Community succession of bacteria and eukaryotes in dune ecosystems of Gurbantünggüt Desert, Northwest China

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Abstract Pyrosequencing and quantitative polymerase chain reaction of small subunit rRNA genes were used to provide a comprehensive examination of bacterial, cyanobacterial, and eukaryotic communities in the biological soil crusts (BSCs) of Gurbantünggüt Desert sand dunes (China). Three succession stages were recognized based on the analyses of eukaryotic communities: a late succession stage of BSCs in a swale with eukaryotes mainly related to the *Bryophyta* clade, an initial succession stage in a slope with barely any eukaryotic phototrophic microorganisms detected, and an intermediate succession type detected from both the swale and slope BSCs dominated by the phylum *Chlorophyta*. Moreover, the cyanobacterial community dominated all of the BSCs (48.2–69.5 % of the total bacteria) and differed among the three succession stages: sequences related to *Microcoleus steenstrupii* and the genus *Scytonema* were abundant in the later succession stage, whereas both the initial and intermediate stages were dominated by *Microcoleus vaginatus*. Compared with swales, BSCs from slopes are exposed to a harsher environment, e.g., higher irradiance and lower water availability, and thus may be restricted from developing to a higher succession stage. Other disturbances

such as wind and grazing may explain the different succession stages observed in swales or slopes. However, no clear differences were detected from non-phototrophic bacterial communities of the three succession stages, and sequences related to Alphaproteobacteria and Actinobacteria were most abundant in all the BSCs. The closest matches for the most frequent non-phototrophic bacterial genera were mainly derived from harsh environments, indicating the robustness of these genera.

Keywords Gurbantünggüt Desert · Dune ecosystem · Cyanobacterial diversity · *Chlorophyta* · *Bryophyta* · Pyrosequencing

Abbreviations

BSCs Biological soil crusts

qPCR Quantitative polymerase chain reaction

Introduction

Phototrophic microorganisms, including cyanobacteria, eukaryotic algae, and *Bryophyta*, are important primary producers in arid areas (Lange et al. 1992; Warren-Rhodes et al. 2007). Together with heterotrophic microorganisms and soil particles, these phototrophic microorganisms can form biological soil crusts (BSCs) in the topmost layers of soils (Garcia-Pichel and Belnap 2001). BSCs have low moisture requirements and high tolerance of extreme temperatures and light, thus enabling their survival in arid, semiarid, and hyperarid regions (Belnap 2006).

Previous studies have classified BSCs into different stages based on the dominant species, crust color, or external morphology, such as initial and stable BSCs

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(Langhans et al. 2009): smooth, rugose, and pediceled BSCs (Rivera-Aguilar et al. 2005); and cyanobacterial, lichen, and moss BSCs (Lan et al. 2013). Early successional cyanobacterial BSCs are light in color, having few ultraviolet (UV)-protective pigments, and are generally found at low biomass (Housman et al. 2006). Compared with early successional cyanobacterial crusts, later successional crusts have a higher biological metabolic efficiency and better stabilized soils against water and wind (Housman et al. 2006).

Initially, large filamentous cyanobacteria such as *Microcoleus vaginatus* often colonize the bare sand, binding the soil particles together by their sheath material and protecting the soil from disturbance (Belnap and Gardner 1993). Subsequently, smaller cyanobacteria and eukaryotic algae will begin to establish (Langhans et al. 2009). Once the soil surface is stabilized, bryophytes colonize the soil and their abundance can increase as long as the climate conditions maintain sufficient moisture (Belnap 2006; Langhans et al. 2009). Besides the important role of these phototrophic communities during the succession of BSCs, some heterotrophic microorganisms, such as *Deltaproteobacteria*, also show resistance to drying, UV radiation, and heat (Abed et al. 2010). Most related studies have been focused on the prokaryotic community in BSCs (Abed et al. 2010, 2012; Garcia-Pichel et al. 2001; Gundlapally and Garcia-Pichel 2006; Li et al. 2013; Nagy et al. 2005; Steven et al. 2013). However, to date, only one whole eukaryotic survey of diatomaceous BSCs has been conducted using molecular methods (Meadow and Zabinski 2012), and knowledge of whole eukaryotic community succession in BSCs is still lacking, particularly of non-phototrophic eukaryotes. In this study, both the bacterial and eukaryotic communities were investigated to study the community succession patterns of BSCs.

Gurbantünggüt Desert is the second largest desert in China. Its BSC community structure in swales and slopes could be affected by various environmental factors (e.g., wind, water, temperature, and radiation), and is thus well suited for studying the patterns of microbial community succession. Therefore, the objectives of the present study were to (1) characterize the microbial communities in BSCs, including the bacterial and eukaryotic communities from swales and slopes, and (2) to assess the community succession of BSCs in Gurbantünggüt Desert.

Materials and methods

Study sites and sample collection

This study was conducted at the south end of Gurbantünggüt Desert (44°84'23"N, 87°11'36"E), in the

hinterland of Junggar Basin in Northwest China. Gurbantünggüt Desert is the largest fixed and semi-fixed desert in China. Because of the 'blocking effect' of the Himalayan range, most air currents from the Indian Ocean fail to reach the area, resulting in vast expansion of arid terrain. The annual rainfall ranges from 70 to 180 mm, falling predominantly in the spring. In sharp contrast, the annual potential evaporation is approximately 1000 mm (Fan et al. 2014). The mean relative air humidity ranges from 50 to 60 % (Zhang et al. 2009). The annual maximum, minimum, and mean temperature is 42.6, −41.6, and 6.6 °C, respectively (Fan et al. 2014). Natural vegetation in the area is dominated by xerophytic shrubs including *Haloxylon ammodendron* and *Haloxylon persicum* (family *Chenopodiaceae*) (Song et al. 2006) with a vegetation cover of less than 30 % (Wu et al. 2009; Zhang et al. 2007).

On the dune crests, the sand shows high eolian activity and there are no obvious crusts on the surface. However, the sand in most of the interdune swales and slopes is stabilized and mainly covered by BSCs. In this study, the BSCs were collected on May 17, 2012 from south to north. Four sites were chosen as follows: Swale 1 (interdune swale), Slope 1 (slope), Swale 2 (interdune swale), and Slope 2 (slope). The distance between adjacent sites was approximately 1 km. From each site, six 9-cm-diameter petri dishes of the topmost 1 cm of soil were collected (Li et al. 2013), and the thickness of crusts was less than 3 mm (Fig. S1). Once the samples were transported to the laboratory, approximately 20-g crust samples from each site were picked out and stored at −20 °C for DNA extraction. The remaining samples from each site were pulverized and mixed thoroughly for analyses of soil properties.

Physicochemical characteristics

The soil water content was measured by determining the weight lost after drying the sample at 105 °C overnight. Soil pH was measured in distilled water (1/10, w/v) using a portable pH meter (Hach, USA). Total organic carbon content was determined by oxidation with potassium dichromate ($K_2Cr_2O_4$) in sulfuric acid (H_2SO_4) medium and excess $Cr_2O_4^{2-}$ was measured using ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$) with an *ortho*-phenanthroline ferrous complex as the indicator (Schumacher 2002). Total nitrogen content was determined using the Kjeldahl procedure (Bremner and Mulvaney 1982). Chlorophyll *a* was extracted in 10 mL 95 % ethanol at 4 °C for 24 h and determined with a spectrophotometer (Chen et al. 2006; Lan et al. 2010).

DNA extraction

For each site, triplicate 0.5 g crust subsamples were used to extract DNA using the FastDNA SPIN kit for soil (Bio101Systems, USA) according to the manufacturer's instructions. The DNA extractions were suspended in a final volume of 100 μL ddH₂O, and then checked by 1.5 % agarose gel electrophoresis. The DNA extracted from each site were mixed and stored at $-20\text{ }^{\circ}\text{C}$ before use.

Pyrosequencing and sequence analysis

To determine the bacterial, cyanobacterial, and eukaryotic community composition of the crusts, barcoded small subunit (SSU) rRNA gene libraries were generated and sequenced with high-throughput pyrosequencing. The bacterial primers, BAC27F/533R (Lane 1991), were used to amplify the V1–V3 region of the bacterial 16S rRNA gene, and primers CYA359F and CYA781R (a+b) (Nubel et al. 1997) were used to amplify the V4 region of the cyanobacterial 16S rRNA gene. The eukaryote-specific primers 3NDf/V4_euk_R2 (Bråte et al. 2010) were used to amplify the V4 region of the 18S rRNA gene. Each primer pair had a barcode sequence of ten nucleotides on the 5' or 3' end, which was unique for each library. Polymerase chain reactions (PCRs) were performed in a total volume of 20 μL containing 5 $\times$ FastPfu buffer, 2.5 Units of FastPfu polymerase, 2.5 mM dNTP mix, 5 μM of each primer, and ~ 50 –100 ng of template DNA. Cycling conditions were 94 $^{\circ}\text{C}$ for 2 min; 25 cycles of 95 $^{\circ}\text{C}$ for 30 s, 55 $^{\circ}\text{C}$ (for bacteria and eukaryotes) or 60 $^{\circ}\text{C}$ (for cyanobacteria) for 30 s, and 72 $^{\circ}\text{C}$ for 30 s, followed by 72 $^{\circ}\text{C}$ for 10 min. Pooled PCR products were purified and then sequenced using a 454 Life Sciences Genome Sequencer FLX instrument (Roche Diagnostics, Indianapolis, IN, USA). The raw SFF files were deposited into the National Center for Biotechnology Information database and can be found under project number SRA099446.

The open-source software Mothur v. 1.30.2 was used for processing, cluster analysis, and classification of the raw barcoded sequences from SSU rRNA gene libraries following the Standard Operating Procedure (http://www.mothur.org/wiki/454_SOP). Sequences with no ambiguous bases and no homopolymer stretches of more than 8 bp were retained (Schloss et al. 2011). Quality-checked sequences were aligned using the bacterial SILVA 16S rRNA gene and the eukaryotic SILVA 18S rRNA gene template (Pruesse et al. 2007). These alignments were then trimmed in Mothur to ensure that reads with the same primer set were aligned to the same exact region. A further screening step (pre-cluster) was applied to reduce sequencing noise by clustering reads differing by 2 bp. Once chimeric sequences were detected and removed, the

remaining reads were clustered into operational taxonomic units (OTUs) at 97 % identity using an average neighbor algorithm (Schloss and Westcott 2011). The sequence and OTU classification were conducted using Mothur with a naïve Bayesian approach (SILVA) at a confidence threshold of 70 % (Pruesse et al. 2007; Wang et al. 2007). From the OTU cluster file, the Chao 1 richness estimator, ACE, Shannon diversity index and Good's coverage were generated in Mothur for each library.

Quantitative PCR

The abundance of total bacterial and cyanobacterial 16S rRNA genes and eukaryotic 18S rRNA genes was estimated by quantitative PCR (qPCR) using the primer pair 338F/518R (Muyzer et al. 1993) for bacteria, CYA 359F/CYA781 (a+b) (Nubel et al. 1997) for cyanobacteria, and 3NDf/V4_euk_R2 (Bråte et al. 2010) for eukaryotes. The standard plasmid-carrying target genes were obtained by TA cloning and were extracted using the TIAN Pure Mini Plasmid kit (Tiagen, China). The 25- μL reactions typically contained 1 $\times$ dye (Fermentas, USA), 200 nM each primer, 0.5 mg mL^{-1} BSA, and 1 μL properly diluted DNA templates. The qPCR amplification was performed in an ABI7300 apparatus (ABI, USA) following the modified protocols as described by the manufacturer, except that annealing temperatures of 57 $^{\circ}\text{C}$ for bacteria, 60 $^{\circ}\text{C}$ for cyanobacteria, and 55 $^{\circ}\text{C}$ for eukaryotes were used. Triplicates of serially diluted standard plasmids were performed to obtain the standard curves, and triplicate sample and negative control reactions were performed for quantification.

Results

Soil crust characteristics

Soil crust properties are listed in Table 1. The highest pH of the crust samples was observed in Slope 2 (7.77) and the lowest was observed in Swale 2 (7.26), indicating slight alkalinity of the crusts in Gurbantünggüt Desert. The levels

Table 1 The physical and chemical properties of crusts

Samples	Slope 1	Slope 2	Swale 1	Swale 2
H ₂ O content (%)	0.19	0.12	0.23	0.20
pH	7.58	7.77	7.44	7.26
Total organic matter (g kg^{-1})	3.91	1.88	5.91	4.81
Total nitrogen (g kg^{-1})	0.20	0.17	0.50	0.65
Chl <i>a</i> ($\mu\text{g cm}^{-2}$)	0.05	0.06	0.58	0.34

Table 2 Calculated diversity indices based on pyrosequencing of microbial communities in different crust samples

Libraries	Sites	Number of quality-checked sequences ^a	OTUs	Chao 1 (lower/upper)	ACE ^b	Shannon diversity ^b	Coverage ^b (%)
Bacteria	Slope 1	10714	1691	3069 (2835–3350)	3827	4.85	91.94
	Slope 2	12438	1358	2597 (2283–2760)	3156	3.91	94.40
	Swale 1	11609	1869	3317 (3085–3593)	4269	4.72	91.70
	Swale 2	9508	1945	3435 (3199–3715)	4334	6.11	89.60
Cyanobacteria	Slope 1	4444	55	65 (58–86)	73	0.71	99.62
	Slope 2	4766	31	48 (35–95)	52	0.34	99.75
	Swale 1	4702	65	97 (77–148)	124	0.49	99.43
	Swale 2	4335	138	296 (221–443)	420	2.79	98.16
Eukaryotes	Slope 1	4940	643	1154 (1019–1337)	1430	4.75	93.58
	Slope 2	5833	603	973 (872–1113)	1218	4.51	95.30
	Swale 1	6483	434	724 (633–856)	888	2.79	96.88
	Swale 2	5805	424	590 (534–674)	599	3.82	97.31

^a The number of quality-checked sequences indicates the number remaining after removing short and low-quality sequences and can be used to generate the diversity measures

^b The values were calculated based on a 97 % similarity threshold

of total organic matter, total nitrogen, and chlorophyll *a* were lower in slope BSCs (Slope 1 and Slope 2) compared to the interdune swales (Swale 1 and Swale 2) (Table 1), and Swale 2 BSCs had the highest total nitrogen content (0.65 g kg⁻¹) (Table 1).

Bacterial community composition

Analysis of the coverage (Table 2) showed that bacterial libraries could not account for all of bacterial species present in the BSCs. One of the swale BSCs (Swale 2) had the highest bacterial diversity with a Shannon diversity index of 6.11 and a Chao 1 index of 3435. However, one of the slopes (Slope 2) showed the lowest bacterial diversity with a Shannon diversity index of 3.91 and a Chao 1 index of 2597 (Table 2).

The obtained bacterial sequences of the four libraries fell into 13 different phyla (Fig. 1). Cyanobacteria dominated all of the BSCs and comprised 48.2–69.5 % of the total bacterial sequences from each library (Fig. 1; detailed cyanobacterial information is provided in the next section). *Proteobacteria* were the second most abundant bacterial community, especially in Swale 2. *Alphaproteobacteria* comprised 11.3–17.7 % of the total bacterial sequences (Slope 1, 16.8 %; Slope 2, 12.3 %; Swale 1, 11.3 %; Swale 2, 17.7 %), while other *Proteobacteria* constituted 0.9–1.4 % of the total (Fig. 1). Bayesian analysis at the 70 % confidence interval indicated that 82.2–86.4 % of the *Alphaproteobacteria* belonged to the orders: *Rhizobiales*, *Rhodobacterales*, *Rhodospirillales*, and *Sphingomonadales*. *Actinobacteria*-related sequences were detected as the third most dominant taxon in all BSCs. The five most

Fig. 1 Relative abundance of sequences evaluated at phylum level (class level for *Proteobacteria* lineages) to the total sequences in each bacterial library (%)

frequently classified non-phototrophic genera (>1.0 % of the total bacteria) are listed in Table 3, with two of them affiliated to *Alphaproteobacteria* and the other three belonging to *Actinobacteria*. The remaining less dominant sequences were distributed among the phyla *Acidobacteria*, *Armatimonadetes*, *Bacteroidetes*, *Chloroflexi*, *Deinococcus-Thermus*, *Firmicutes*, *Gemmatimonadetes*, *Nitrospirae*, *Planctomycetes*, and *Verrucomicrobia* (Fig. 1).

Cyanobacterial community composition

Similar to the bacterial libraries, cyanobacterial diversity indices of the OTUs, Shannon diversity, ACE, and Chao 1 (Table 2), showed that the Swale 2 BSCs had the highest cyanobacterial diversity and the Slope 2 BSCs showed the lowest diversity. The coverage of the four cyanobacterial

Table 3 The five most frequent non-phototrophic bacterial genera and their relative abundance in each bacterial library and the total libraries. The matches showed the sequences that have the highest similarity to the represent clones

	Slope 1	Slope 2	Swale 1	Swale 2	Total	Represent clones ^a	Matches GenBank	Identity (%)	Isolation sites
<i>Methylobacterium</i> (Alphaproteobacteria)	1.47	0.80	0.76	1.61	1.13	H9XAIBP01ENQ3 H9XAIBP01ALNI0 H9XAIBP01EHE4H	JQ402734 KF974283 AB859255	100 100 98	Desert of Colorado Plateau, USA Permafrost-affected soils, Arctic Spring sediments, China
<i>Rubellimicrobium</i> (Alphaproteobacteria)	3.76	3.34	2.77	0.90	2.77	H9XAIBP01BQIG H9XAIBP01C3LM3 H9XAIBP01A2YMF	JQ375224 JQ386530 JQ384935	99 100 99	Ecosystem with elevated CO ₂ , USA Ecosystem with elevated CO ₂ , USA Ecosystem with elevated CO ₂ , USA
<i>Blastococcus</i> (Actinobacteria)	2.92	1.13	4.96	4.11	3.21	H9XAIBP01CV5HH H9XAIBP01EP435 H9XAIBP01DZ9VH	NR_109679 FR865889 KF974288	100 99 99	Sediment of eutrophic lakes, China Stone in arid environments, Italy Permafrost-affected soils, Arctic
<i>Cryptosporangium</i> (Actinobacteria)	2.26	0.90	1.08	0.28	1.14	H9XAIBP01DIM5X H9XAIBP01DFI4 W	JQ379110 JX667797	100 100	Ecosystem with elevated CO ₂ , USA Sediment of cold springs, China
<i>Friedmanniella</i> (Actinobacteria)	1.97	1.51	1.53	0.75	1.46	H9XAIBP01EEKN3 H9XAIBP01ARPY5 H9XAIBP01BLPM0	JQ379190 HQ910328 JQ291033	100 99 100	Ecosystem with elevated CO ₂ , USA MDRS, USA Meltwater in two glaciers, China
						H9XAIBP01C3KT6	AB696028	100	Soil, Japan

MDRS mars desert research station

^a Three represent clones for one genus

libraries surpassed 98 %, suggesting high sequencing coverage.

The cyanobacterial sequences derived from bacterial and cyanobacterial libraries were integrated to analyze the cyanobacterial composition in the four crusts. The cyanobacterial sequences could be further divided into the orders *Oscillatoriales* (genera *Microcoleus*, *Leptolyngbya*, *Symploma*, and *Crinalium*), *Nostocales* (genera *Scytonema*, *Nodularia*, *Nostoc*, *Aphanizomenon*, *Tolypothrix*, and *Calothrix*), and *Pleurocapsales* (genus *Chroococcidiopsis*) (Fig. 2). Both the bacterial and cyanobacterial libraries confirmed the dominance of the genus *Microcoleus* in the BSCs, except for Swale 2, representing 88.6–95.7 % of the total cyanobacterial sequences in Slope 1, Slope 2, and Swale 1, and 28.4–42.9 % of the total in Swale 2 (Fig. 2). The nitrogen-fixing genus *Scytonema* was the second most abundant cyanobacterial genus in Swale 2, comprising 20.3–28.8 % of the total cyanobacterial sequences (Figs. 2, 3). Chloroplast sequences were also detected from the cyanobacterial libraries and constituted 9.0–18.1 % of the total cyanobacterial sequences in Swale 2. However, chloroplast sequences comprised less than 1.0 % of the total cyanobacterial sequences in the other three BSCs (Fig. 2).

The 10 most dominant phylotypes in each cyanobacterial library were determined, indicating that *Microcoleus vaginatus* was the most dominant phylotype in all the cyanobacterial libraries (Fig. 3). *Microcoleus steenstrupii* were abundant in Swale 2 (>20.8 %), whereas they were not ranked within the 10 most dominant phylotypes in the other three BSCs (<0.3 %, Fig. 3).

Eukaryotic community structure

A total of 20361 high-quality eukaryotic sequences were recovered from the BSC samples. The coverage showed that eukaryotic libraries could not reveal all of the eukaryotic species in the crust samples (Table 2). The diversity of eukaryotes was higher in the slopes than that in the interdune swales (Table 2).

Sequences were affiliated to 10 clades (Fig. 4), with *Chlorophyta*, *Bryophyta*, *Cercozoa*, and *Ascomycota* representing the majority of sequences (>97 %) in each site. *Chlorophyta* constituted the major eukaryotic community with the highest abundance detected in Swale 1 (75.6 %), whereas this group constituted only 2.6 % of the eukaryotic sequences in Swale 2. Most (>50 %) of the detected *Chlorophyta* sequences were affiliated with uncultured *Chlorophyta* (Fig. 5), while the rest of the sequences were related to the genera *Chlorococcum*, *Chlamydomonas*, *Chlorosarcinopsis*, *Neochlorosarcina*, *Chlorella*, *Diplospira*, *Lobosphaera*, and *Acrosiphonia* (Fig. 5). In total, the composition of *Chlorophyta* was very similar across the

Fig. 2 Relative abundance of cyanobacterial sequences at genus level to total cyanobacterial sequences in each bacterial and cyanobacterial library (%)

four sites, and the genus *Lobosphaera* (order Trebouxiophyceae) was classified as the most abundant taxon in all four sites. Nonetheless, some differences were noted among the sites: the genus *Acrosiphonia* was only found in Swale 1 and the genera *Chlamydomonas* and *Chlorosarcinopsis* were not found in Swale 2.

Bryophyta were the most abundant eukaryotic phototrophic microorganisms in Swale 2 (representing 84.1 % of eukaryotic sequences), whereas they only comprised <2 % of the total in the other crust samples. All of the detected *Bryophyta*-related sequences were affiliated to unclassified *Bryophyta*. The protozoan phylum *Cercozoa* was much more abundant in Slope 1 and 2 than in Swale 1 and 2 (27.5 and 60.6 % vs 2.3 and 0.3 %, respectively). *Ascomycota*-related sequences were detected in all crusts with an abundance of 12.4–20.9 % of total eukaryotic sequences. The remaining less dominant groups were distributed among the *Alveolata*, *Nematoda*, *Spermatophyta*, *Amoebozoa*, *Bacillariophyta*, and *Chrysophyta*.

Microbial abundance

Quantitative PCR was performed to assess the abundance of bacteria, cyanobacteria, and eukaryotes in crusts. Total bacterial and cyanobacterial 16S rRNA gene abundance in crusts ranged from 1×10^{11} to 3×10^{11} copies g^{-1} soil (Fig. 6), which is congruent with a previous study conducted in Colorado (Bates et al. 2010). The ratio of cyanobacteria to bacteria was above 60 % and was highest in the Swale 1 sample (97.6 %). However, the average abundance of the eukaryotic 18S rRNA gene was approximately 10^7 – 10^9 copies g^{-1} soil (Fig. 6), which was two to four orders of magnitude lower than the abundance of bacterial and cyanobacterial 16S rRNA genes.

Discussion

In this study, all the BSCs analyzed from slopes and swales were found to be cyanobacteria dominated based on pyrosequencing and qPCR. Whereas bacteria are the numerically dominant population in cyanobacterial BSCs, eukaryotic phototrophic communities vary greatly in different BSC succession stages, as revealed using cultivation-dependent approaches (Büdel et al. 2009; Langhans et al. 2009); therefore it is important to investigate the whole bacterial and eukaryotic communities to accurately characterize the community succession of cyanobacterial BSCs.

Eukaryotic community succession

To our knowledge, this is the first report of the whole eukaryotic community succession in BSCs using pyrosequencing. Although the abundance of eukaryotes was significantly lower than that of bacteria and cyanobacteria in cyanobacterial BSCs, great differences in eukaryotic community structure were observed among the four BSCs. Most of the eukaryotic sequences from Swale 2 were affiliated to the *Bryophyta* clade (>80 %), which appears to characterize the later phase of succession (Langhans et al. 2009). *Bryophyta* have special structures to store and conduct water and also contain revolute (curled-under) leaf margins to reduce water loss through transpiration (Belnap 2003; Lange et al. 1992). Thus, they play an important role in the stabilization of the surface through the presence of rhizoids and protonemata (Lange et al. 1992).

Surprisingly, few phototrophic microorganisms could be retrieved from the eukaryotic community of one of the slopes (Slope 2). In this slope, eukaryotic sequences were mainly affiliated to heterotrophic microorganisms, including members of the *Cercozoa* (60.6 %) and *Ascomycota* clades (19.85 %). A study of protist distribution revealed that the *Cercozoa* were widespread and tended to be more abundant in arid soils (Bates et al. 2012). These findings are congruous with the results of our study because the water content was the lowest in Slope 2. The pH was highest in Slope 2, which likely served to increase the abundance of *Cercozoa* while limiting the growth of *Bryophyta* and *Chlorophyta* (Lange et al. 1992). Consequently, BSCs in this slope appeared to be at a lower succession stage compared with the other crusts.

From Slope 1 and Swale 1 BSCs, the *Chlorophyta* clade was found to be the dominant eukaryotic community. The high proportion of unclassified *Chlorophyta* suggested that novel *Chlorophyta* exist in the Gurbantünggüt Desert. Among the classified *Chlorophyta*, sequences of the order *Trebouxiophyceae* were most abundant, which are also known to be dominant on building facades and other crusts

Slope 1	Slope 2	Swale 1	Swale 2	ID	% identity	GenBank	Match	Order
87.40	94.29	92.80	20.03	H9XAIBP02HBV17	98	HF678512	<i>Microcoleus vaginatus</i>	Oscillatoriales
NA	NA	NA	15.82	H9XAIBP02I32E0	98	JQ697044	<i>Scytonema javanicum</i>	Nostocales
NA	NA	NA	10.8	H9XAIBP02GIYN1	98	AF355395	<i>Microcoleus steenstrupii</i>	Oscillatoriales
NA	NA	NA	8.23	H9XAIBP02IUGF0	100	DQ531697	<i>Scytonema</i> sp	Nostocales
3.95	0.10	0.21	NA	H9XAIBP02IGZAY	94	AJ871987	<i>Microcoleus steenstrupii</i>	Oscillatoriales
2.38	2.43	0.63	NA	H9XAIBP02JNV64	99	CP003620	<i>Crinalium epipsammum</i>	Oscillatoriales
0.36	NA	0.36	4.34	H9XAIBP02HG9M	97	JQ083659	<i>Scytonema</i> sp	Nostocales
NA	NA	NA	3.71	H9XAIBP02JU8XH	93	AY301000	<i>Chroococcidiopsis</i> sp	Pleurocapsales
NA	NA	NA	3.68	H9XAIBP02GR4HJ	97	AF355382	<i>Microcoleus steenstrupii</i>	Oscillatoriales
NA	NA	NA	3.16	H9XAIBP02HH9PD	97	AJ871987	<i>Microcoleus steenstrupii</i>	Oscillatoriales
0.47	NA	NA	NA	H9XAIBP02HMHXB	98	JX494785	<i>Chroococcidiopsis thermalis</i>	Pleurocapsales
NA	NA	NA	2.84	H9XAIBP02F8QYO	97	AJ871983	<i>Microcoleus steenstrupii</i>	Oscillatoriales
NA	NA	NA	2.41	H9XAIBP02FR0KF	96	AF279107	<i>Chroococcidiopsis</i> sp	Pleurocapsales
0.83	0.17	0.49	NA	H9XAIBP02HLHMM	99	EU586741	<i>Microcoleus</i> sp	Oscillatoriales
0.38	0.10	NA	NA	H9XAIBP02H4GH4	96	DQ914863	<i>Chroococcidiopsis</i> sp	Pleurocapsales
NA	NA	0.21	NA	H9XAIBP02J12G2	99	EU068737	<i>Phormidium corium</i>	Oscillatoriales
NA	NA	0.49	NA	H9XAIBP02HQKWC	100	AY493595	<i>Nostoc</i> sp	Nostocales
NA	0.76	NA	NA	H9XAIBP02GB0YW	96	GU018032	<i>Phormidium autumnale</i>	Oscillatoriales
NA	NA	0.70	NA	H9XAIBP02FSZDT	95	AF355356	<i>Microcoleus vaginatus</i>	Oscillatoriales
0.29	0.44	NA	NA	H9XAIBP02IJW2B	94	KC924437	<i>Lyngbya arboricola</i>	Oscillatoriales
NA	0.50	NA	NA	H9XAIBP02JN8S9	97	NR_102461	<i>Crinalium epipsammum</i>	Oscillatoriales
NA	NA	0.42	NA	H9XAIBP02JTNDP	95	AY702111	chloroplast	Trebouxiophyceae
0.31	0.21	NA	NA	H9XAIBP02JYHOA	95	JQ712635	<i>Microcoleus vaginatus</i>	Oscillatoriales
NA	NA	0.46	NA	H9XAIBP02IF5TH	100	GQ451414	<i>Phormidium autumnale</i>	Oscillatoriales
NA	0.15	NA	NA	H9XAIBP02I34UJ	96	GU018032	<i>Phormidium autumnale</i>	Oscillatoriales
0.25	NA	NA	NA	H9XAIBP02HU8KH	93	NR_102461	<i>Crinalium epipsammum</i>	Oscillatoriales

Fig. 3 Heat map showing the 10 most dominant phylotypes in each cyanobacterial library and their relative abundance (as percentages of all cyanobacterial 16S rRNA genes recovered). Color tones moving from red to yellowish white indicate the highest to lowest relative

abundance values. NA indicates that the phylotype was not included within the 10 most dominant phylotypes for that site. The matches showed the best culturable strains that have highest similarity with the phylotypes

Fig. 4 Relative abundance of specific clade sequences (%) to the total sequences in each eukaryotic library

(Büdel et al. 2009; Hallmann et al. 2013). Most green algae of *Trebouxiophyceae* can synthesize high concentrations of anti-stress metabolites, which could act as antioxidants, heat protectants (protein stabilizers), and rapidly available respiratory substrates (Karsten et al. 2010). UV-absorbing substances have only been identified in members of *Trebouxiophyceae* and are absent in other members of *Chlorophyta* (Karsten et al. 2005). These characteristics could ensure the survival of *Trebouxiophyceae* in Slope 1 and Swale 1 BSCs.

Bacterial community succession

Cyanobacteria constitute the major component of the bacterial community in Gurbantünggüt Desert BSCs. The *Microcoleus* cluster within the order *Oscillatoriales* was clearly the most abundant cyanobacterial group in the BSCs. Both of the slopes and one of the swales (Swale 1) exhibited an absolute predominance of *M. vaginatus*, as was observed in BSCs from the Oman and Colorado Plateau deserts (Abed et al. 2010; Belnap and Gardner 1993). These large, highly mobile filamentous cyanobacteria secrete exopolysaccharide sheaths in ample quantities that glue and weave soil particles together, thereby forming the soil crusts and creating a microhabitat for other biotic components (Belnap and Gardner 1993; Gundlapally and Garcia-Pichel 2006). Members of *Microcoleus* can tolerate some burial and are generally the first colonizers of unstable soils (Belnap 2003). However, in one of the Gurbantünggüt swales (Swale 2), the dominance of *M. vaginatus* was weakened owing to the abundant presence of *M. steenstrupii* and *Scytonema*. *M. steenstrupii* was found to be more thermo tolerant than *M. vaginatus* (Garcia-Pichel et al. 2013), and thus their higher abundance in Swale 2 could imply a higher succession stage compared to other BSCs. In general, *Scytonema*-like cyanobacteria only begin to establish after *Microcoleus* move out in the direction of the crust surface, forming a 1-mm-diameter layer on the

Fig. 5 Relative abundance of *Chlorophyta* sequences at genus level to the total *Chlorophyta* sequences. Color tones moving from red to yellow indicate the highest to lowest relative abundance values

Chlorococcum (Chlorophyceae)
Chlamydomonas (Chlorophyceae)
Chlorosarcinopsis (Chlorophyceae)
Neochlorosarcina (Chlorophyceae)
Chlorella (Trebouxiophyceae)
Diplosphaera (Trebouxiophyceae)
Lobosphaera (Trebouxiophyceae)
Acrosiphonia (Ulvophyceae)
 Unclassified genus

Slope 1	Slope 2	Swale 1	Swale 2
0.30	0.76	0.12	1.31
1.14	2.06	0.04	0.00
0.04	0.32	0.04	0.00
3.20	0.97	0.14	2.61
0.91	0.32	0.14	6.54
7.49	3.03	0.47	1.31
35.11	37.23	1.51	19.61
0.00	0.00	0.02	0.00
51.81	55.31	97.52	68.62

Fig. 6 Bacterial, cyanobacterial and eukaryotic SSU rRNA genes copy number

surface of the crusts (Lange et al. 1992). These cyanobacteria are highly adapted for life in light-intensive arid environments through the production of the UV sunscreen pigment scytonemin (Abed et al. 2008; Belnap et al. 2008), which also serves to protect the microorganisms behind them. Moreover, *Scytonema*-like cyanobacteria are also known to contribute significantly to nitrogen fixation (Abed et al. 2010; Belnap 2002), which was evident by the elevated nitrogen content observed in Swale 2, as shown in Table 1. These characteristics suggested that BSCs in the swale (Swale 2) develop into a higher successional community than other BSCs, as proposed by Büdel et al. (2009).

In contrast to the cyanobacterial communities, the non-phototrophic bacterial community was similar among the four BSCs, and the sequences were mainly related to *Alphaproteobacteria* and *Actinobacter*. Not surprisingly, the closest matches for the most abundant genera were mainly derived from harsh environments (Table 3). *Rubellimicrobium* was the most abundant alphaproteobacterial genus identified in this study, and bacteria in this genus have been derived from several harsh environments, e.g., elevated CO₂ ecosystems (Table 3) (Dunbar et al. 2012), desert soil crusts (Abed et al. 2010), and air samples (Weon et al.

2009). The abundance of aerobic, anoxygenic phototrophs (*Methylobacterium*) in the crusts implied a higher efficiency of light harvesting by soil crust organisms than previously realized (Csotonyi et al. 2010). *Methylobacterium*, as a plant growth-promoting bacteria (Joe et al. 2013), might also be beneficial for plant colonization on the BSCs. *Actinobacteria* are known to dominate alkaline soils, particularly arid soils, and have been found in abundance in previous surveys of neutral-alkaline soils (Lauber et al. 2009), similar to the environment in this study. The most abundant actinobacterial genus was *Blastococcus*, which is known for its resistance to environmental hazards and has morphological features that facilitate adaptation to dry biotopes, including the formation of spores (Gtari et al. 2012). Other actinobacterial genera, such as *Cryptosporangium* and *Friedmanniella*, have been derived from arid soil crusts in the Turpan Basin and the Colorado Plateau (Gundlapally and Garcia-Pichel 2006).

Succession patterns of cyanobacterial BSCs and possible impact factors

Previously, Büdel et al. (2009) distinguished seven BSC types on the basis of morphology and taxonomic composition, and three of them were found to be cyanobacteria dominated. Some other researchers differentiated BSCs into initial crusts and stable crusts, and cyanobacteria-dominated crusts were classified as initial crusts (Langhans et al. 2009). In the present study, eukaryotic communities differed greatly among the four BSCs, and the succession stage of BSCs from Gurbantünggüt Desert sand dunes was evaluated on the basis of eukaryotic communities. In addition, the bacterial communities were also integrated to interpret the succession patterns of the cyanobacterial BSCs.

Four BSCs may represent three succession stages. Initial crusts (stage 1) were found in a slope area (Slope 2) that is dominated by the *M. vaginatus* (cyanobacteria),

with a high proportion of non-phototrophic eukaryotes detected. According to Hu et al. (2003), the higher abundance of *Chlorophyta* in Swale 1 and Slope 1 suggests that these areas are at a higher succession stage compared to Slope 2. Thus, the intermediate succession crusts (stage 2) were detected both in swales (Swale 1) and slopes (Slope 1), with *M. vaginatus* and the phylum *Chlorophyta* dominating the cyanobacterial and eukaryotic communities, respectively. The later succession stage (stage 3) was identified in a swale area (Swale 2) with *Bryophyta* dominating the eukaryotic community and abundant *Scytonema* and *M. steenstrupii* present in the cyanobacterial communities.

It is reasonable to assume that the succession stage in swales was higher than that in slopes because of their different environmental factors. Late spring, summer, and early fall are times of higher irradiation and UV exposure for organisms on the soil surface. BSCs that occur in slope areas are subjected to a longer radiation exposure time than those in swale areas. *Microcoleus* avoids high radiation exposure by residing below the soil surface and gliding to the surface only when soils are sufficiently moistened for photosynthesis (Belnap 2003; Garcia-Pichel and Belnap 2001). Unlike *Microcoleus*, *Nostoc* and *Scytonema* are relatively non-motile cyanobacteria and cannot seek refuge. These organisms could produce UV-protective pigments and polysaccharides for protection from radiation exposure. However, such pigment production would dramatically decline under conditions of long-term radiation and UV exposure (Belnap et al. 2004). Thus, under these conditions, the presence of *Nostoc* and *Scytonema* is expected to be limited, which could explain the low presence of *Scytonema* in the slope crusts. Water distribution over dune slopes is also affected by the slope position, with meager quantities in the midslope sections and larger amounts detected at the swales (Jacobs et al. 2000; Kidron et al. 2010). Crusts are metabolically active when wet, and the periods of soil wetness are infrequent and short in slopes (Belnap et al. 2004). Thus, the crusts in swales have higher metabolic activity and, in consequence, have increased potential to develop into later succession crusts. Disturbances such as wind and grazing may also affect the development of soil crusts in Gurbantünggüt Desert. The development of BSCs in one of the swales (Swale 1) may be more affected by disturbance, and thus was at a lower stage than the other swale (Swale 2). This situation might also apply to the slopes, with Slope 1 being at a higher succession stage than Slope 2.

In conclusion, our study expands on previous studies by providing the whole eukaryotic community structure in desert sand dune BSCs. The harsher environment in slopes than swales might restrict the ability for *Scytonema* and *Bryophyta* to thrive in slope BSCs, resulting in a lower

succession stage in slopes; non-phototrophic eukaryotes were found to be the major eukaryotic community in the lowest succession stage. Besides differences in environmental factors, disturbances may also influence the establishment of phototrophic communities both in swales and slopes, which require long-term measurements of the different degrees of disturbances in these areas.

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