower than algal crusts and lichen crusts. However, the relative abundance of Actinobacteria and Acidobacteria in moss crusts were greatly higher than algal crusts and lichen crusts.

NMDS showed that the bacterial communities in the same types of BSCs tended to cluster together (Fig. S2) and differed significantly among the three types of BSCs (Adonis, Table S2a, $R^2 = 0.9835$, $P = 0.006$). Furthermore, fungal and archaea phyla from the same BSC types also tended to cluster together (Fig. S2b,c) and had significant differences among the three BSC types ($P < 0.05$; Table S2).

Among all the gene categories in KEGG level 1, a relatively higher gene abundance of metabolism function was observed in BSCs (Fig. 3). Hierarchical clustering of the microbial functional compositions clustered the three BSC types into three major groups, and the distance decreased with the same BSC types (Fig. 3). The overall composition and functional gene structures of the microbial communities in different BSC types were highly homologous (Fig. 4a, Fig. 4b), and microbial community (X axis) and functional structure (Y axis) were highly positively correlated (Fig. 4c). In addition, the Mantel test showed that the microbial community functional composition and taxonomic structure were significantly shaped by several key soil physical and chemical variables, including SOC, pH, SWC, MBC, MBN and TN ($P = 0.016$, Table 4).

Putative genes in the C cycle

In the KEGG level 3, genes involved in C fixation including the C4-dicarboxylic acid (*ppc*), Calvin Cycle (*rbcL* and *rbcS*), reductive tricarboxylic acid (rTCA) (*porA*, *porB*, *porD*, *IDH1*, *korA*, *korB* and *korD*), 3-hydroxypropionate (*accA*, *accB*, *accC* and *accD*), and reductive acetyl CoA cycles (*fdhA* and *fdhB*) were detected (Fig. 5). A significantly greater relative abundance of genes involved in C fixation cycles were found in lichen crusts; the *rbcS* genes involved in the Calvin cycle, the *IDH1*,and *korB* genes involved in the rTCA cycle, and the *accA* and *accC* genes involved in the 3-hydroxypropionate cycle showed the highest relative abundance ($P < 0.05$).

In the KEGG level 3, the most of relative abundance of genes involved in C degradation (both labile and recalcitrant carbohydrate) were significantly higher in moss crusts ($P < 0.05$; Fig. 6), and included genes that coded for L-arabinose, arabinan, rhamnose and D-xylose involved in monosaccharide degradation; pectin involved in polysaccharide and disaccharide degradation; xylan, hemicellulose, cellulose, chitin, and lignin involved in cell wall constituent degradation; butyrate involved in carboxylic acid degradation, and glycerophpspholipid and sphingolioid involved in lipid degradation. In addtion , the genes of xylulose, D-galactonate and glyoxylate were higher in lichen crusts ($P < 0.05$).

Putative genes associated with the N cycle

In the KEGG level 3, genes involved in the N cycle including nitrification (1.91%, average specific pathway compered to all N cycling pathway), denitrification (9.46%), dissimilatory nitrate reduction (24.38%), assimilatory nitrate reduction (23.05%), anammox (1.05%), N fixation (0.46%), and N mineralization (39.68%)were detected.

Genes involved in different N cycles differed greatly between BSC types (Fig. 7). Genes involved in N loss (nitrification and denitrification) were significantly higher in moss crusts than in algal and lichen crusts and related to N fixation potential and N mineralization were significantly greater in lichen crusts when compared with algal and moss crusts. For example, *nifh* involved in N fixation, and *ureC* involved in N mineralization showed the highest relative abundance in lichen crusts ($P < 0.05$). *nxrA* involved in nitrification, and *narG* involved in denitrification showed the highest relative gene abundance in moss crusts ($P < 0.05$).

Discussion

4.1. Microbial community and functional structure in BSCs

Our results show that the microbial community structure greatly changed across the three BSC types (Fig 4a; Fig S2; Table 2; Table 3; Table S2). Previous studies have found that bacteria instead of archea and fungi were the dominant microbes in the BSCs (Belnap et al., 2016). Nevertheless, similar to previous studies (Abed et al. 2010; Bates et al. 2011; Liu et al. 2017a, b, 2018; Maier et al. 2014; Steven et al. 2013; Zhang et al. 2016), the most common phyla in three types of BSCs in the Mu Us Desert were Proteobacteria, Actinobacteria, Cyanobacteria, and Acidobacteria. However, our results differed from those found in northern Arizona, the Colorado Plateau, and the Gurbantunggut Desert, in which larger relative abundances of Gemmatimonadetes were found than those observed in the Mu Us Desert (Dunbar et al. 1999; Steven et al. 2014; Zhang et al. 2016). The reason for this difference in bacterial relative abundance in BSCs from different regions might be due to the higher Gemmatimonadetes abundance in the natural habitat around the Mu Us Desert, where Gemmatimonadetes was the most dominant phylum after Actinobacteria, Proteobacteria, and Acidobacteria (Liu et al. 2018). Moreover, Chloroflexi and Firmicutes showed a lower relative abundance than those in the Tengger and Gurbantunggut Deserts (Li et al. 2017a, b; Zhang et al. 2016). Some of Chloroflexi and Firmicutes are thermophilic phyla (Moquin et al. 2012; Nagy et al. 2005), which are beneficial adaptation to a drought environment (Lacap et al. 2011; Wang et al. 2015). We supposed that the lower relative abundance of Chloroflexi and Firmicutes in our research site was primarily due to the higher precipitation in the Mu Us Desert (annual mean annual rainfall is about 287 mm) relative to that in the Tengger Desert (103 mm, Liu et al. 2017a, b) and the Gurbantunggut Desert (150 mm, Zhang et al. 2016).

Furthermore, our result show that the relative abundance of dominate phylum n three types of BSCs were changed greatly, such as Proteobacteria, Actinobacteria, Cyanobacteria, Acidobacteria and Bacteroidetes.We predicted that the different physiochemical environments of BSCs would result in selective microbial growth, which would be seen as a shift in microbial community structure and accompanying gene content. In our study, the microbial community structure of BSCs were highly changed by SOC ($P = 0.006$, Table 3), consistent with the results of Wardle (2002), who found that the local scale spatial patterns of soil biota were determined by changes in the SOC. Bacteroidetes were considered to be a copiotrophic phylum categories commonly associated with

substrates of rich organic C (Fierer et al. 2007). These findings were supported by our results of a relatively lower abundance of Bacteroidetes and the lowest SOC in algal crusts than those in the lichen and moss crusts. Acidobacteria were viewed as oligotrophic groups whose abundance decreased in soil with high organic C concentrations (Fierer et al. 2007). However, the relative abundance of Acidobacteria and SOC were significantly higher in moss crusts than algal and lichen crusts. Similar results were obtained by Marilley and Aragno (1999), who found the relative abundance of Acidobacteria was lower in rhizosphere soil than in bulk soil, which considering that microorganism C availability should be higher in the rhizosphere. Jones et al. (2009) found that certain Acidobacteria subgroups were negatively correlated with pH and concluded that pH is the best predictor of Acidobacteria composition. Therefore, we conclude that this difference was possibly caused by the lower pH in moss crusts (Fig. 2).

Furthermore, the BSC type markedly shifted the functional structures of microbial communities, as indicated by PCoA and PERMANOVA analyses (Fig. 4b); these findings suggested that the functional structures were significantly different among the three different BSC types. Linear regression analyses showed that the microbial function were significantly correlated with microbial community composition (Fig. 4c), which indicated that the different functional potential was changed by different microbial community structures in different BSC types. In addition, the Mantel test showed that the microbial community functional composition and taxonomic structure were significantly shaped by several key soil physical and chemical variables, including SOC, SWC, TN, MBN, and MBC, which indicated that the microbial function structure of BSCs were mainly changed by TN ($P = 0.016$, Table 4). Together, these results indicated that the composition, structure, and potential functional activity of the microbial communities in the three BSC types were significantly different, and these differences were strongly controlled by microbial community structure and soil nutrient conditions. To the best of our knowledge, this is the first comprehensive study at the whole-community level to clearly demonstrate the changes in the composition and functional structure of microbial communities in response to different types of BSCs in a desert ecosystem.

C metabolic potential in different BSC types

Our results show that lichen crusts possessed a higher C fixation potential relative to the moss and algae crusts, as they showed a significantly greater relative abundance of C fixation genes (Fig. 5), especially in the 3-hydroxypropionate pathway and rTCA cycle. Several mechanisms would account for such different C fixation pathway potentials in BSCs, including soil environment, energy sources, and oxygen conditions (Bar-Even et al. 2011; Magnabosco et al. 2016). First, the 3-hydroxypropionate pathway prefers to fix C in an alkaline soil environment (Bar-Even et al. 2011). The pH in lichen crust is significantly higher than that of the moss crust, which indicated that lichen crusts may provide more adaptable conditions for microbes than moss crusts that exercise the 3-hydroxypropionate pathway. Second, since there are different energy requirements for the different carbon fixation pathways, microbes will employ the pathways with a low ATP requirement in desert ecosystem with poor energy sources. The higher SOC and TN content in moss crusts could provide substantial energy for C fixation, as compared with those in the lichen crusts, and may be by the primary reason for the different expression levels of genes involved in the rTCA cycle (2 ATP requirement, Bar-Even et al. 2011). However, while the SOC and TN content in algal crusts were significantly lower than those in the moss crusts, the relative abundance of rTCA cycle genes did not differ between algal and moss crusts. We suspected that some microbes that operate the rTCA cycle possess special biochemical adaptations to aerobic conditions (Hügler et al. 2007; Pieulle et al. 1997; Yamamoto et al. 2006). With the continuous succession of BSCs, the soil pore network of its profile was expand. At the same time, soil pore connectivity also increases with the increase in the number of macropores (Wang et al., 2011). Therefore, the porous property of moss crusts allows them to provide sufficient oxygen for microbes to fix C using the rTCA cycle, while microbes in algal crusts are able to function in conditions with lower oxygen using

biochemical adaptations.

A previous study in our research station found that the net photosynthesis is higher in moss crusts than those in lichen and algal crusts (Feng et al. 2014). We supposed that C fixation in BSCs is the result of a joint action between microbes and plants, such as cyanobacteria and moss. The main reason for this difference may be due to the different chlorophyll content in the three BSC types. Studies have shown that chlorophyll content can directly reflect the C fixation capacity of BSCs (Palmqvist 2000; Tuba et al. 1996). It is generally believed that moss has a higher chlorophyll content than lichen and algae (Lan et al., 2011), which could be used to fix more photosynthetic C. These findings were supported by our results of a relatively lower abundance of Cyanobacteria, and which indicated that moss could replace Cyanobacteria in moss crusts to fix C. Furthermore, C fixation capacity is potentially responsible or one of several potential factors, for example the water holding capacity and other in moss crusts (Grote et al. 2010; Yoshitake et al. 2010). BSCs have photosynthetic physiological activity only under moist conditions (Lange 1980). Our results show that the SWC in moss crusts were higher than those in algal and lichen crusts could be another important reason for higher C fixation in moss crusts, but lower C fixation genes.

Taken together, these results indicate that C fixation capacity in BSCs was not only shaped by the composition and abundance of genes that operate the specific carbon fixation pathways, but was also influenced by the corresponding desert soil conditions of each BSC. In short, the C fixation genes were higher in lichen crusts than algal crusts, indicating that lichen crust microbes could fix C in early development of BSCs, which could persistent drought conditions which were viewed as early BSCs are existed environment. Therefore, the higher C fixation genes in lichen crusts could resulted in the blockage of photosynthetic carbon sequestration, a factor that is of great significance to the nutrient input processes of desert ecosystems. After BSCs development, soil nutrient conditions were better than before, moss would replace Cyanobacteria in BSCs, which could play the major role in C fixation.

Different types of BSCs also significantly impacted a number of microbial functional genes that were important for C decomposition. Specifically, most of the genes from labile and recalcitrant C degradation were significantly higher in moss crusts than in algal and lichen crusts, including genes involved in producing monosaccharides, cell wall constituents, carboxylic acid, and lipids (Fig. 6). We suggested that the mainly reason for the difference of genes involved in C degradation maybe due to the difference between fungi. A previous study found that BSCs can establish a hyphal network though fungal communities in semiarid conditions to participate in C transformation and decomposition (Collins et al. 2008). These findings were maybe supported by our observations of a relatively higher relative abundance of fungi, and genes involved in degrading recalcitrant C in moss crusts than in algal and lichen crusts (Table 2). Vascular plants produce the bulk of the organic matter to supply soil organic matter in desert ecosystems that lack large reserves (Rudgers et al. 2018). The higher relative abundance of fungi in moss crusts may control C decomposition metabolism owing to the abundance of substrate in areas with abundant plants where moss crusts frequently exist. Conversely, there was low plant cover in algal and lichen crusts relative to those in the moss crusts, and certain fungi may be less important in algal and lichen crusts (McLain and Martens 2006; Porras-Alfaro et al. 2007; Strauss et al. 2012). Nevertheless, some fungal populations are more adaptable than bacterial populations in arid and semiarid conditions for they can active in lower levels of soil moisture than bacteria (Allen 2007), and can decompose the recalcitrant organic molecules that are unavailable to bacteria to obtain nutrients and alter the stability of soil organic matter (Porras-Alfaro et al. 2008, 2011).

N metabolic potential in different BSC types

The different BSC types displayed different genes involved in N cycling processes including nitrification, denitrification, N fixation, and N mineralization, suggesting that different BSC types had different microbial N metabolism potential in the desert ecosystem (Fig. 7). First, the relative abundance of most key genes involved in N loss (nitrification and denitrification) was significantly higher in moss crusts than in algal and lichen crusts.

Previous studies suggested that the nitrification process is mainly regulated by pH; higher pH could increase the solubility of organic matter to provide C and N substrates for microbes (Curtin et al. 1998; Mladenoff 1987). However, in our study, the relative abundance of genes involved in the nitrification process was higher in moss crusts, where pH was significantly lower, than in the algal and lichen crusts. This discrepancy may be due to allelopathic effects, and allelochemicals inhibiting the nitrification process in the algal and lichen crusts (Liu et al. 2013). The higher nitrification process in moss crusts provided more NO_3^-, which is the primary denitrification substrate involved in promoting the denitrification process. Nevertheless, studies have shown that nitrification is the dominant mechanism influencing N_2O emission (Klemedtsson et al., 1988; Ryden, 1981; Yang et al., 2014). As a result, the increasing of N_2O emissions also negatively affect soil NO_3^- content, which in turn increases the abundance of denitrification genes (Yang et al. 2014). Recent work has suggested that fungi in some arid-land soil have a vital role as denitrifiers in the N cycle (Marusenko et al. 2013). This finding is supported by our observations, in that a higher relative abundance of fungi and genes involved in denitrification were found in moss crusts than in algal and lichen crusts.

Our results show that lichen crusts possessed a higher N fixation potential and N mineralization potential owing to their significantly greater relative abundance of N fixation and N mineralization genes when compared with algal and moss crusts (Fig. 4). N fixation and N mineralization were the two major processes to supply and transfer available N in soil (Tu et al., 2017). Studies have suggested that BSCs obtain N from atmosphere was the predominant method to acquire N in desert ecosystems (Belnap 2001; Veluci et al. 2006). In desert ecosystem, Cyanobacteria were the major bacteria to fix N in BSCs, which was supported by the highest relative abundance of Cyanobacteria and *nifH* in lichen crusts. The *nifH* expression of algal and moss crusts did not differ significantly; however, the relative abundance of Cyanobacteria were greatly changed in our study. We assume that BSCs could obtain atmospheric N from other bacteria, such as Planctomycetes and Proteobacteria, that are proven N fixers in other ecosystems (Delmont et al. 2018). Considering that BSCs are N sink in desert, we indicated that the increasing N fixation and N availability in lichen crusts would offset the N loss in moss crusts, as well as maintain BSC development in desert ecosystems.

Our study provided an overall picture of microbial community and functional structures and the potential critical roles of microorganisms in C and N cycles in different BSC types. Our result could supply an useful information for understanding biochemical processes and nutrient cycling in desert. However, although significant differences in the relative abundance of genes for C and N cycling processes in different BSC types may lead to play different nutrient cycling potentially. More systematic and in-depth studies are needed to clarify the rates and extent of various stimulated nutrient cycling processes, and their impacts on the over-all BSCs nutrient dynamics in this ecosystem.

Conclusions

We investigated the microbial community, functional structure, and the relative abundance of C and N cycles in three types of BSCs in the Mu Us Desert in northern China using metagenomics. The results show that the microbial community structure and microbial function structure changed in different BSCs types, which are driven largely by soil nutrient conditions. In addition, lichen crusts possessed higher C and N fixation potential and moss crusts owed higher C metabolic and N loss potential, which could resulting positive soil nutrient feedback and improving BSCs development. Although we cannot accurately evaluate each portion and the amount of the C and N cycle based on metagenomics, our results indicate that BSCs microbial-mediated ecological function depends on which microbial communities are stimulated by each BSCs type and environmental condition. Thus, to improve the predictions of ecosystem feedback in different BSCs types, it is important to consider the various types of feedback mechanism resulting from the changes in microbial composition and functional structure.

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Figure and Table Legends

Fig. 1 Photographs of the three BSCs types ; (a) algal crust, (b) lichen crust, and (c) moss crust

Fig. 2 Soil property contents in the algal, lichen, and moss crust BSCs types. Data represent the mean ± SE (n = 3). Letters a, b, and c indicate a significant difference among the three sampling sites (Tukey's HSD; $P < 0.05$), respectively. SOC, soil organic carbon; SWC, soil water content; DOC, soil dissolved organic carbon; TN, total nitrogen; MBC and MBN, microbial biomass carbon and nitrogen. Algal crust, lichen crust, moss crust

Fig. 3 Heat map and clustering of relative microbial functional gene structure abundance of different BSCs types in KEGG level 1

Fig. 4 Differences in the algal, lichen, and moss crust BSCs types on the taxonomic (a) and functional (b) composition of soil microbial communities, and the relationship between the taxonomic and functional compositions (c). Algal crust, lichen crust, moss crust

Fig. 5 Carbon fixation in the algal, lichen, and moss crust BSCs types. The heat map shows the metagenome relative abundance reads of genes involved in the carbon fixation cycle, based on normalized sequencing data obtained from the three BSCs types. Letters a, b, and c indicate significant differences in the relative abundance of nitrogen cycle genes among the three BSCs types (Dunn's test, n = 3; $P < 0.05$), and no letter means no difference between in the relative abundance of nitrogen cycle genes among the three BSCs types. Algal crust, lichen crust, moss crust

Fig. 6 Carbon degradation in the algal, lichen, and moss crust BSCs types. The heat map shows the metagenome relative abundance reads of genes involved in carbon degradation, based on normalized sequencing data obtained from the three BSCs types. Letters a, b, and c indicate significant differences in the relative abundance of nitrogen cycle genes among the three BSCs types (Dunn's test, n = 3; $P < 0.05$), and no letter means no difference between in the relative abundance of nitrogen cycle genes among the three BSCs types. Algal crust, lichen crust, moss crust

Fig. 7 Nitrogen cycle in the algal, lichen, and moss crust BSCs types. (a) Heat map showing the metagenome relative abundance reads of genes involved in the nitrogen cycle based on sequencing data obtained from three types of BSCs. Letters a, b, and c indicate significant differences in the relative abundance of nitrogen cycle genes among the three BSCs types (Dunn's test, n=3; $P < 0.05$), and no letter means no difference between in the relative abundance of nitrogen cycle genes among the three BSCs types. Algal crust, lichen crust, moss crust

Fig. S1 The length distribution of samples

Fig. S2 Ordination of Bacterial (a), Fungal (b) and Archaea (c) community structure, based on the relative abundance Bacteria, Fungi, and Archaea phyla performing nonmetric multidimensional scaling (NMDS) using the Bray-Curtis similarity index (n = 3). Algal crust, lichen crust, moss crust

Table 1 Relative abundance of the microbial community composition in the algal, lichen, and moss crust BSCs types (n = 3). Letters a, b, and c in the same row indicate a significant difference at α = 0.05 (n = 3). Algal crust, lichen crust, moss crust

Table 2 Relative abundance of the main microbial phyla in the algal, lichen, and moss crust BSCs types (n = 3). Letters a, b, and c in the same row indicate a significant difference at α = 0.05 (n = 3). Algal crust, lichen crust, moss crust

Table 3 Correlation between microbial community structure and soil properties as shown by the Mantel test (n=3)

Table 4 Correlation between microbial function structure and soil properties as shown by the Mantel test (n=3)

Table S1 Data statistics of sequencing result (in megabases). Q20, Q30 represents the percentage of the number of bases in the Clean Data with a sequencing error rate of less than 0.01 (mass value greater than 20) and 0.001 (mass value greater than 30); GC (%) represents the percentage of GC base content in Clean Data; Effective Rate represents the percentage of valid data (Rean Data) to Raw Data.

Table S2 Results of analysis of variance using distance matrices (Adonis) for comparing the distributions of the microbial communities present in the algal, lichen, and moss crust BSCs types (n = 3)