# 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 (BSC) 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 BSC in the revegetation of Shapotou in the Tengger Desert. The most dominant phyla of bacterial communities shifted with the changed types of BSC in the successional stages, from Firmicutes in mobile sand and physical crusts to Actinobacteria and Proteobacteria in BSC, 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 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 BSC. The results suggested that bacterial communities of BSC 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 BSC in desert ecosystems.

**Key words** biological soil crusts (BSC), successional stages, bacterial community, revegetation, desert ecosystem

## 1 Introduction

Biological soil crusts (BSC) 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, BSC 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 BSC play critical roles in the structure and function of semi-arid 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). Due to the species concept is relatively well-defined in BSC organisms, BSC may act as 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 BSC. BSC exhibit primary successional stages 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 BSC vary in their ecological function (Belnap, 2006; Bowker and Belnap, 2007; Li, 2012; Moquin et al., 2012).

During successional stages of BSC, physical crusts in mobile sand contain the lowest C and N contents (Zhang et al., 2009). Algal crust is the earliest biocrust stage. It shows a surface thin layer which composed by aeolian-born 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 with 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 compared to the early successional crusts (Housman et al., 2006; Lan et al., 2012b). In the successional process of BSC, 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 most important driving force in most terrestrial ecosystems, largely due to control of conversion rates and mineralization of organic matter (Albiach et al., 2000; Baldrian et al., 2010).

Bacteria have a highest proportion of the microbial biomass in BSC (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 successional process of BSC. They can decompose organic material and release nutrients, mediating geochemical processes necessary for ecosystem functioning in the persistence of BSC (Balser and Firestone, 2005). Species composition and community structure of bacteria change greatly during the successional process of BSC (Gundlapally et al., 2006; Moquin et al., 2012; Zhang et al., 2016). Most research on prokaryotic diversity of BSC has focused on cyanobacteria-dominated biocrusts in arid and semi-arid 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 nitrogen (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 BSC during the development process in desert zones, and these only in the Sonoran (Nagy et al., 2005) and Gurbantunggut Deserts (Zhang et al., 2016). What changes occur in bacterial community composition and their potential roles in improving soil properties in different successional stages of BSC? What is the significance of these changes on BSC succession in the recovery process of desert revegetation in temperate zones?

Bowker (2007) examines the role of BSC in primary succession (vs secondary succession) where their role may exist during a time when resources are made available (e.g. light). However,

they fade into the background once higher vegetation takes over. On the other hand, in some environments of high abiotic stress (e.g. deserts), BSC play a role in succession yet exist as a permanent component. Bowker's review and discussion is supported by work carried out in southern Africa (Büdel et al., 2009) where different successional BSC are described. Büdel et al. (2009) also describes 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 BSC (Liu et al., 2017). To better understand these questions, we must analyze in detail the bacterial community composition of BSC at all levels of classification and their corresponding function in the recovery process of BSC. In the present study, bacterial community composition and potential function were analyzed in BSC along a chronosequence of over 50-year-old revegetation. We want to know: what are the drivers of bacterial composition over time? What are the micro-processes that drive bacterial composition and function? Do bacteria drive changes to soil physicochemical properties or alternatively do the larger BSC organisms drive these changes which in turn has a direct influence on bacterial composition and function?

# 2 Materials and methods

## 2.1 Study site description

The study site is located at Shapotou, 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 inter-annual variation. The mean wind speed is 3.5 m/s, and the average days with dust events are 122 d per year. The revegetation protection system for Bao–Lan railway in this area was established initially in 1956, and was expanded in 1964, 1973, 1981 and later through the plantation of the xerophilous shrubs. This unirrigated revegetation system works quite well to protect the railroad line from sand bury and dust hazard during past sixty years. Also, the experimental plots of less than one hectare were established with the same plantation techniques by the Shaptou 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 for studying the variation of environmental

117 factors. As mentioned in other literature, the initial state of BSC began to form following the
118 stabilization of sand dunes and developed with the colonization of cryptogam (Liu, et al, 2006). The
119 BSC could be divided into four types, such as physical crusts, algal-dominated, lichen-dominated
120 and moss-dominated crusts. In this study, we selected the whole BSC layer from the revegetation
121 established in 1964, 1981, 1987, 2000 and 2010, and non-fixed mobile sand (MS) as the control
122 (Figure 1). BSC were sampled in early November 2015, and named according to the fixed-sand time
123 as 51YR (51 years of revegetation), 34YR, 28YR, 15YR, 5YR and MS, respectively. The main
124 types of BSC were algae–lichen- and moss-dominated crusts from 15YR to 51YR.

125 ## 2.2 BSC sampling

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

134 ## 2.3 DNA extraction and Illumina MiSeq sequencing

135 Microbial DNA was extracted from BSC samples using E.Z.N.A Soil DNA (Omega Bio-tek,
136 Norcross, GA, U.S.) according to the manufacturer's protocols. The extracted DNA was diluted in
137 TE buffer (10 mM Tris–HCl and 1 mM EDTA at pH 8.0) and stored at –20 °C until use. An aliquot
138 of the extracted DNA from each sample was used as a template for amplification. The bacteria 16S
139 ribosomal RNA gene was amplified by PCR (95 °C for 3 min, followed by 25 cycles at 95 °C for
140 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
141 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′).
142 PCRs were performed in triplicate 20-μL mixture containing 2 μL of 5 × FastPfu Buffer, 2 μL of
143 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.2 μL of FastPfu Polymerase and 10 ng of template
144 DNA. This was conducted according to Wang et al. (2015). Amplicons were extracted from 2%
145 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′-GTGCCAGCMGCCGCGGTAA-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 20 μL reaction mixtures contained 10 μL of 2 × SYBR Mix (with ROX) (DBI Bioscience, Ludwigshafen, Germany), 0.4 μL each of 10 μM forward and reverse primers, 1 μL of total DNA template (1 ng/μL) and 8.2 μL of RNase-free dd$H_2O$. The reaction was conducted on a Stratagene Mx3000P Real-time PCR system (Stratagene, Agilent Technologies Inc., Santa Clara, CA, USA) using the following program: 94 °C for 3 min followed by 40 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s, then 72 °C for 2 min. The detection signal was collected at 72 °C for 30 s and analyzed. The melting curve was obtained to confirm that the amplified products were of the appropriate size. For each soil sample, the qPCRs were repeated six times.

## 2.5 Processing of sequencing data

Raw fastq files were demultiplexed, 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) exact barcode matching, two nucleotide mismatch in primer matching, 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://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 IEG pipeline (http://ieg.ou.edu). The average data were calculated for BSC 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 BSC and top soil physicochemical properties by permutation test analysis (Zhang et al., 2016). Phylogenetic analysis of the top abundance genus were 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 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 BSC in successional stages for revegetation in Shapotou. Total 18 libraries of bacterial 16S rRNA were constructed, at least 37,332 effective sequences in each sample were obtained, and an average length of 437 bp. 1197–2307 OTUs were generated using a threshold of 0.97 (Table S1). 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. Rarefaction 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 (Figure 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 (Figure 3 A) and PCA (Figure 3 B) showed that the

triplicate samples of each age of BSC 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 they clustered into four groups according to their relative abundance (Figure 4). Of the total sequences, 4.48% were not classified at the phylum level. The percentages of major phyla for each age of BSC are shown in Figure 5. The most abundant phylum shifted from Firmicutes (72.8%) in MS and 5YR to Actinobacteria in BSC (minimum 27.4% in 15YR and maximum 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 BSC. 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; and Actinobacteria was the predominant class from 15YR to 51YR. In addition to subdivisions of Proteobacteria, other major classes included Acidobacteria, Cyanobacteria, Chloroflexi, Clostridia, Cytophagia, Deinococci, Gemmatimonadetes, Ktedonobacteria, Sphingobacteria and Thermomicrobia. The percentages of high (> 10% of total OTUs) and low abundance (1% < of total OTUs <10%) classes decreased from 98% in 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 samples, 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 samples) (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 BSC are summarized in Figure 6. *Bacillus*, *Enterococcus* and *Lactococcus* were the primary genera and 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 BSC compared with MS and 5YR, including RB41_norank, JG34-KF-361_norank, Acidimicrobiales_uncultured, JG34-KF-161_norank, JG30-KF-CM45_norank, *Microvirga*, Actinobacteria_norank and *Rubrobacter* (relative abundance > 2%).

The phylogenetic relationships of the 30 most abundant genera are shown in Figure 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% sequences in MS (Table S4). An unclassified species in this genus reached nearly 30% relative abundance in MS (Figure 8). In the *Enterococcus* genus, another core component, there was also an unclassified species with high abundance. In the core species (Figure 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, and represented the highest proportion in 51YR (2.62%). The relative abundance of the primitive species in MS and physical crusts decreased in BSC (from 15YR to 51YR) because of the increased numbers of species. There was little difference in numbers of genera and species among biocrusts (from 15YR to 51YR), only in sequence numbers.

## 3.4 Relationships between bacterial community structure and soil

## physicochemical properties

RDA (Figure 9) and hierarchical clustering analysis (Figure 3) were used to discern the correlations between bacterial communities and soil physicochemical properties. Taking into account the likely changes in the soil properties from samples with the same successional stages in the same experimental site, we selected soil biogeochemical data collected from 2005 in the RDA (data from Li et al., 2007a; Table S5). The BSC grouping patterns of bacterial communities at the phylum and genus levels were similar to the OTU level, with all divided into two groups. Group I contained two members, MS and 5YR, which dominated the physical crusts and algal crusts (Figure 1 A and B), and had the lowest diversities with Shannon indexes of 3.3 and 4.61, and Simpson indexes of 0.139 and 0.0531, respectively (Table S1). The remaining BSC comprised the largest branch of Group II, which dominated BSC composed of algae, lichens or mosses (Figure 1 C–F), and had higher diversity with Shannon indexes > 6.0 (Table S1).

From Figure 9, it can be inferred that BSC development was associated with soil physicochemical properties. The development of microbial community structure was positively correlated with the physicochemical index except for soil bulk density. The total variation in OTU data explained by the first four axes in the RDA (as constrained by the measured environmental variables) was 82.16%, with the first axis explaining 75.27% and the second axis explaining 4.42%. Of all the environmental factors, silt+clay content and total K were most strongly related to axis 1, with highest correlated variable (silt+clay: -0.91; total K:-0.90). Therefore, silt+clay content and total K were closely related to bacterial community development of BSC, shown by the positions of cluster groups along axis 1. Eight soil physicochemical variables were all significant verified by the permutation test analysis ($P < 0.05$): pH; silt and clay content; organic C; total phosphorus (P), nitrogen (N) and potassium (K); electrical conductivity (EC) and water-holding capacity (WHC).

## 3.5 Quantification of bacterial abundance

The averaged bacterial abundance in MS was $1.12 \times 10^6$ copies (16S rRNA gene) per gram of soil (Table 2). Similar to the shift of bacterial richness, gene copies increased quickly in the initial 15 years of BSC development, and reached the approximate highest level of $2.70 \times 10^8$ copies in 15YR. There were no significant differences among 28YR, 34YR and 51YR.

290 ## 4 Discussion

291 On a landscape scale and in high stress environments, the role of diversity hot spots of BSC
292 microbes is crucial to establishing stability, regulating moisture and nutrient cycling (Bowker, 2007).
293 Additionally, bacteria are the conduits between the larger BSC organisms and plants facilitating
294 micro-processes (Castillo-Monroy et al., 2011), and thus bacteria as key contributors to the BSC
295 primary succession process and no doubt in terms of secondary succession as well.

296 ### 4.1 Impact of BSC age on bacterial community composition

297 In the present study, we gained information concerning the diversity of bacterial communities
298 in BSC of different ages in restored vegetation at Shapotou in the Tengger Desert. The 16S rRNA
299 gene-based amplicon survey revealed the dominance of Actinobacteria, Proteobacteria, Chloroflexi,
300 Acidobacteria and Cyanobacteria in all BSC, with Firmicutes dominating MS (72.8%) and
301 decreasing to 3.05% in 51YR, and Actinobacteria increasing from 15YR (27.4%) to 51YR (30.7%).
302 Due to different arid conditions, comparisons with other studies of BSC should be viewed with
303 caution. Cyanobacteria, Actinobacteria, Proteobacteria and Acidobacteria are ubiquitous in soils
304 and sediments everywhere, in arid as well as wet landscapes (Fierer et al. 2012), and Proteobacteria
305 are very common and diverse among all BSC. We observed that Actinobacteria were the most
306 abundant phylum in the developing (15YR, 28YR and 34YR) and relatively developed (51YR) BSC,
307 similar to BSC from the Colorado Plateau and the Sonoran Desert, where Actinobacteria were
308 dominant (Gundlapally and Garcia-Pichel 2006; Nagy et al. 2005; Steven et al. 2013).
309 Actinobacteria and Proteobacteria are usually predicted to be copiotrophic groups which increase
310 in high C environments (Fierer et al., 2007). These results differ from those reported in BSC from
311 Oman and the Gurbantunggut Desert (Abed et al. 2010; Moquin et al., 2012; Zhang et al., 2016),
312 and even from BSC of natural vegetation at the edge of the Tengger Desert (Wang et al., 2015),
313 where Proteobacteria were the most abundant phylum followed by Cyanobacteria, Actinobacteria
314 and Chloroflexi. Unexpectedly, Cyanobacteria had a high proportion in the developed BSC,
315 although they were prevalent in early successional stages of BSC (5YR) and play crucial roles in
316 initial crust development (Belnap and Lange, 2001). This is relatively similar to that in the natural
317 habitat around the Tengger Desert, where Cyanobacteria (19.5%) and Actinobacteria (19.4%) were
318 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 Chlorflexi, an unexplained presence of thermophilic phyla (Gundlapally and Garcia-Pichel, 2006; Moquin et al., 2012; Nagy et al., 2005) that display good adaptation to drought conditions together with important roles in the development of BSC in arid zones (Lacap et al., 2011; Wang et al., 2015).

## 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 BSC 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 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 BSC (Belnap and Gardner, 1993). They are thought to serve as pioneers in the stabilization process of soils (Garcia-Pichel and Wojciechowski 2009), of which genus *Phormidium* is significantly more abundant in surface soils (0–1 cm depth), and genus *Microcoleus* is globally dominant as biocrust-forming microorganisms in most arid lands and their production of polysaccharide sheaths aids in formation of cm-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*, *Mastigocladopsis* and *Trichocoleus* were also in the 30 most abundant genera of BSC in Shapotou, and mainly harvest energy from light. *Pseudonocardia*, a mycelial genus of Actinobacteria, were dominant and are 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 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 community play various roles in improving soil properties in different successional stages of BSC, 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 BSC 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, organic C increased with years since revegetation (Li et al., 2007a, b). The annual recovery rates of soil properties were greater in the initial revegetated sites (0–14 years) than that in the old revegetated sites (43–50 years) (Li et al., 2007a). These results suggest that bacterial communities of BSC 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 BSC, vegetation composition, soil temperature and soil moisture. Because they 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 BSC 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 BSC, plant, soil biochemical properties and climate conditions, and the microorganisms in turn have the positive influence on soil improvement (Li et al., 2007b, 2010).

Many reports have interpreted correlations among soil properties and BSC as an indicator that BSC 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 (Bowker et al., 2006, Rivera-Aquilar et al., 2009, Bowker and Belnap 2008, Root and McCune 2012, Concostrina-Zubiri et al., 2013, Belnap et al., 2014, Weber et al., 2016). These are important questions and parsing out the interactions of BSC and soil biogeochemical properties remains an important frontier in BSC research. However, further work to identify controlled experimental approaches are required to answer this question as field correlations leave us wondering about the directionality of controls over time.

### 4.4 The role of BSC to succession

In temperate desert regions, BSC 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 seres, suggesting that assisted recovery of BSC could speed up succession (Bowker, 2007). Because BSC are ecosystem engineers in high abiotic stress systems, loss of BSC may be synonymous with crossing degradation thresholds. Whether or not BSC are deemed facilitative or inhibitory for later successional vegetation may depend on how exhaustively the interaction between plants and BSC. On fixed-sand areas, BSC may in some cases reduced infiltration (inhibitory effect) (Mitchell et al., 1998), but they also increased soil stability and served as an N source for surviving and recolonizing trees (facilitative effects) (Uchida et al., 2000; Tateno et al., 2003). BSC bacterial communities recovered in the successional stages may help establishing stability, regulating nutrient and biogeochemical cycling. Castillo-Monroy et al. (2011) found that BSC richness matrix has 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. It provides valuable insights on semi-arid 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 BSC at all levels of classification was related to their corresponding function in the BSC recovery process. BSC bacteria are crucial to establish stability and nutrient cycling in desert ecosystem, and are the conduits between the larger BSC organisms and plants facilitating micro-processes. These results confirmed our hypothesis that bacteria as key contributors to the BSC succession process.

***Data availability***. Raw data for Illumina MiSeq sequencing of 18 samples was deposited in the NCBI Sequence Read Archive database (https://www.ncbi.nlm.nih.gov/sra/?term=SRP091312).

***Author contributions***. Lichao Liu and Yubing Liu designed the research. Peng Zhang, Guang Song and Rong Hui collected samples from the field. Yubing Liu and Jin Wang performed DNA extraction and quality detection. Yubing Liu analyzed the high-throughput data and prepared the manuscript with consistent contributions from Lichao Liu. Zengru Wang analyzed the soil biogeochemical data and made the RDA figure.

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

***Acknowledgments.*** This work was financially supported by the Creative Research Group Program of National Natural Science Foundation of China (grant No. 41621001) and the National Natural Science Foundation of China (grant No. 41371100 and 41401112).

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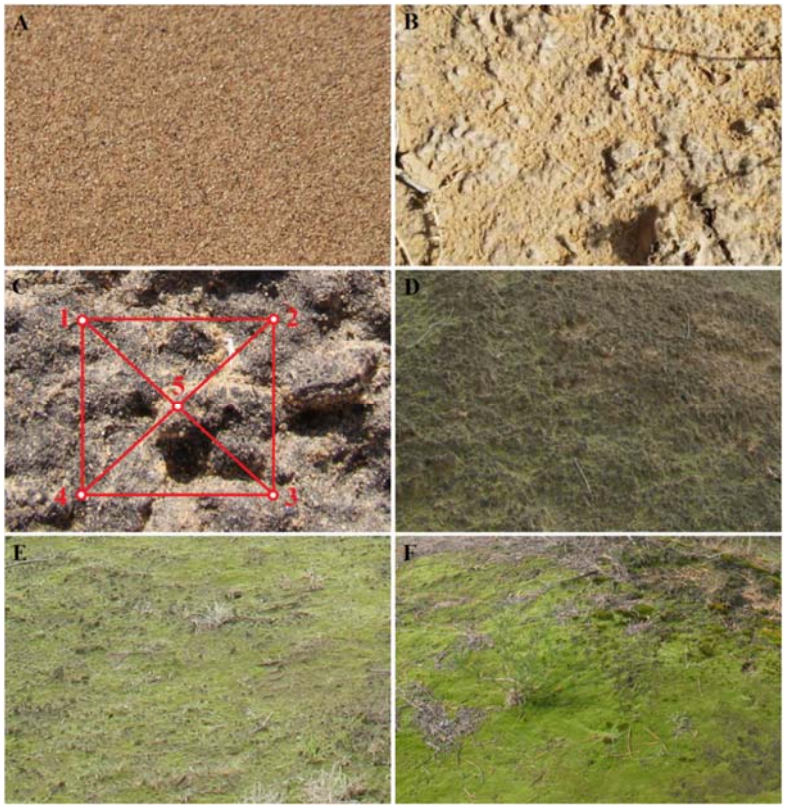
**Table 1**. Percentages of the major classes in each age of BSC. MS, 5YR, 15YR, 28YR, 34YR and 51YR represent mobile sand, 5, 15, 28, 34 and 51-year-old BSC, respectively.

| Dominant | MS | 5YR | 15YR | 28YR | 34YR | 51YR |
|---|---|---|---|---|---|---|
| **Bacilli** | 68.73281 | 32.6217 | 10.87003 | 18.88014 | 14.65767 | 2.809922 |
| **Actinobacteria** | 10.25572 | 17.22651 | 27.36705 | 28.34208 | 29.31533 | 30.65824 |
| **Alphaproteobacteria** | 4.058181 | 12.26026 | 19.93375 | 16.30594 | 18.98282 | 21.11772 |
| **Acidobacteria** | 1.404514 | 2.372406 | 11.75488 | 8.32619 | 7.703847 | 9.022644 |
| **Chloroflexia** | 0.886639 | 2.423301 | 4.006393 | 2.962606 | 3.367977 | 3.857281 |
| **Cyanobacteria** | 0.112504 | 16.13272 | 3.943891 | 2.275974 | 2.367049 | 9.32444 |
| **Clostridia** | 4.091218 | 1.661666 | 0.517876 | 1.017893 | 0.704489 | 0.15447 |
| **Cytophagia** | 0.265188 | 1.223258 | 0.93039 | 0.739312 | 1.022358 | 1.579521 |
| **Deinococci** | 0.048216 | 1.255402 | 0.342869 | 0.372335 | 0.249116 | 0.20715 |
| **Deltaproteobacteria** | 0.447337 | 0.740205 | 1.150934 | 0.993785 | 1.087539 | 1.255402 |
| **Gammaproteobacteria** | 5.715383 | 2.632237 | 1.011643 | 1.890246 | 1.417015 | 0.425908 |
| **Gemmatimonadetes** | 0.645559 | 2.400979 | 2.406336 | 2.646523 | 2.75992 | 2.40455 |
| **Ktedonobacteria** | 0.053573 | 0.113397 | 1.75542 | 1.121469 | 2.072395 | 1.657202 |
| **Sphingobacteriia** | 0.262509 | 0.666095 | 1.200043 | 0.897353 | 0.995571 | 0.889317 |
| **Thermomicrobia** | 0.449123 | 1.351834 | 3.24208 | 3.414408 | 3.008143 | 2.810815 |
| **Betaproteobacteria** | 0.572342 | 0.789314 | 0.939319 | 1.021465 | 1.073253 | 1.11254 |
| **Minor** | 0.018688 | 0.039555 | 0.080851 | 0.08194 | 0.081753 | 0.085887 |
| **Unclassified** | 0.000911 | 0.00142 | 0.005018 | 0.005822 | 0.009866 | 0.02084 |

**Table 2**. Absolute abundances of bacteria (copies of ribosomal genes per gram of soil) in BSC 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 BSC, respectively.

| Dominant | MS | 5YR | 15YR | 28YR | 34YR | 51YR |
|---|---|---|---|---|---|---|
| **Bacteria abundance** | $1.12 \times 10^6 \pm 4.19 \times 10^5$ a | $3.94 \times 10^7 \pm 2.21 \times 10^6$ b | $2.70 \times 10^8 \pm 1.91 \times 10^7$ c | $5.44 \times 10^8 \pm 4.23 \times 10^7$ c | $7.61 \times 10^8 \pm 8.5 \times 10^7$ c | $9.03 \times 10^8 \pm 2.55 \times 10^7$ c |

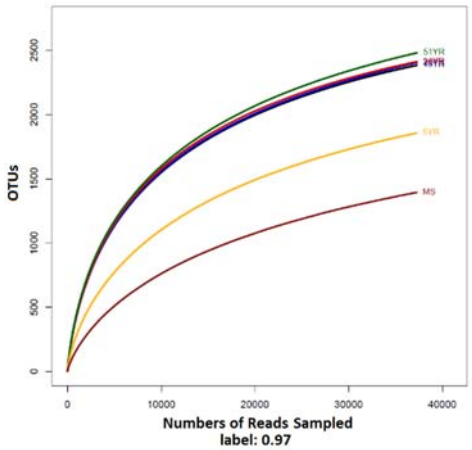
Means with different letters are significantly different ($P < 0.05$).




631

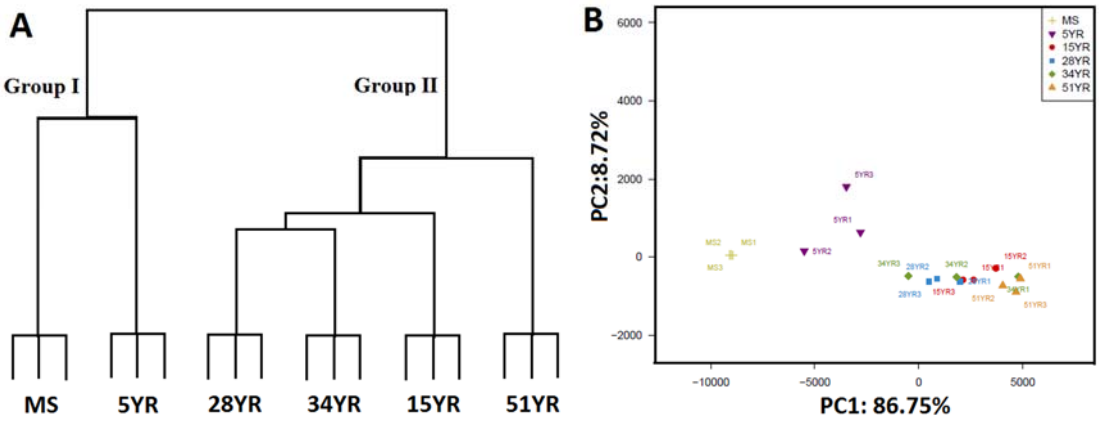
632 **Figure 1**. Sand dune landscape before (MS, A) and after establishing sand-binding vegetation with physical crusts
633 dominated by few algae, revegetated in 2010 (5YR, B); with BSC dominated by algae and lichens, revegetated in
634 2000 (15YR, C); with BSC dominated by lichens and few mosses, revegetated in 1987 (28YR, D); with BSC
635 dominated by few lichens and mosses, revegetated in 1981 (34YR, E); and with BSC dominated by mosses,
636 revegetated in 1964 (51YR, F). Five soil cores (3.5-cm diameter) with crust layers from four vertices of a square
637 (20-m length) and a diagonal crossing point in each plot were sampled individually (as shown in C).

638




639

640 **Figure 2**. Rarefaction results of the 16S rDNA libraries based on 97% similarity in different age of BSC. MS, 5YR,
641 15YR, 28YR, 34YR and 51YR represent mobile sand, 5-, 15-, 28-, 34- and 51-year-old BSC, respectively.



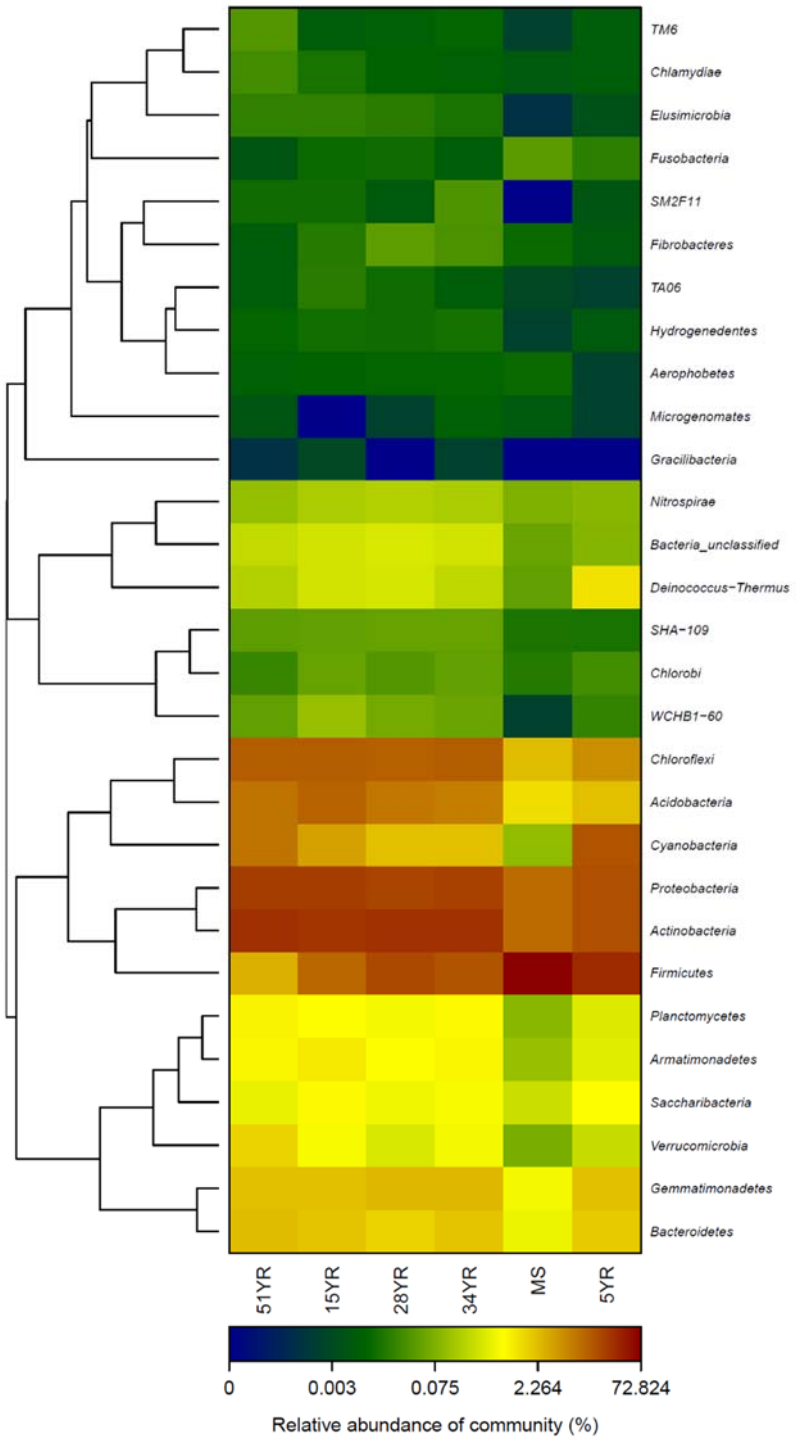

642

643 **Figure 3**. Hierarchical clustering analysis and PCA of bacterial communities in six different ages of BSC at OTU

644 level based on 97% similarity (triplicate samples for each age). MS, 5YR, 15YR, 28YR, 34YR and 51YR represent

645 mobile sand, 5-, 15-, 28-, 34- and 51-year-old BSC, respectively.

646




647

648 **Figure 4**. Heatmap of bacterial communities in different ages of BSC at phylum level. MS, 5YR, 15YR, 28YR,

649 34YR and 51YR represent mobile sand, 5-, 15-, 28-, 34- and 51-year-old BSC, respectively.

650

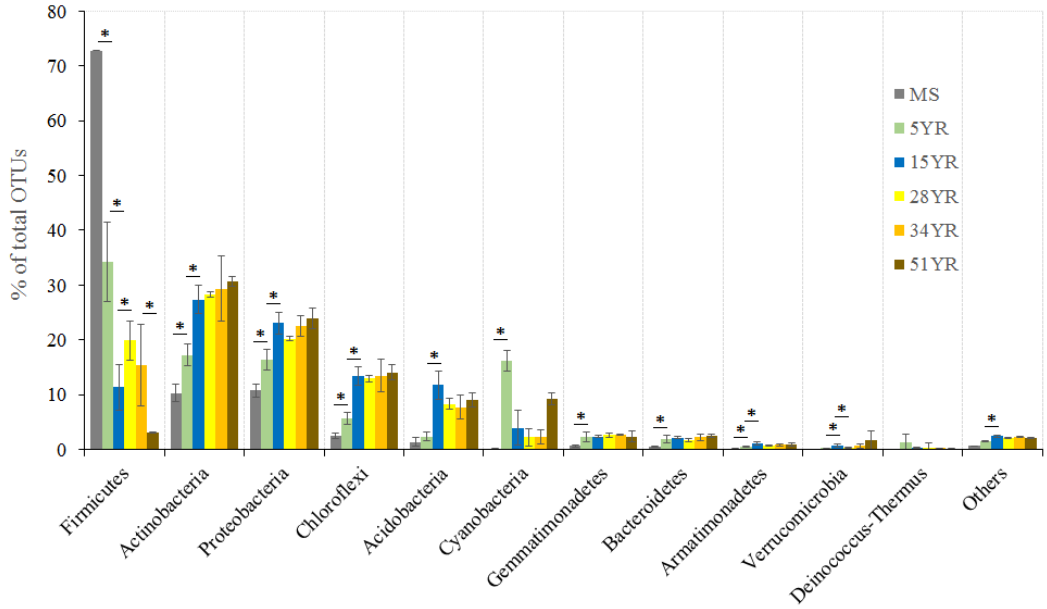



**Figure 5**. Abundant phyla (> 10% of total OTUs) and low-abundance phyla (1% < of total OTUs < 10%) of bacteria distributed in different ages of BSC. Data are defined at a 3% OTU genetic distance. Data are presented as mean ± standard deviation; n = 3 per BSC sample. Paired t-test (BSC samples) was used to assess the significance between adjacent ages of BSC. *P ≤ 0.05, **P ≤ 0.001. MS, 5YR, 15YR, 28YR, 34YR and 51YR represent mobile sand, 5, 15, 28, 34 and 51-year-old BSC, respectively.

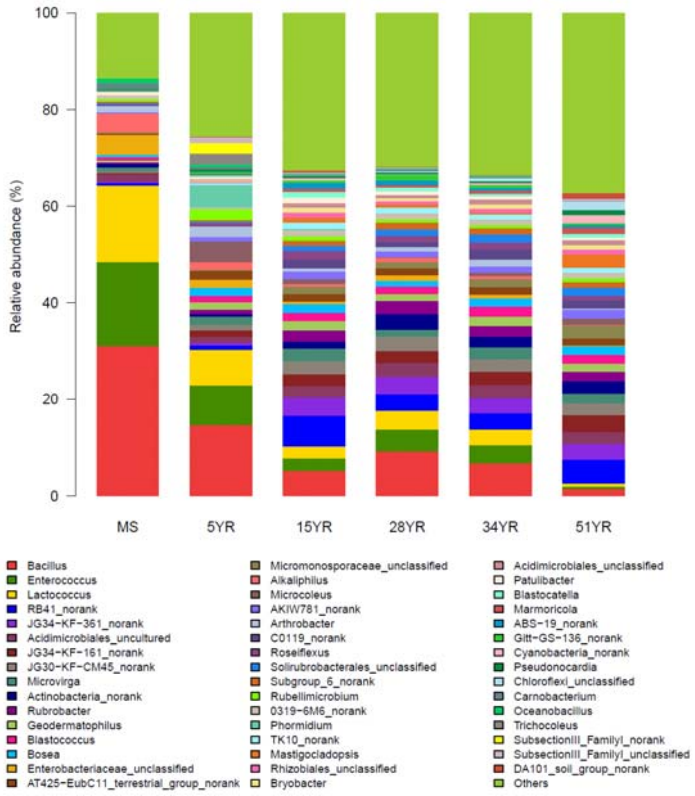


**Figure 6**. Bacterial community composition in six different ages of BSC at the genus level. Data are defined at a 3% OTU genetic distance. MS, 5YR, 15YR, 28YR, 34YR and 51YR represent mobile sand, 5, 15, 28, 34 and 51-year-old BSC, respectively.

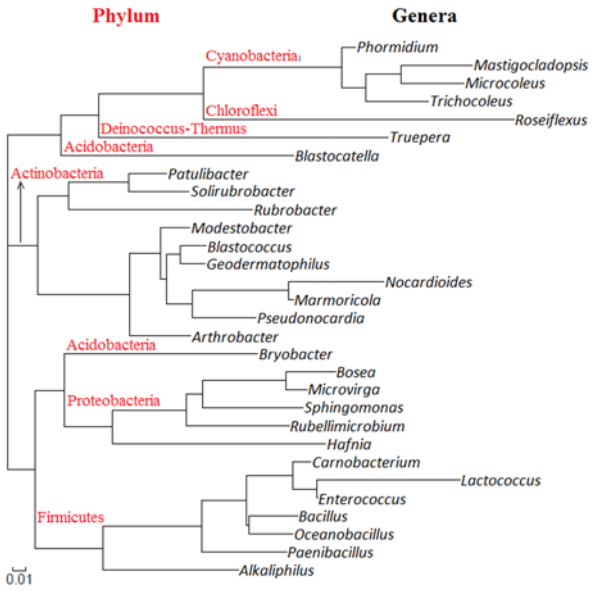



**Figure 7**. Phylogenetic relationship of the 30 most abundant genera in bacterial composition of BSC.

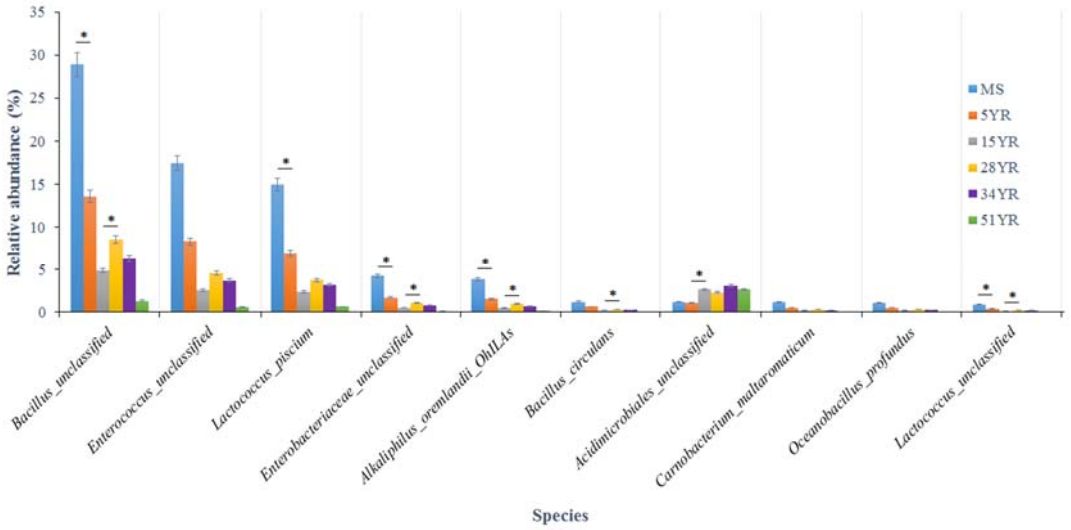
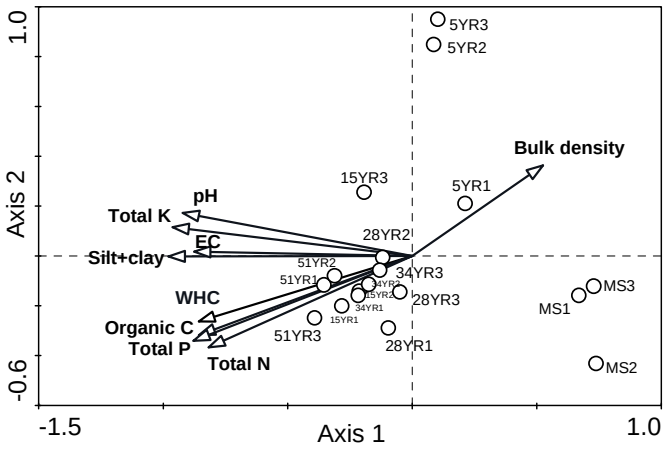



**Figure 8**. Abundant species (> 10% of total OTUs) and low-abundance species (1% < of total OTUs < 10%) of bacteria distributed in different ages of BSC. Data are defined at a 3% OTU genetic distance. Data are presented as mean ± standard deviation; n = 3 per BSC samples; Paired t-tests (BSC samples) were used to assess the significance between the adjacent ages of BSC. *P ≤ 0.05, **P ≤ 0.001. MS, 5YR, 15YR, 28YR, 34YR and 51YR represent mobile sand, 5, 15, 28, 34 and 51-year-old BSC, respectively.




672

673 **Figure 9**. Redundancy analysis (RDA) of bacterial community structures in relation to soil physiochemical
674 properties. Arrows indicate the direction and magnitude of soil physiochemical index associated with bacterial
675 community structures. The length of arrows in the RDA plot correspond to the strength of the correlation between
676 variables and community structure. Each circle represents the bacterial community structure for each sample.