Effects of Sand Burial on Bacterial Community Structure and Diversity in Bryum Crusts of Arid Desert Regions: Postprint

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Date: 2017-04-18T00:00:00+00:00

Abstract

As a common disturbance in arid sandy regions, sand burial significantly affects the structure and function of biological soil crusts, but the underlying biological mechanisms remain unclear. Using high-throughput sequencing technology, this study investigated the effects of sand burial on the structure and diversity of bacterial communities in Bryum argenteum crust layers in the Shapotou region at the southeastern edge of the Tengger Desert, by determining the species composition and abundance of bacterial communities after sand burial treatments of 0 (control), 0.5 (shallow), 2, and 10 mm (deep). The results showed that: (1) A total of 38 phyla, 106 classes, and 181 genera of bacteria were detected in the B. argenteum crust layer in the Shapotou region, with Actinobacteria, Proteobacteria, Cyanobacteria, Planctomycetes, Bacteroidetes, and Acidobacteria being the dominant groups (accounting for 78.4%–83.0% of the bacterial community); (2) PCA analysis indicated that sand burial caused significant changes in the community structure and composition of bacteria in the B. argenteum crust layer in this region. Without sand burial, Cyanobacteria had the highest relative abundance (18.6%) in the bacterial community of the B. argenteum crust layer. With increasing sand burial thickness, the dominant group successively changed to Proteobacteria (21.5%, sand burial thickness 0.5 mm), Planctomycetes (21.5%, sand burial thickness 2 mm), and Actinobacteria (23.3%, sand burial thickness 10 mm). Shallow sand burial significantly increased the abundance of key functional bacteria such as photosynthetic bacteria, nitrogen-fixing bacteria, and mycelium-producing bacteria in the B. argenteum crust layer bacterial community, but deep sand burial decreased their abundance; (3) Sand burial significantly increased both the diversity ($P<0.05$) and species richness ($P<0.05$) of the bacterial community in the B. argenteum crust layer. The bacterial community richness index was highest after 0.5 mm sand burial, while the crust layer bacterial community diversity index was highest after 2 mm sand burial. This study reveals the effects of sand burial on the

structure and diversity of bacterial communities in B. argenteum crust layers in arid sandy regions, providing a theoretical basis for deepening our understanding of the biological mechanisms through which sand burial affects the structure and ecological functions of biological soil crusts in sandy areas.

Full Text

Preamble

ACTA ECOLOGICA SINICA ChinaXiv Partner Journal Vol. 37, No. 7 Apr., 2017 DOI: 10.5846/stxb201601010002

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Teng J L, Jia R L, Zhao Y. Impact of sand burial on bacterial community structure and diversity within biocrusts dominated by *Bryum argenteum*. *Acta Ecologica Sinica*, 2017, 37(7): 2179–2187.

Impact of Sand Burial on Bacterial Community Structure and Diversity within Biocrusts Dominated by *Bryum argenteum* in Arid Sandy Regions

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Abstract

As a key component of biocrusts that constitute up to or more than 70% of living cover in arid and semiarid lands worldwide, bacteria play a primary role in carbon and nitrogen inputs in deserts. Thus, changes in bacterial community structure and diversity can significantly alter their ecological processes and the functions of biocrusts. Sand burial is a common environmental stress for biocrusts in arid and semiarid areas, yet little information is available regarding the effects of sand burial on bacterial community structure and diversity within biocrusts. Therefore, we adopted high-throughput sequencing techniques to investigate the effects of sand burial on bacterial community structure and diversity of biocrusts dominated by *Bryum argenteum* following sand burial treatments of 0 (control), 0.5, 2, and 10 mm in Shapotou, southeastern edge of the Tengger Desert. Bacterial community species composition, abundance variation, and diversity indices including the Shannon-Wiener diversity and richness indices Chao and abundance-based coverage estimator were compared among biocrusts that suffered sand burial at various depths.

The results showed the following: (1) In total, 38 phyla, 106 classes, and 181 genera were identified within biocrusts dominated by *B. argenteum*, of which the dominant bacterial phyla included Actinobacteria, Proteobacteria, Cyanobacteria, Planctomycetes, Bacteroidetes, and Acidobacteria, which comprised 78.4%–83.0% of the whole community. (2) Principal component analysis showed that, compared to the control, the bacterial community structure was the most variable in soils at 2 and 10 mm depths. Sand burial induced significant changes in bacterial community composition; with the highest abundance were Cyanobacteria, Proteobacteria, Planctomycetes and Actinobacteria following 0, 0.5, 2 and 10 mm burial depths, respectively. With the increase in burial depth, the relative abundance of Actinobacteria, Proteobacteria, Planctomycetes, Bacteroidetes, Verrucomicrobia, Gemmatimonadetes, and FBP increased, while that of the phyla Cyanobacteria, Acidobacteria, and Chloroflexi decreased. In addition, the abundance of photosynthetic bacteria, nitrogen-fixing bacteria, and mycelial genera of Actinobacteria all increased largely at 0.5 mm burial depth and decreased sharply at 2 and 10 mm burial depths. (3) The total count of bacteria, species richness, and microbial diversity of biocrusts dominated by *B. argenteum* increased following sand burial, among which the biocrusts subjected to 0.5 mm burial depth had the highest richness indices and biocrusts subjected to 2 mm burial depth had the highest diversity indices.

The study demonstrated that various depths of sand burial had significant effects on bacterial community and diverse features within biocrusts dominated by *B. argenteum* in Shapotou and thus offered the theoretical foundation for further understanding of the influence mechanism of sand burial on the structure and ecological functions of biocrusts in arid desert areas.

Keywords: *Bryum argenteum*; high-throughput sequencing techniques; sand burial; bacteria; community structure and diversity

Introduction

Biocrusts are complex composites formed by the cementation of soil surface particles with cryptogams such as cyanobacteria and mosses, soil bacteria, rhizoids, and secretions. As an important feature of sandy ecosystem composition and surface landscape, biocrusts play crucial roles in sandy landscape patterns, soil biological and geochemical cycling processes, and ecological restoration in arid and semiarid regions. The structure and function of biocrusts are easily affected by environmental and disturbance factors. Wind-sand burial is one of the common disturbances that biocrusts frequently suffer in arid sandy areas. Due to their surface habitat characteristics, low stature, and poikilohydric nature, previous studies have shown that sand burial not only significantly changes the growth and succession of moss crusts, reduces the extracellular polysaccharide content and total sugar reserves of cyanobacterial crusts, but also affects the photosynthetic rate of crusts, gas fluxes, and the bioavailability of nitrogen in cyanobacterial crusts. However, the underlying biological mechanisms of how sand burial affects the ecological functions of crusts remain unclear.

Current research has found that sand burial can alter environmental conditions such as soil temperature, soil moisture, water vapor condensation, and light intensity. However, beyond these environmental factor changes, the above physiological processes all involve microbial participation. At the community level, some studies have found that sand burial may increase soil microbial numbers and change microbial community structure. Whether sand burial affects these biocrust functions by influencing microbial community structure and diversity within the biocrust layer remains an open question.

Bacteria, as the most abundant microbial population in the biocrust layer, play key roles in carbon and nitrogen fixation and organic matter decomposition. Their community structure and diversity can sensitively reflect ecological functions and environmental changes in sandy areas. Photosynthetic bacteria participate in the carbon cycle process of crusts, which can improve ecosystem productivity in sandy areas. Nitrogen-fixing bacteria increase the nitrogen fixation capacity of crusts, making them more adapted to nitrogen-poor sandy ecosystems. Extracellular polysaccharide-producing bacteria have multiple functions, including increasing soil carbohydrate reserves, preventing attacks by bacteriophages and protozoa, cementing sand particles, and stabilizing soil aggregates. Some bacteria that can be preyed upon by protozoa such as amoebae in biocrusts are also an important part of the soil food web. Studying the bacterial community structure and diversity of biocrust layers can provide important information for a deeper understanding of biocrust ecological functions.

Currently, research on bacterial communities in biocrust layers mainly focuses on cyanobacterial crusts and their primary producers in arid and semiarid regions. Studies on other types of crusts, heterotrophic organisms in crusts, and the effects of disturbances on crust microbial communities are relatively scarce. With the development of molecular biology theory and technology in microbial ecology research, new breakthroughs have been made in soil microbial diversity studies, and a large number of previously unrecognized microbial species and their new functions have been identified and applied. High-throughput sequencing technology, as a revolutionary technological innovation in nucleic acid sequencing research, provides new scientific methods and solutions for more comprehensive, accurate, and in-depth exploration of microbial community composition, ushering in a new chapter for research on microbial community structure and function in biocrust layers. However, most current studies focus on exploring microbial communities in biocrust layers under different habitats.

For example, Maier et al. used pyrosequencing to study the prokaryotic microbial community structure and diversity of lichen crusts in the Tabernas Basin of Spain and compared the differences in microbial community structure between moss crust layers and crust-covered soils. Abed et al. compared microbial communities in aeolian dust from saline lake sediments and adjacent biocrust layers in southern Australia. Angel et al. explored the resuscitation process of microbial communities in crust layers after simulated precipitation. Dojani et al. investigated the diversity of cyanobacteria in biocrust layers of arid grasslands

in South Africa. However, reports on the effects of sand burial on bacterial communities in biocrust layers are rare.

This study focuses on the dominant moss crust *Bryum argenteum* Hedw. growing in the artificial vegetation area of Shapotou on the southeastern edge of the Tengger Desert. Using high-throughput sequencing technology, we investigated the effects of different thicknesses of sand burial treatment on the bacterial community structure and diversity of the *B. argenteum* crust layer, aiming to reveal the characteristics of microbial community diversity and structural changes in the *B. argenteum* crust layer after sand burial disturbance in this region. This research provides a scientific basis for further understanding the impact of sand burial on microbial community functions in sandy areas and for deepening our comprehension of the mechanisms underlying the effects of sand burial on biocrust structure and ecological functions.

1. Study Area Overview

The test biocrusts were collected from the artificial vegetation area north of the Baolan Railway at the Shapotou Desert Research and Experimental Station, Chinese Academy of Sciences (37°32 N, 105°02 E). The site has an elevation of 1330 m, with a multi-year average temperature of 9.6°C and average annual precipitation of 186.5 mm, concentrated between July and September. The artificial sand-fixing vegetation was established in 1964 and 1981, with multiple expansions. The main sand-fixing shrubs and semi-shrubs are *Artemisia ordosica*, *Caragana korshinskii*, *Hedysarum scoparium*, *Salix psammophila*, and *Salsola ruthenica*. Dominant herbaceous plants include *Didymodon vinealis*, *Syntrichia caninervis*, *Eragnostis poaeoides*, and *Bassia dasyphylla*. Algae and lichens mainly include *Bryum argenteum*, *Microcoleus vaginatus*, *Lyngbya cryptovaginatus*, *Scytonema javanicum*, *Navicula cryptocephala*, and *Endocarpon pusillum*. The soil stable water content is 2%–3%.

2. Sand Burial Treatment

The experiment used *Bryum argenteum* crusts distributed in the windward slope of the artificial vegetation area established in 1964. In early April, experimental samples were selected for sand burial treatment within the study area. In the windward slope, *B. argenteum* crust-covered areas were randomly selected, and PVC pipes with a diameter of 10 cm and height of 20 cm were inserted into the crust soil. Before sand burial treatment, other herbaceous plants and litter outside the crust were manually removed. Then, the volume-surface area method was used for sand burial, with the upper edge of the pipe 0.5 cm above the internal *B. argenteum* crust or sand surface.

Four thicknesses of sand burial treatment were applied: 0 (control), 0.5, 2, and 10 mm, with 3 replicates per treatment. During sand burial, care was taken to ensure sand was evenly sprinkled on the crust. Each sample was labeled, and during the experiment, if sand cover changed dramatically (e.g., after windy

weather), sand was added or leveled with a soft brush to restore the original condition as much as possible.

3. Sample Collection

Samples were collected in early July. During collection, sterile brushes were used to gently sweep away the sand covering the *B. argenteum* crust surface. Petri dishes were inverted on the crust surface to avoid edge effects, and biocrust samples about 3–5 mm thick were collected from the center of the PVC pipe. For each sand burial treatment, three *B. argenteum* crust samples were collected and mixed evenly as one sample, then passed through a 2 mm soil sieve and placed in sterile ziplock bags. Samples were stored on dry ice, transported back to the laboratory, and stored at -80°C.

5. High-Throughput Sequencing

Soil total DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio, USA). The concentration and purity were determined by UV spectrophotometer, and integrity was detected by agarose gel electrophoresis. The samples were stored at -20°C for later use. Bacterial universal primers 520F (5 -AYTGGGYDTAAAGNG-3) and 802R (5 -TACNVGGGTATCTAATCC-3) were selected to amplify the V4 high-variability region fragments of bacterial 16S rRNA. PCR amplification was performed as follows: 98°C for 5 min; 30 cycles of 98°C for 30 s, 50°C for 30 s, 72°C for 30 s; and final extension at 72°C for 5 min. After agarose gel electrophoresis detection, the products were sequenced on the Illumina MiSeq platform. The sequencing was commissioned to Shanghai Personal Biotechnology Co., Ltd.

6. Bioinformatics Analysis

First, the raw data underwent quality filtering and paired-end sequence connection. The connected sequences were filtered and chimeras removed. Then, high-quality sequences were subjected to Operational Taxonomic Unit (OTU) clustering and annotation analysis at 97% similarity level, where each OTU represents a specific species. Dilution curves were plotted, and diversity index analysis was performed. Alpha diversity refers to the diversity within a specific region or ecosystem. The mothur software was used to generate species abundance tables at different classification levels and multi-sample species distribution maps, calculate biodiversity indices (Chao, Ace, Shannon), and perform principal component analysis (PCA) on genus-level classification and species abundance. One-way ANOVA was used to compare differences in bacterial abundance at different classification levels and diversity indices (Chao, Ace, and Shannon) of *B. argenteum* crust bacterial communities under different sand burial thickness treatments. Significance tests used the least significant difference (LSD) method. QIIME was used for OTU clustering and annotation.

2. Results Analysis

2.1 Effects of Sand Burial on Bacterial Community Diversity in the *Bryum argenteum* Crust Layer

After quality control processing of all raw sequences, including connection and chimera removal, low-quality sequences were deleted, resulting in 2858–3079 high-quality sequences per sample for subsequent analysis. The average library size was 3509.78 ± 4.01 to 3774.06 ± 2.64. Alpha diversity analysis showed that sand burial significantly increased the species richness and diversity indices of bacterial communities in the *B. argenteum* crust layer. The richness indices of crust bacterial communities showed the pattern: 0.5 mm > 2 mm > 10 mm > 0 (control), and the increase in species richness decreased with increasing sand burial thickness. The diversity indices (Shannon) showed the pattern: 2 mm > 10 mm > 0.5 mm > 0 (control). Sand burial significantly increased the species richness and diversity indices of bacterial communities in the *B. argenteum* crust layer ($P < 0.05$).

Table 1 Effects of sand burial on richness and diversity indices of bacterial communities within biocrusts

Treatments	Richness Index	Diversity Index (Shannon-Wiener)	Coverage
0 (Control)	3464.59 ± 21.16 a	5.62 ± 0.02 a	0.98 ± 0.01
0.5 mm	3715.12 ± 1.39 b	6.21 ± 0.01 b	0.98 ± 0.01
2 mm	3613.62 ± 8.59 c	6.54 ± 0.04 c	0.98 ± 0.01
10 mm	3554.45 ± 11.80 d	6.48 ± 0.16 c	0.98 ± 0.01

Note: Different letters indicate significant differences at $P < 0.05$ level.

2.2 Effects of Sand Burial on Bacterial Community Structure in the *Bryum argenteum* Crust Layer

Community composition analysis was performed at phylum and class levels for each sample. The results showed that at the phylum level, the dominant bacterial groups in the *B. argenteum* crust layer included Actinobacteria (15.3%-23.3%), Proteobacteria (17.0%-21.5%), Cyanobacteria (0.7%-18.6%), Planctomycetes (8.8%-21.5%), Bacteroidetes (9.0%-16.9%), and Acidobacteria (6.5%-12.5%). These six bacterial groups accounted for 78.4%-83.0% of the bacterial community in the *B. argenteum* crust layer. The highest relative abundance was Cyanobacteria (18.6%) in the control, which successively changed to Proteobacteria (21.5%) at 0.5 mm, Planctomycetes (21.5%) at 2 mm, and Acidobacteria (12.5%) at 10 mm burial depth.

At the class level, the most abundant bacteria included Alphaproteobacteria (13.4%-15.5%), Actinobacteria (8.6%-11.4%), Chloroplast (0.3%-15.5%), Planctomycetia (4.2%-11.5%), and Phycisphaerae (3.2%-9.9%). With increasing sand burial thickness, the most abundant bacterial group at the class level changed from Chloroplast of Cyanobacteria to Alphaproteobacteria. The bacterial community composition at phylum and class levels showed that sand-buried *B. argenteum* crust bacterial communities were more similar to each other, with higher similarity among sand-buried crust bacterial communities (71.49% similarity between 0.5 mm and 10 mm treatments).

Figure 1 [Figure 1: see original paper] Relative abundances of different phyla and classes in the bacterial communities within biocrusts following sand burial

Principal component analysis (PCA) revealed similar results regarding community variation. The bacterial communities of crusts under different sand burial thickness treatments all showed obvious positional changes compared to the control and were divided into two groups: shallow sand burial treatments (0.5 mm) clustered at one end, while 2 mm and 10 mm sand burial treatments clustered at the other end, indicating that sand burial caused significant changes in the bacterial community of the *B. argenteum* crust layer.

Figure 2 [Figure 2: see original paper] Principal components analysis (PCA) of the bacterial communities within biocrusts following sand burial

2.3 Effects of Sand Burial on Phylum-Level Species Abundance in the *Bryum argenteum* Crust Layer

Phyla with increased abundance after sand burial included Actinobacteria, Planctomycetes, Proteobacteria, Bacteroidetes, Verrucomicrobia, Gemmatimonadetes, and FBP. Among these, the increase in relative abundance of Planctomycetes reached significant levels after 10 mm sand burial ($P < 0.05$). Phyla with decreased abundance after sand burial included Cyanobacteria, Acidobacteria, Chloroflexi, and FBP. Only the relative abundance of Cyanobacteria decreased significantly after 10 mm sand burial ($P < 0.05$), while the relative abundance of FBP decreased significantly after 10 mm sand burial ($P < 0.05$).

Figure 3 [Figure 3: see original paper] Effects of sand burial on abundant phyla (>1% of OTUs) of bacterial communities within biocrusts

Among Actinobacteria, mycelium-producing genera *Geodermatophilus*, *Nocardioides*, and *Pseudonocardia* showed significantly increased abundance after 0.5 mm sand burial ($P < 0.05$), but their relative abundance decreased significantly when sand burial thickness increased. The highest abundance cyanobacterium in the Cyanobacteria phylum was oxygenic photosynthetic algae *Phormidium*, whose abundance increased significantly after 0.5 mm sand burial but decreased sharply after deeper burial. Non-oxygenic photosynthetic bacteria such as

Methylobacterium and *Belnapia* increased significantly after 0.5 mm sand burial but showed a decreasing trend after deeper burial. Filamentous cyanobacteria were not detected in any of our samples. *Microcoleus*, *Nostoc*, and *Scytonema* are the dominant cyanobacterial genera in mature biocrusts. Although *Microcoleus* is widely present in crusts and is the most abundant filamentous cyanobacterium in *B. argenteum* crusts, it was not detected in our samples. Different regions may select different filamentous cyanobacteria as dominant bacterial members. Studies have shown that biocrust functional recovery after disturbance depends on filamentous cyanobacteria for structural reconstruction and photosynthetic capacity restoration.

In addition to dominant bacterial groups, sand burial significantly increased the abundance of nitrifying spirilla in the *B. argenteum* crust layer, which increased significantly after 0.5 mm sand burial but showed a decreasing trend after deeper burial. This may help improve the crust' s resistance to disturbance. The increase in photosynthetic and nitrogen-fixing bacteria abundance in the *B. argenteum* crust layer after shallow sand burial (0.5 mm) means increased carbon and nitrogen input to the crust, but the decrease after deeper burial may reduce the biocrust' s recovery ability after sand burial removal.

3. Discussion

The dominant bacterial groups in the *B. argenteum* crust layer of the Shapotou area were Actinobacteria, Proteobacteria, and Cyanobacteria, which is basically consistent with previous research results. However, the dominant bacterial groups differ among different study areas. Our research on the *B. argenteum* crust in the Shapotou area and studies on the Sonoran Desert, Colorado Plateau, and Helan Mountain areas found that Actinobacteria and Proteobacteria are ubiquitous bacterial groups in biocrusts of various study areas. However, differences exist: Proteobacteria dominated in the Hunshandake Sandy Land crusts, while Cyanobacteria had the highest abundance in crusts of the Colorado Plateau and Helan Mountain. Previous studies have shown that soil microbial community composition is influenced by specific habitat environmental factors such as soil moisture content, carbon availability, and salinity. These differences in bacterial community structure among different regions may be caused by differences in environmental factors of different habitats.

Sand burial changes the environmental conditions of biocrusts, such as soil temperature, soil moisture, water vapor condensation, and light intensity. Due to the death of crust organisms and the self-dissolution of cyanobacterial cells and the resulting release of extracellular nitrogen, long-term sand burial may increase the bioavailable nitrogen content of algal crusts. Numerous studies have shown that soil physical and chemical properties, such as nutrient availability, and climatic conditions like temperature and sunshine duration, have extensive effects on bacterial diversity and community structure. Therefore, sand burial is bound to affect the microbial community structure and diversity of the crust layer.

This study shows that sand burial had significant effects on both the bacterial community structure and diversity of the *B. argenteum* crust layer. All sand burial thicknesses increased the bacterial community richness of the crust layer. The reason for this change may be that sand burial reduces solar radiation intensity and increases soil moisture, thereby providing a more suitable living environment for bacteria in the community. The bacterial community richness of crusts after 0.5 mm sand burial was significantly higher than that of crusts after 10 mm sand burial. This may be because 0.5 mm sand burial, while moderately reducing evaporation, does not isolate the crust from air and light, providing relatively suitable conditions for photosynthetic bacteria such as cyanobacteria and aerobic bacteria, while also providing suitable living conditions for certain bacterial populations that were not originally adapted to grow here, increasing their dominance in the community and thus increasing community richness.

The bacterial community diversity of the *B. argenteum* crust layer also increased significantly after sand burial. The community diversity of 0.5 mm sand-buried crusts was significantly lower than that of the other two thicknesses (2 mm and 10 mm). Community diversity includes both species richness and evenness. Although 0.5 mm sand burial increased species richness in the crust bacterial community, it may have reduced the evenness of species distribution. Shallow sand burial provides a relatively suitable environment for microorganisms in the biocrust layer, promoting the development of various bacterial groups, but limited resources inevitably cause competition for nutrients among bacterial groups, leading to differences in abundance among species and thus reducing the evenness of species distribution.

Actinobacteria and Proteobacteria are the most abundant bacterial groups in the *B. argenteum* crust layer of the Tengger Desert artificial vegetation area. However, with increasing sand burial thickness, the abundance of Cyanobacteria gradually decreased, while the abundance of Actinobacteria increased with sand burial and successively became the dominant species under 0.5, 2, and 10 mm sand burial treatments. Although Cyanobacteria can survive for long periods in arid environments, they are also prone to autolysis during drought periods. The humidity increase caused by sand burial may regulate bacterial respiratory metabolism, enhance decomposition, and lead to extracellular polysaccharide (EPS) degradation. Both aspects ultimately cause the abundance of Cyanobacteria in the community to decline due to insufficient light to activate photosynthesis after sand burial.

The increase in abundance of mycelium-producing bacteria, nitrogen-fixing bacteria, and non-oxygenic photosynthetic bacteria after shallow sand burial indicates that shallow sand burial is beneficial for the physiological and ecological functions of moss crusts. However, with increasing sand burial thickness, the decrease in abundance of specific functional bacterial groups such as nitrogen-fixing bacteria, photosynthetic bacteria, and mycelium-producing bacteria may mean reduced recovery ability of biocrusts after disturbance, affecting biocrust function performance and succession processes. Deep sand burial creates a mi-

croenvironment without light or gas exchange for biocrusts. The sharp decline in Cyanobacteria abundance may cause a decrease in biomass and nutrients in the crust layer, thereby reducing the recovery capacity of biocrusts after sand burial removal.

Sandy areas have exceeded one-third of the Earth' s land surface area. Soil microorganisms in sandy areas serve as pioneers in ecological environment restoration, and their community structure and diversity can sensitively reflect sandy area ecological functions and environmental changes. Bacteria, as important components of biocrusts in sandy areas, have significant impacts on the maintenance and performance of biocrust structure and function. Sand burial affects biocrust structure and ecological functions by altering bacterial and other microbial community structures. The increase in heterotrophic bacterial abundance caused by deep sand burial may increase bacterial decomposition rates and accelerate carbon and nitrogen loss in sandy soils.

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Note: Figure translations are in progress. See original paper for figures.