Development of bacterial communities in biological soil crusts along a revegetation chronosequence in the Tengger Desert, northwest China

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Abstract. Knowledge of structure and function of microbial communities in different successional stages of biological soil crusts (BSCs) is still scarce for desert areas. In this study, Illumina MiSeq sequencing was used to assess the compositional changes of bacterial communities in different ages of BSCs in the revegetation of Shapotou in the Tengger Desert. The most dominant phyla of bacterial communities shifted with the changed types of BSCs in the successional stages, from Firmicutes in mobile sand and physical crusts to Actinobacteria and Proteobacteria in BSCs, and the most dominant genera shifted from Bacillus, Enterococcus and Lactococcus to RB41_norank and JG34-KF-361_norank. Alpha diversity and quantitative real-time polymerase chain reaction (PCR) analysis indicated that bacterial richness and abundance reached their highest levels after 15 years of BSC development. Redundancy analysis showed that silt + clay content and total K were the prime determinants of the bacterial communities of BSCs. The results suggested that bacterial communities of BSCs recovered quickly with the improved soil physicochemical properties in the early stages of BSC succession. Changes in the bacterial community structure may be an important indicator in the biogeochemical cycling and nutrient storage in early successional stages of BSCs in desert ecosystems.

1 Introduction

Biological soil crusts (BSCs) are assemblages of cryptogamic species and microorganisms, such as cyanobacteria, green algae, diatoms, lichens, mosses, soil microbes and other related microorganisms that cement the surface soil particles through their hyphae, rhizines/rhizoids and secretions (Eldridge and Greene, 1994; Li, 2012; Pointing and Belnap, 2012; Weber et al., 2016). Due to their specialized structures and complicated assemblages of their members, BSCs constitute one of the most important landscapes and make up 40 % of the living cover of desert ecosystems, even exceeding 75 % in some special habitats (Belnap and Eldridge, 2003).

It is well known that BSCs play critical roles in the structure and function of semiarid and arid ecosystems (Eldridge and Greene, 1994; Li, 2012). They provide ecological services such as soil stabilization, reduction of wind and water erosion, and facilitation of higher plant colonization (Belnap, 2003; Belnap and Lange, 2001; Maier et al., 2014; Pointing and Belnap, 2012). BSCs are functionally important and variable, and may be a useful model system for diversity-function research. Their functional attributes are relatively well known, and estimation and manipulation of biodiversity in experiments are feasible, at least within some groups of BSC biota (Bowker et al., 2010). This relationship is more easily interpreted in artificially constructed BSCs. There are primary successional stages for BSCs in desert ecosystems: mobile sand, algal crust, lichen crust and moss crust (Lan...
et al., 2012a; Liu et al., 2006). The different successional stages of BSCs vary in their ecological function (Belnap, 2006; Bowker et al., 2006b; Li, 2012; Moquin et al., 2012).

During BSC succession, physical crusts in mobile sand contain the lowest carbon (C) and nitrogen (N) contents (Zhang et al., 2009). Alg al crust is the earliest stage; it has a thin surface layer composed of eolian-borne materials and an organic layer formed by filamentous cyanobacteria associated with sand particles (Housman et al., 2006; Zhang, 2005; Zhang et al., 2009). Lichen and moss appear following stabilization of the algal filaments on the soil surface. The C and N fixation rates are increased in lichen crust (Evans and Lange, 2003; Lan et al., 2012b; Zhang et al., 2010), and there is higher photosynthesis, exopolysaccharide and nitrogenase activity in moss crust than in the early successional crusts (Housman et al., 2006; Lan et al., 2012b). In the BSC successional process, the microbial composition and community structure change greatly (Hu and Liu, 2003; Zhang et al., 2009). Crust succession is positively correlated with phospholipid fatty acid content and microbial biomass (Liu et al., 2013). The microbial biomass of soils is the greatest driving force in most terrestrial ecosystems, largely due to control of conversion rates and mineralization of organic matter (Albianch et al., 2000; Baldrian et al., 2010).

Bacteria present the highest proportion of the microbial biomass in BSCs (Bates et al., 2010; Green et al., 2008; Gundlapally and Garcia-Pichel, 2006; Maier et al., 2014; Wang et al., 2015) and thus have important roles in the BSC successional process. They can decompose organic material and release nutrients, mediating geochemical processes necessary for ecosystem functioning in the persistence of BSCs (Balser and Firestone, 2005). Species composition and community structure of bacteria change greatly during the successional process of BSCs (Gundlapally et al., 2006; Moquin et al., 2012; Zhang et al., 2016). Most research on prokaryotic diversity of BSCs has focused on cyanobacteria-dominated biocrusts in arid and semiarid regions (Abed et al., 2010; Garcia-Pichel et al., 2001; Nagy et al., 2005; Steven et al., 2013; Yeager et al., 2004). Recent studies of the bacterial community structure of bryophyte- or lichen-dominated crusts indicate that lichen-associated communities encompass a wide taxonomic diversity of bacteria (Bates et al., 2011; Cardinale et al., 2008; Maier et al., 2014). Heterotrophic bacteria may perform a variety of roles such as nutrient mobilization and N fixation and could be of considerable importance for the stability of lichen-dominated soil communities. However, there have been few studies on changes of bacterial diversity and their function in BSCs during the development process in desert zones, and these have only focused on the Sonoran (Nagy et al., 2005) and Gurbantunggut deserts (Zhang et al., 2016). What changes occur in bacterial community composition and in their potential roles in improving soil properties for different BSC successional stages? What is the significance of these changes to BSC succession in the recovery process of desert revegetation in temperate zones?

Bowker (2007) examined the role of BSCs in primary succession (vs. secondary succession) during a time when resources were available (e.g., light); however, they became less important once higher vegetation took over. In some environments of high abiotic stress (e.g., deserts), BSCs play a role in succession but remain a permanent component. Bowker’s review and discussion is supported by work performed in southern Africa (Büdel et al., 2009) in which different successional BSCs are described. Büdel et al. (2009) also describe in detail crust types that were representative of successional stages. Castillo-Monroy et al. (2011) showed few BSC effects on ecosystem function could be ascribed to bacteria.

A recent study on crusts in the Tengger Desert, China, showed that bacterial diversity and richness were highest after 15 years, and at least 15 years might be needed for recovery of bacterial abundance of BSCs (Liu et al., 2017). To better understand these questions, we must analyze in detail the bacterial community composition of BSCs at all levels of classification and their corresponding function in the recovery process of BSCs. In the present study, bacterial community composition and potential function were analyzed in BSCs along a chronosequence of over 50-year-old revegetation. We investigated the following questions. What are the drivers of bacterial composition over time? What are the micro-processes that drive bacterial composition and function? Do bacteria drive changes in soil physicochemical properties which in turn have a direct influence on bacterial composition and function?

2 Materials and methods

2.1 Study site description

The study site is located in Shapotou, at the southeast fringe of the Tengger Desert, northwest China. The natural landscape is characterized by the reticulated chains of barchan dunes with a vegetation cover of less than 1%. The mean annual precipitation is about 180 mm with large seasonal and interannual variation. The mean wind speed is 3.5 m s⁻¹, and on average 122 d yr⁻¹ days with dust events. The revegetation protection system for Bao–Lan railway in this area was established initially in 1956 and was expanded away from the railway in 1964, 1973, 1981 and 1987 through establishment of straw checkerboards and plantation of xerophilous shrubs. This unirrigated revegetation system has worked well in protecting the railroad line from sand burial and dust hazards for the past 60 years. Also, the experimental plots of less than 1 ha were established with the same plantation techniques by the Shapotou Desert Research and Experiment Station in 1987, 2000 and 2010 in the nearby sand dunes. These fixed-sand areas provide an ideal temporal succession sequence.
Figure 1. Sand dune landscape before (MS, a) and after establishing sand-binding vegetation with physical crusts dominated by few algae, revegetated in 2010 (5YR, b); with BSCs dominated by algae and lichens, revegetated in 2000 (15YR, c); with BSCs dominated by lichens and few mosses, revegetated in 1987 (28YR, d); with BSCs dominated by few lichens and mosses, revegetated in 1981 (34YR, e); and with BSCs dominated by mosses, revegetated in 1964 (51YR, f). Five soil cores (3.5 cm diameter) with crust layers from four vertices of a square (20 m length) and a diagonal crossing point in each plot were sampled individually (as shown in c).

for studying the variation in environmental factors. As mentioned in the literature, the initial state of BSCs begins to form following the stabilization of sand dunes and develop with the colonization of cryptogam (Liu et al., 2006). BSCs can be divided into four types: physical, algae-dominated, lichen-dominated and moss-dominated crusts. In this study, we selected the whole BSC layer from the revegetation established in 1964, 1981, 1987, 2000 and 2010, and non-fixed mobile sand (MS) as the control (Fig. 1). BSCs were sampled in early November 2015 and named according to the fixed-sand time as 51YR (51 years of revegetation), 34YR, 28YR, 15YR, 5YR and MS, respectively. The main types of BSCs were algae-, lichen- and moss-dominated crusts from 15YR to 51YR.

2.2 BSC sampling

The detailed sampling method is shown in Fig. 1c, and BSCs were sampled individually using a sterile trowel. To decrease spatial heterogeneity, each BSC sample was taken from six individual plots (at least 20 m between two adjacent plots) from each revegetation time. Therefore, we obtained 30 BSC samples in total (5 cores × 6 individual plots), and these were mixed together to form one composite BSC sample. Triplicate composite samples for each revegetation time were collected, and the BSC samples were preserved in an ice box. Samples were then taken back to the laboratory, immediately sieved (by 1 mm) to remove stones and plant roots, homogenized thoroughly and stored at −70 °C for subsequent analyses.
2.3 DNA extraction and Illumina MiSeq sequencing

Microbial DNA was extracted from BSC samples using E.Z.N.A. Soil DNA (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer’s protocols. The extracted DNA was diluted in TE buffer (10 mM Tris-HCl and 1 mM EDTA at pH 8.0) and stored at −20°C until use. An aliquot of the extracted DNA from each sample was used as a template for amplification. The bacteria 16S ribosomal RNA (rRNA) gene was amplified by polymerase chain reaction (PCR) (95°C for 3 min; followed by 25 cycles at 95°C for 30 s, 55°C for 30 s and 72°C for 45 s; and a final extension at 72°C for 10 min) using primers 338F (5’-ACTCCTACGGGAGGCAGCA-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’). PCR analyses were performed in triplicate 20 µL mixture containing 2 µL of 5× FastPfu Buffer, 2 µL of 2.5 mM dNTPs, 0.8 µL of each primer (5 µM), 0.2 µL of FastPfu Polymerase and 10 ng of template DNA. This was conducted according to Wang et al. (2015). Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer’s instructions and quantified using QuantiFluor™-ST (Promega Corporation, Madison, WI, USA).

Purified amplicons were pooled in equimolar and paired-end sequenced (2×300) on an Illumina MiSeq platform according to the standard protocols at Majorbio Bio-Pharm Technology Co. Ltd., Shanghai, China (http://www.majorbio.com). The raw reads were deposited in the NCBI Sequence Read Archive database (accession number: SRP091312).

2.4 Quantitative real-time PCR (qPCR)

qPCR was performed to determine the absolute 16S rRNA gene abundance. We used the primer sets of 515F (5’-GTGCCAGCMGCCCAGTTA-3’) and 806R to quantify the total bacterial populations. The standard templates were made from 10-fold dilutions of linearized plasmids containing the gene fragment of interest that was cloned from amplified pure culture DNA. The reaction was conducted according to the standard protocols at Majorbio Bio-Pharm Technology Co. Ltd., Shanghai, China (http://www.majorbio.com). The raw reads were deposited in the NCBI Sequence Read Archive database (accession number: SRP091312).

2.5 Processing of sequencing data

Raw FASTQ files were demultiplexed and quality-filtered using QIIME (version 1.17) with the following criteria: (i) the 300 bp reads were truncated at any site receiving an average quality score < 20 over a 50 bp sliding window, discarding the truncated reads shorter than 50 bp; (ii) there was exact barcode matching, with at most two mismatched nucleotides in primer matching, and reads containing ambiguous characters were removed; and (iii) only sequences that overlapped > 10 bp were assembled according to their overlap sequence. Reads that could not be assembled were discarded.

Operational taxonomic units (OTUs) were clustered with 97% similarity cut-off using UPARSE (version 7.1 http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://www.rdp.cme.msu.edu/) against the SILVA (SSU115) 16S rRNA database using a confidence threshold of 70%. Hierarchical clustering analysis was performed using Cluster and visualized using TreeView, and other statistical analyses were performed with the Institute for Environmental Genomics (IEG) pipeline (http://ieg.ou.edu). The average data were calculated for BSCs of each revegetation before analyzing the unique and shared OTUs/genera. The figures were generated with OriginPro 9.1 and Excel 2013. Alpha-diversity analysis was used to reflect the richness and diversity of microbial communities. In order to investigate the overall differences in community composition among the samples, principal component analysis (PCA) was performed using unweighted UniFrac distance (Lozupone and Knight, 2005). Redundancy
analysis (RDA) was used to assess the relationship between bacterial compositions of BSCs and top soil physicochemical properties by permutation test analysis (Zhang et al., 2016). Phylogenetic analysis of the top abundance genus was aligned with closely related 16S rRNA gene sequences, previously selected according to initial BLAST analyses and downloaded from the NCBI website (http://www.ncbi.nlm.nih.gov), using CLUSTAL W (Gundlapally and Garcia-Pichel, 2006). Phylogenetic trees were constructed using the approximately maximum likelihood routine by FastTree (version 2.1.3 http://www.microbesonline.org/fasttree/).

3 Results

3.1 Overview of sequencing and bacterial diversity

Illumina MiSeq sequencing was used to assess the bacterial community composition and diversity of BSCs in successional stages for revegetation in Shapotou. In total, 18 libraries of bacterial 16S rRNA were constructed, and at least 37,332 effective sequences in each sample were obtained, with an average length of 437 bp. A total of 1,197,2307 OTUs were generated using a threshold of 0.97 (Table S1 in the Supplement); 394 OTUs were shared and occupied a relatively high proportion among all samples (17.07–32.92 %) (Table S2), and these OTUs accounted for 41.96–84.88 % of the total sequences (Table S2). This indicated a high coherence of community among these soil crusts. Alpha-diversity analysis revealed the microbial richness and diversity. Raretation curves showed that the most bacterial OTUs were found in 51YR crust, whereas MS contained the fewest. The number of OTUs was almost the same from 15YR to 51YR (Fig. 2). Community richness estimation using ACE and Chao revealed a similar trend to that for community diversity, which was further supported by Shannon’s indexes (Table S1). Hierarchical clustering analysis (Fig. 3a) and PCA (Fig. 3b) showed that the triplicate samples of each age of BSCs were clustered, verifying that the sequencing results were reliable and the samples were reproducible.

3.2 Bacterial community composition at high taxonomic levels

In the bacterial community, a total of 28 phyla were retrieved at genetic distances of 3 % and clustered into four groups according to their relative abundance (Fig. 4). Of the total sequences, 4.48 % were not classified at the phylum level. The percentages of major phyla for each age of BSCs are shown in Fig. 5. The most abundant phylum shifted from Firmicutes (72.8 %) in MS and 5YR to Actinobacteria in BSCs (minimum of 27.4 % in 15YR and maximum of 30.7 % in 51YR). The following major phyla were at high abundance (> 10 % of total OTUs): Proteobacteria, Chloroflexi, Acidobacteria and Cyanobacteria. The low-abundance phyla (1 % < of total OTUs < 10 %) were Gemmatimonadetes, Bacteroidetes, Armatimonadetes, Verrucomicrobia and Deinococcus–Thermus. The percentages of Proteobacteria, Chloroflexi and Acidobacteria were nearly the same after 15 years of development of BSCs. Cyanobacteria, in addition to the high proportion for 15YR (16.13 %), also had a high proportion in 51YR (9.32 %). The other 17 phyla were all < 1 % of total OTUs and so were removed from further analysis.

At the class level (Table 1), 95.61 % of sequences were assigned, and there was considerable consistency in dominant classes among the crusts. Bacilli was the largest class in MS and 5YR with sequence percentages of 68.73 and 32.62 %, respectively; Actinobacteria was the predominant class from 15YR to 51YR. In addition to subdivisions of Proteobacteria, other major classes included Acidobacteria, Cyanobacteria, Chloroflexi, Clostridia, Cytophaga, Deinococcaceae, Gemmatimonadetes, Ktedonobacteria, Sphingobacteria and Thermomicrobia. The percentages of high-abundance (> 10 % of total OTUs) and low-abundance (1 % < of total OTUs < 10 %) classes decreased from 98 % in

Figure 3. Hierarchical clustering analysis and PCA of bacterial communities in six different ages of BSCs at OTU level based on 97 % similarity (triplicate samples for each age). MS, 5YR, 15YR, 28YR, 34YR and 51YR represent mobile sand, 5-, 15-, 28-, 34- and 51-year-old BSCs, respectively.

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Table 1. Percentages of the major classes in each age of BSCs. MS, 5YR, 15YR, 28YR, 34YR and 51YR represent mobile sand, 5-, 15-, 28-, 34- and 51-year-old BSCs, respectively.

<table>
<thead>
<tr>
<th>Dominant</th>
<th>MS</th>
<th>5YR</th>
<th>15YR</th>
<th>28YR</th>
<th>34YR</th>
<th>51YR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacilli</td>
<td>68.73</td>
<td>32.22</td>
<td>10.87</td>
<td>18.88</td>
<td>14.66</td>
<td>2.81</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>10.26</td>
<td>17.23</td>
<td>27.37</td>
<td>12.84</td>
<td>28.34</td>
<td>29.32</td>
</tr>
<tr>
<td>Acidobacteria</td>
<td>4.04</td>
<td>2.37</td>
<td>11.75</td>
<td>8.32</td>
<td>7.03</td>
<td>7.02</td>
</tr>
<tr>
<td>Chloroflexia</td>
<td>0.89</td>
<td>2.42</td>
<td>4.01</td>
<td>1.94</td>
<td>2.74</td>
<td>0.84</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>0.11</td>
<td>16.13</td>
<td>3.94</td>
<td>2.28</td>
<td>2.37</td>
<td>9.32</td>
</tr>
<tr>
<td>Clostridia</td>
<td>4.09</td>
<td>1.66</td>
<td>0.52</td>
<td>1.02</td>
<td>0.70</td>
<td>0.15</td>
</tr>
<tr>
<td>Cytophagia</td>
<td>0.27</td>
<td>1.22</td>
<td>0.94</td>
<td>0.74</td>
<td>1.02</td>
<td>1.58</td>
</tr>
<tr>
<td>Deinococci</td>
<td>0.05</td>
<td>1.26</td>
<td>0.34</td>
<td>0.37</td>
<td>0.25</td>
<td>0.21</td>
</tr>
<tr>
<td>Deltaproteobacteria</td>
<td>0.44</td>
<td>0.74</td>
<td>1.15</td>
<td>1.01</td>
<td>1.41</td>
<td>0.43</td>
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<tr>
<td>Gammaproteobacteria</td>
<td>5.71</td>
<td>2.63</td>
<td>1.01</td>
<td>1.89</td>
<td>1.41</td>
<td>0.43</td>
</tr>
<tr>
<td>Gemmatimonadetes</td>
<td>0.65</td>
<td>2.41</td>
<td>2.41</td>
<td>2.65</td>
<td>2.75</td>
<td>2.41</td>
</tr>
<tr>
<td>Ktedonobacteria</td>
<td>0.05</td>
<td>0.11</td>
<td>1.76</td>
<td>1.12</td>
<td>2.07</td>
<td>1.66</td>
</tr>
<tr>
<td>Sphingobacteriia</td>
<td>0.26</td>
<td>0.66</td>
<td>1.20</td>
<td>0.89</td>
<td>0.99</td>
<td>0.89</td>
</tr>
<tr>
<td>Thermomicrobia</td>
<td>0.45</td>
<td>1.36</td>
<td>3.24</td>
<td>3.44</td>
<td>3.08</td>
<td>2.81</td>
</tr>
<tr>
<td>Betaproteobacteria</td>
<td>0.57</td>
<td>0.79</td>
<td>0.93</td>
<td>1.02</td>
<td>1.07</td>
<td>1.12</td>
</tr>
<tr>
<td>Minor</td>
<td>0.02</td>
<td>0.04</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Unclassified</td>
<td>0.00</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.02</td>
</tr>
</tbody>
</table>

MS to 89.29% in 51YR, and minor and unclassified classes increased from 1.96% in MS to 10.67% in 51YR.

At the family level, there were 133 identified families (data not shown), with the most abundant families being Bacillaceae, Enterococcaceae and Streptococcaceae (Table S3). Other dominant families were Geodermatophilaceae, JG34-KF-161, JG34-KF-361, Methylobacteriaceae, Micromonosporaceae, Bradyrhizobiaceae and Enterobacteriaceae.

3.3 Characterization of major genera and species

A large proportion of sequences were not assigned to any genera. Even for genera with relative abundance >1% in any sample, unclassified sequences occupied a high proportion (4.87–8.59%). Moreover, higher percentages of total sequences (from 13.51% in MS to 37.28% in 51YR) were found in low-abundance genera (<1% in any sample) (Table S4). A total of 460 genera were found in the crusts, of which 201 were shared by all BSC samples (data not shown). The major genera in each age of BSCs are summarized in Fig. 6. Bacillus, Enterococcus and Lactococcus were the primary genera; they represented 64.31% of the total sequences in MS, and decreased to 30.20% in 5YR and only 2.63% in 51YR, indicating that these three genera were predominant in mobile sand or physical crusts. Enterobacteriaceae_unclassified and Alkaliphilus were low-abundance genera in MS. With the decrease in the three primary genera from MS to 51YR, a series of genera increased in BSCs compared with MS and 5YR, including RB41_norank, JG34-KF-361_norank, Acidimicrobiales_uncultured, JG34-KF-161_norank, JK30-KF-45_norank, Microvirga, Actinobacteria_norank and Rubrobacter (relative abundance >2%).

The phylogenetic relationships of the 30 most abundant genera are shown in Fig. 7. They clustered into three groups at the phylum level: Actinobacteria formed one group and included 10 genera; another group was Firmicutes and Proteobacteria; and Cyanobacteria, Chloroflexi and Deinococcus–Thermus formed the third group. The genera Bryobacter and Blastocatella in phylum Acidobacteria were divided into two different groups.

Bacillus was the primary genus and represented 31% of the sequences in MS (Table S4). An unclassified species in this genus reached nearly 30% relative abundance in MS (Fig. 8). In the Enterococcus genus, another core component, there was also an unclassified species with high abundance. In the core species (Fig. 8), Bacillus_unclassified, Enterococcus_unclassified, Lactococcus_piscium, Enterobacteriaceae_unclassified and Alkaliphilus_oremlandii OhILAs were predominant and decreased from MS to 51YR; only Acidimicrobiales_unclassified increased, with the highest proportion in 51YR (2.62%). The relative abundance of the primitive species in MS and physical crusts decreased in BSCs (from 5YR to 51YR) because of the increased numbers of species. There was little difference in numbers of genera and species among biocrusts (from 5YR to 51YR), only in sequence numbers.

3.4 Relationships between bacterial community structure and soil physicochemical properties

RDA (Fig. 9) and hierarchical clustering analysis (Fig. 3) were used to discern the correlations between bacterial community structure and soil physicochemical properties.
Figure 4. Heat map of bacterial communities in different ages of BSCs at phylum level. MS, 5YR, 15YR, 28YR, 34YR and 51YR represent mobile sand, 5-, 15-, 28-, 34- and 51-year-old BSCs, respectively.

Figure 4. Heat map of bacterial communities in different ages of BSCs at phylum level. MS, 5YR, 15YR, 28YR, 34YR and 51YR represent mobile sand, 5-, 15-, 28-, 34- and 51-year-old BSCs, respectively.

Bacteri are key contributors to the BSC primary succession process and no doubt also in terms of secondary succession.

4.1 Impact of BSC age on bacterial community composition

In the present study, we gained information concerning the diversity of bacterial communities in BSCs of different ages in restored vegetation in Shapotou in the Tengger Desert. The 16S rRNA gene-based amplicon survey revealed the dominance of Actinobacteria, Proteobacteria, Chloroflexi, Acidobacteria and Cyanobacteria in all BSCs, with Firmi-
Figure 5. Abundant phyla (>10% of total OTUs) and low-abundance phyla (1% < of total OTUs < 10%) of bacteria distributed in different ages of BSCs. Data are defined at a 3% OTU genetic distance. Data are presented as mean ± standard deviation; n = 3 per BSC sample. A paired t test (BSC samples) was used to assess the significance between adjacent ages of BSCs. * P ≤ 0.05; ** P ≤ 0.001. MS, 5YR, 15YR, 28YR, 34YR and 51YR represent mobile sand, 5-, 15-, 28-, 34- and 51-year-old BSCs, respectively.

Table 2. Absolute abundances of bacteria (copies of ribosomal genes per gram of soil) in BSCs quantified by qPCR (means ± standard deviation, n = 6). MS, 5YR, 15YR, 28YR, 34YR and 51YR represent mobile sand, 5-, 15-, 28-, 34- and 51-year-old BSCs, respectively.

<table>
<thead>
<tr>
<th>Dominant</th>
<th>MS</th>
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<th>28YR</th>
<th>34YR</th>
<th>51YR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria abundance</td>
<td>1.12 × 10⁶ ± 4.19 × 10⁵a</td>
<td>3.94 × 10⁷ ± 2.21 × 10⁶b</td>
<td>2.70 × 10⁸ ± 1.91 × 10⁷c</td>
<td>5.44 × 10⁸ ± 4.23 × 10⁷c</td>
<td>7.61 × 10⁸ ± 8.5 × 10⁷c</td>
<td>9.03 × 10⁸ ± 2.55 × 10⁷c</td>
</tr>
</tbody>
</table>

Means with different letters are significantly different (P < 0.05).

cutes dominating MS (72.8%) and decreasing to 3.05% in 51YR, and Actinobacteria increasing from 15YR (27.4%) to 51YR (30.7%). Due to different arid conditions, comparisons with other studies of BSCs should be viewed with caution. Cyanobacteria, Actinobacteria, Proteobacteria and Acidobacteria are ubiquitous in soils and sediments everywhere, in arid as well as wet landscapes (Fierer et al., 2012), and Proteobacteria are very common and diverse among all BSCs. We observed that Actinobacteria were the most abundant phylum in the developing (15YR, 28YR and 34YR) and relatively developed (51YR) BSCs, similar to BSCs from the Colorado Plateau and the Sonoran Desert, where Actinobacteria were dominant (Gundlapally and Garcia-Pichel, 2006; Nagy et al., 2005; Steven et al., 2013). Actinobacteria and Proteobacteria are usually predicted to be copiotrophic groups which increase in high-C environments (Fierer et al., 2007). These results differ from those reported in BSCs from Oman and the Gurbantunggut Desert (Abed et al., 2010; Mouquin et al., 2012; Zhang et al., 2016), and even from BSCs of natural vegetation at the edge of the Tengger Desert (Wang et al., 2015), where Proteobacteria were the most abundant phylum, followed by Cyanobacteria, Actinobacteria and Chloroflexi. Unexpectedly, Cyanobacteria had a high proportion in the developed BSCs, although they were prevalent in early successional stages of BSCs (5YR) and play crucial roles in initial crust development (Belnap and Lange, 2001). This is relatively similar to that in the natural habitat around the Tengger Desert, where Cyanobacteria (19.5%) and Actinobacteria (19.4%) were the most dominant phyla after Proteobacteria (25.0%). Moreover, the results did not resemble those from arid Arizona soils (Dunbar et al., 1999) or the Gurbantunggut Desert (Zhang et al., 2016) due to the high proportion of Chloroflexi, an unexplained presence of thermophilic phyla (Gundlapally and Garcia-Pichel, 2006; Mouquin et al., 2012; Nagy et al., 2005) that display good adaptation to drought conditions and the important roles in the development of BSCs in arid zones (Lacap et al., 2011; Wang et al., 2015).
Figure 6. Bacterial community composition in six different ages of BSCs at the genus level. Data are defined at a 3% OTU genetic distance. MS, 5YR, 15YR, 28YR, 34YR and 51YR represent mobile sand, 5-, 15-, 28-, 34- and 51-year-old BSCs, respectively.

4.2 Function of BSC bacteria

More recent information about BSC bacteria has been reported with the convenience of culture-independent sequencing methods, and studies of their function and classification in BSCs are increasingly detailed. The main function of these dominant bacteria involves the cycling and storage of C and N in desert ecosystems, which is vital to the functioning of arid land (Weber et al., 2016). Firmicutes are more frequently detected in below-biocrust soils (1–2 cm depth) (Elliott et al., 2014) and dominated in MS and 5YR, with the vast majority of abundant species being in Firmicutes in the Tengger Desert. Cyanobacteria are the main contributors to C and N fixation in soils during successional processes of BSCs (Belnap and Gardner, 1993). They are thought to serve as pioneers in the stabilization process of soils (Garcia-Pichel and Wojciechowski, 2009), of which the genus Phormidium is significantly more abundant in surface soils (0–1 cm depth), and the genus Microcoleus is globally dominant as biocrust-forming microorganisms in most arid lands, and their production of polysaccharide sheaths aids in the formation of centimeter-long filament bundles (Belnap and Lange, 2003; Boyer et al., 2002; Garcia-Pichel et al., 2001; Pointing and Belnap, 2012). In addition to the filamentous bacteria of Microcoleus and Phormidium, Mastigocladosiplis and Trichocoleus were also in the 30 most abundant genera of BSCs in Shapotou and mainly harvest energy from light. Pseudonocardia, a mycelial genus of Actinobacteria, was dominant and is likely important during BSC formation (Weber et al., 2016). Proteobacteria and Bacteroidetes can produce exopolysaccharides, so they could also play roles in soil stabilization and BSC formation (Gundlapally and Garcia-Pichel, 2006).

Owing to limited culture collections and curated sequence databases of BSC bacteria, most non-cyanobacterial se-
 sequences from DNA-based bacterial surveys cannot be reliably named or taxonomically defined, especially in relatively abundant genera in Actinobacteria and Proteobacteria, such as Bosea, Microvirga, Rubellimicrobium, Patulibacter, Solirubrobacter, Blastococcus and Arthrobacter in the present study. Different compositions of bacterial communities play various roles in improving soil properties in different BSC successional stages, suggesting their positive potential function in soil biogeochemical cycle and ecosystem process. Further discovery and characterization of the functions of these dryland-adapted bacteria is a challenging area for future study.

4.3 Relationship between bacterial community shift and soil physicochemical properties

PCA and RDA showed that bacterial community compositions of MS and 5YR significantly differed from those of BSCs of more than 15 years in age and were positively correlated with soil physicochemical properties. Combined with the results of alpha-diversity analysis and qPCR, this means that the species richness and abundance reached their highest levels at 15 years of BSC development and then maintained similar levels thereafter. Similar trends were found in recovery of soil properties and processes after sand binding at five different-aged revegetated sites – proportions of silt and clay, and organic C increased with years since revegetation (Li et al., 2007a, b). The annual recovery rates of soil properties were greater at the initial revegetated sites (0–14 years) than at the old revegetated sites (43–50 years) (Li et al., 2007a).

These results suggest that bacterial communities of BSCs recovered quickly in the fastest recovery phase of soil properties (the initial 15 years), and the bacterial biomass increased with the improvement of soil texture and nutrients, especially silt, clay and total K content in the Tengger Desert. A significant positive correlation was found between silt and clay and the number of BSC types in southern Africa (Büdel et al., 2009), suggesting that fine grain size promotes BSC succession and their biomass content. This may be attributed to the diversity of BSCs, vegetation composition, soil temperature and soil moisture, because these are key factors regulating soil microbial composition and activity (Butenschoen et al., 2011; De Deyn et al., 2009; Sardans et al., 2008), soil nutrient uptake and release (Peterjohn et al., 1994; Rustad et al., 2001), especially in the BSCs of top soil. It would be good to understand more of the factors that together influenced the composition and function of BSC bacteria in long-term revegetation, including BSCs, plants, soil biochemical properties and climate conditions, and the microorganisms that in turn have the positive influence on soil improvement (Li et al., 2007b, 2010).

Many reports have interpreted correlations among soil properties and BSCs as an indicator that BSCs are drivers of soil fertility and development (Chamizo et al., 2012; Delgado-Baquerizo, 2013; Yu et al., 2014; Zhang et al., 2010); some have reported the opposite and suggest a direct influence of soil properties on BSC development (Belnap et al., 2014; Bowker et al., 2006a; Bowker and Belnap, 2008; Concostrina-Zubiri et al., 2013; Rivera-Aquilar et al., 2009; Root and McBride, 2012; Weber et al., 2016). These are important questions, and parsing out the interactions of BSCs and soil biogeochemical properties remains an important frontier in BSC research. However, further work to identify controlled experimental approaches is required because field correlations do not explain the directionality of causality over time.

4.4 The role of BSCs in succession

In temperate desert regions, BSCs are not well investigated regarding community structure and diversity. Furthermore, studies on succession are rare (Langhans et al., 2009). Most evidence indicates that BSC facilitate succession to later series, suggesting that assisted recovery of BSCs could speed up succession (Bowker, 2007). Because BSCs are ecosystem engineers in high-abiotic-stress systems, loss of BSCs may be synonymous with crossing degradation thresholds. Whether BSCs are deemed facilitative or inhibitory for later successional vegetation may depend on how exhaustively the interaction between plants and BSCs is investigated. In fixed-sand areas, BSCs may in some cases reduce infiltration (inhibitory effect) (Mitchell et al., 1998), but they also increase soil stability and serve as an N source for surviving and recolonizing trees (facilitative effects) (Tateno et al., 2003; Uchida et al., 2000). The BSC bacterial communities in the

Figure 7. Phylogenetic relationship of the 30 most abundant genera in bacterial composition of BSCs.
successional stages may help establish stability and regulate nutrient and biogeochemical cycling. Castillo-Monroy et al. (2011) found that the BSC richness matrix had the greatest direct effect on the ecosystem function matrix. Despite this result, very few of the BSC effects on ecosystem function could be ascribed to changes within the bacterial community. This provides valuable insights concerning semiarid ecosystems where plant cover is spatially discontinuous and ecosystem function in plant interspaces is regulated largely by BSCs.

5 Conclusions

Illumina MiSeq sequencing showed that changes of BSC bacterial diversity and richness in BSC succession were consistent with the recovery phase of soil properties in vegetation succession of Shapotou in the Tengger Desert. The shift of bacterial community composition in BSCs at all levels of classification was related to their corresponding function in the BSC recovery process. BSC bacteria are crucial to establishing stability and nutrient cycling in desert ecosystem, and they are the conduits between the larger BSC organisms and plants facilitating micro-processes. These results have confirmed that bacteria are key contributors to the BSC succession process.

Data availability. Raw data for Illumina MiSeq sequencing of 18 samples were deposited in the NCBI Sequence Read Archive database (https://www.ncbi.nlm.nih.gov/sra/?term=SRP090538).

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tributions from LL. ZW analyzed the soil biogeochemical data and made the RDA figure.

Competing interests. The authors declare that they have no conflict of interest.

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