2012

Bacterial Diversity in Biological Soil Crusts from Extrazonal Mountain Dry Steppes in Northern Mongolia

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Erforsch. biol. Ress. Mongolei (Halle/Saale) 2012 (**12**): 437–449

Bacterial diversity in biological soil crusts from extrazonal mountain dry steppes in northern Mongolia

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Abstract

Biological Soil Crusts (BSCs), consisting of prokaryotes, microalgae, lichens, mosses and eventually small vascular plants, cover wide areas in arid and semi-arid environments. In the present study, the microbial diversity of these crusts was explored at extrazonal mountain steppe sites in the western Khentej (Northern Mongolia). At the study site the Siberian taiga borders on the Mongolian-Daurian forest steppe, resulting in a unique intermixture of the dark taiga, the light taiga, and forest steppe (DULAMSUREN 2004). Due to the presence of boreal, temperate and dauric elements the forest steppe is eminently rich in species (MÜHLENBERG et al. 2004).

BSCs in the western Khentej only occur in mainly non-forest areas of the *Carex amgunensis*-, *Festuca lenensis*-, *Pulsatilla ambigua*- and *Artemisia frigida*-mountain-dry-steppe and *Ulmus pumila*-open woodland (DULAMSUREN 2004). BSCs at these sites contain small vascular plants, mosses, lichens, and different microorganisms in varying ratios. In this communication, the diversity of BSCs, especially with respect to bacterial phylotypes in two different sites with a diverging degree of disturbance is presented. According to results based on 16S rDNA analysis, the relative abundance of Cyanobacteria decreases on disturbed sites, whereas abundances of other large groups increase. Generally, the more disturbed site appears to be more diverse.

Key words: biological soil crusts, biodiversity, arid environment, western Khentej, Mongolia

Introduction

Biological soil crusts (BSCs), also known as microbiotic, cryptogamic, cryptobiotic or biogenic soil crusts, are highly complex systems. Intriguingly, crust structure and morphology is determined by prokaryotes, eukaryotic microalgae and multicellular cryptograms. They occur on the upper soil layer and are some millimeters to several centimeters thick. BSCs of this study are composed by mosses, microfungi, lichen, diatoms, green algae, cyanobacteria, and other prokaryotes. In addition, the BSCs provide a habitat for higher organisms like small vascular plants and insects. They are ubiquitous and appear generally at locations where higher vegetation is disturbed or is completely lacking (e.g. parts of cold and hot deserts, dunes and mountain dry steppe sites).

Climate in Mongolia is marked by extreme continentality as the region is surrounded by mountain ranges in the centre of the Asian continent. The absolute thermal fluctuation is 90°C (T_{max} = 40° C; T_{min} = -50°C), whereas the daily fluctuation in summer can reach about 25°C. The vegetation period lasts just 90–120 days. Frost may occur as late as May and as early as August, snowfall in late July. Precipitation averages between 250-350 mm per year. The yearly fluctuations are high. 75-90 % of the annual precipitation occurs as summer rain, especially in July and August. Thus, the main growing season is in summer. Winters are cold and dry; the thickness of the snow cover does not exceed 10-15 cm.

Khan Khentej is a low mountain range that occupies 48.000 km^2 and borders to Siberia in the North. It is part of the transbaicalic mountain system, which mostly consists of proterozoic and paleozoic geological formations, mainly granite. The elevations range from about 2800 m above sea level in the Central Khentej to about 600 m in the Northeastern foothills. Khonin Nuga is located Northwest of the center. The river valleys are approximately 900 m above sea level, the highest elevations are at around 1600 m. The soils in Khentej are strongly affected by permafrost.

The climatic factors and the resulting cryogenic processes lead to soil podzolization (SCHEFFER & SCHACHTSCHABEL 1995, WYSS 2006). Generally, it should be expected that the podzolic soils, depleted in organic carbon, favor development of BSCs.

Valleys of the Khan Khentej mountain range are mostly oriented from East to West, the mountain ridges are mostly even or rounded. The main wind direction perpendicular to the mountain ridges (north to northwest) leads to a multitude of mesoclimatic conditions, with considerable variations depending on elevation and exposition. There are big differences in the mesoclimate of northern and southern slopes. Northern slopes of the mountains represent the upwind flank. Slowdown and cooling down of the ascending air masses lead to increased precipitation at the northern slopes. The lee side (southern slopes) accumulates less precipitation (OGORODNIKOV 1981). With the distance to the mountain ridge at the southern slope the temperature increases, precipitation and air moisture decrease (VIPPER 1953). The exposition also affects radiation. The northern slopes are normally afforested, while on southern slopes the different types of mountain dry steppe occur (DULAMSUREN 2004). Between the sparse cover of mostly low vascular plants is enough space for the settlement of BSCs.

The appearance of a BSC strongly depends on its degree of succession. In many regions, even in temperate latitudes, initial states of BSCs form after a disturbance by destruction of the original vegetation. Just in moist sites, the BSCs are overgrown by fast growing and more competitive ruderal plant species. The BSC in its initial state is often dominated by Cyanobacteria, which in many cases are mobile by gliding. These Cyanobacteria secrete polysaccharide sheaths, and spread quickly due to their mobility, often leaving behind the empty sheaths (MAZOR et al. 1996). These sheaths are holdfasts and organic substrate for other microorganisms. The polysaccharide absorbs and stores water for longer time periods (KEMMLING et al. 2001). Based on the ability of many Cyanobacteria to fix nitrogen, even on poor soils more fastidious organisms will settle in this microhabitat. Over time, horizontally layered structures may develop within complex BSCs, including anaerobic zones (e.g. GARCIA-PICHEL & BELNAP 1996). More complex crusts that contain mosses or lichens, may only form where BSCs are not outcompeted by faster growing vascular plants. In their initial stages, BSCs are often pioneer settlers at disturbed locations, but their further development depends on the existence or absence of disturbances like natural physical erosion, or fire. Thus BSCs may be considered as indicators for fast changing ecosystems.

Little knowledge on BSCs or prokaryotic diversity of dryland soils for Northern Asia is available, including the Mongolian steppe (BELNAP et al. 1999, rev. by BÜDEL 2001), which differs greatly from Khan Khentej. For Siberia, only a few observations were documented, implying the presence of diverse BSCs. Studies by NOVICHKOVA-IVANOVA (1977, 1980,1988) dealt with the diversity of soil algae in several deserts and steppes of Turkmenistan and the Gobi desert. All in all, more than 400 species of algae were discovered and it was observed that the microbial biomass strongly depends on soil conditions and moisture content of the soil.

In this study, we compare two sites with different disturbance histories and hence specific types or stages of BSCs. Here, we use 16S rDNA, amplified from isolated environmental DNA to compare the microbial communities of BSCs at two different sites (cf. AMANN et al. 2003).

Material and methods

Sample collection and DNA extraction

The samples were collected *in July* 2007 at two different sites (see fig. 1 and table 2) in the northwestern part of Khan Khentej near Khonin Nuga research station (49°05' N, 107°17' E). In both sites, dry specimens of BSCs were collected at three different sampling sites. The specimens of each site were combined, macerated and intensively mixed by shaking. 0.25 g of the resulting powder was used for the following DNA extraction. DNA was extracted with the *Power Soil™ DNA-Kit* according to the manufacturers protocol (MoBio, Carlsbad, California, USA).

Clone library construction

The PCR (polymerase chain reaction) mixture (50 µl) for amplification of 16S rRNA genes contained 5 µl of Mg-free polymerase buffer (MBI Fermentas, St. Leon-Rot, Germany), 200 µM of each of the four deoxynucleoside triphosphates, 1.75 mM $MgCl_2$, as well as 2 µM of each of the primers, 1 U of Taq DNA polymerase (MBI Fermentas), and 25 ng of isolated DNA as a template. The PCR was performed in a *Mastercycler Gradient thermal cycler* (Eppendorf, Hamburg, Germany) at 94°C for 2 min (for initial denaturation and activation of the polymerase); followed by 28 cycles at 94°C for 1 min of denaturation at 94°C for 1 min, annealing at 58°C for 1.5 min, extension at 72°C for 1.3 min and final extension at 72°C for 10 min. For both clone libraries 16S rRNA genes were amplified with universal reverse oligonucleotide primer 1114R and forward primer 8F (see table 1).

Table 1: PCR-primers

primer	sequence (5`-3‘)	specifity	reference
8F	GGATCCAGACTTTGATYMTGGCTCAG	Bacteria	BEN-DOV et al. (2006)
1114R	GGGTTGCGCTCGTTRC	Bacteria	modificated from REYSENBACH & PACE (1995)

The amplified 16S rDNA was further checked and purified by electrophoresis in a 1 % (wt/vol) agarose gel. DNA fragments of expected sizes were excised and recovered from the agarose using the *PeqGold Gel Extraction Kit* TM (PeqLab Biotechnologie GmbH, Erlangen, Germany) according to the manufacturers protocol. 100 µg DNA was ligated with a topoisomerase into the vector pCR2.1-TOPO® (Invitrogen, Karlsruhe, Germany) and afterwards cloned into heat shock-competent *E.coli*-TOP10®-cells according to the manufacturers protocol. After plating on LB/kanamycin agar plates and incubation over night at 37°C, positive clones were picked into liquid LB-medium and incubated over night at 37°C. Subsequently, plasmid preparation with the *Qiagen Plasmid Midi Kit* (Qiagen, Hilden, Germany) was conducted. Plasmids were checked for inserts by digestion with *Eco*RI restriction endonuclease (MBI Fermentas, St. Leon-Rot, Germany).

Sequencing and phylogenetic analysis of bacterial libraries

16S rRNA gene sequencing was performed by the Göttingen Genomics Laboratory (Göttingen, Germany). The Sanger sequencing reaction was conducted with the primer pair 8F and 1114R. The raw sequences were then edited with the *Staden Package* (STADEN et al., 2000). Initially, *Pregap4* (BONFIELD et al., 1995) was applied to remove vectorial contaminations and less-quality sequences. After *Pregap4* processing the sequences were assembled with the program *Gap4*. This was done via the PHRAP algorithm (http://bozeman.mbt.washington.edu/phrap.docs/phrap.html), for detection of sequence overlaps. Assembly mistakes were corrected manually. Afterwards all sequences were adjusted into sense-direction with *Orientation Checker* (http://www.bioinformatics-toolkit.org/). Chimera were detected with *Bellerophon chimera detection program* (http://foo.maths.uq.edu.au/~huber/bellerophon.pl.; HUBER et al. 2004) and *Mallard* (ASHEL-FORD et al. 2006) and removed from the datasets. Operational taxonomical units (OTU’s; SCHLOSS & HANDELSMANN 2005) were determined using *DOTUR*. For species-level phylotypes, genetic distances of 0.03 (3 %) were defined. Distances of 0.2 (20 %) were defined as phylum level. Shannon-Weaver and Simpson indices (as a description of the diversity within a dataset), abundance-based coverage estimator (*ACE*) as well as nonparametric abundance estimator based on one- and two-elemental masses *(Chao1)* and rarefaction curves were also calculated with *DOTUR*. Sequences generated in this study have been deposited to GenBank under the accession numbers JX254918-JX255375.

Soil chemistry and other habitat conditions

50 g samples of dry soil taken from the O-horizon were collected at three different sampling sites within the two sites and mixed afterwards. The soil samples were sieved with a 2-mm-sieve. The soil chemical parameters (pH, pH_{KCl}, C/N) were determined according to FENDER et al. (2012).

The maximum water holding capacity (WHC_{max}) was measured gravimetrically using 20 g dry soil. The soil was saturated for 30 hours with water and was weighted again. The resulting mass difference represents WHC_{max} in grams stored water per gram dry soil.

The mean temperature of the ground was measured according to PALLMANN (1940) for 10 days during the sampling period on topsoil, on the basis of sucrose hydrolysis to "invert sugar" (glucose and fructose) which was quantified using a polarimeter (Zeiss, Oberkochen, Germany).

Results and discussion

The structure of BSCs depends to a high degree on their age. It is known that generally filamentous cyanobacteria play a key role in crust growth and dispersal. BELNAP et al. (2008) defined six stages in development of BSCs dominated by cyanobacteria. For this they used the observation that the cyanobacterial crust progressively darkens during succession (HOUSMAN et al. 2006), thereby providing UV protection for less pigmented species (BOWKER et al. 2002). In some regions, instead of Cyanobacteria, mosses are the important colonizers of the BSCs (e.g. READ et al. 2011). According to our own field observations, putatively young BSCs are dominated by Cyanobacteria and mosses. Lichen-containing BSCs were interpreted as later stages in crust development: first lichens of low complexity like *Collema* sp., then assumedly *Cladonia* sp. and *Psora* sp. and later the complex leafy lichen *Xanthoparmelia* sp. Based on this, we assume that the BSCs in site A in average are older, because they contain complex leafy lichens like *Xanthoparmelia* spec. We also found thinner crusts of the initial type.

It should be expected that the BSCs are particularly affected by trampling of livestock. Non-anthropogenic effectors are erosion by wind, rain, wild animals or slope movement. The last one especially applies at the study site B. Crust damage through trampling cause loss of crust biomass and a reduction of the cryptogamic species diversity (rev. by ELDRIDGE & KOEN 1998). Other factors such as air pollution, application of herbicides or motor vehicles may be excluded for our particular site.

The capacity of regeneration for BSCs varies, depending on species composition, season, and organic carbon contents. Dry BSCs are mostly brittle and heavily affected by mechanical compression and shear forces, other than moistened BSCs (HARPER & MARBLE 1988). Moreover, the crust-building organisms are only active in a wet state; regeneration of the dry crust is not possible (LANGE 2001), which restricts the metabolic activity, crust regeneration and growth mainly to July and August.

In order to find determinants that may influence structure and composition of BSCs, some soil chemical parameters were determined. Table 2 shows an overview of the topological, soil chemical and plant ecological differences of both study sites. The soil pH value of the O-horizon of site A is between 6.7 and 6.8 and that of site B at 7.7 (neutral – slightly alkaline). The pH_{KCl} as indicator for the potential acidity of the soil is quite low for both sites (-0.39 [A] and -0.17[B]). The C/N-ratio of site A is between 11.24 and 11.64 and of site B at 10.98. Reference values for the C/N-ratio are approximately 11 for grassland, 14 for farmland soils, and 28-50 for forests (SCHEFFER & SCHACHTSCHABEL 1992). The maximum water holding capacity of the organic soil components (WHC_{max}) depends on the width of the pores (compaction parameters) and therefore on particle size. The finer the soil particles, the more water can be absorbed and stored (BOSSEL 1997). The maximum water holding capacities of the upper soil of the both sites are equal.

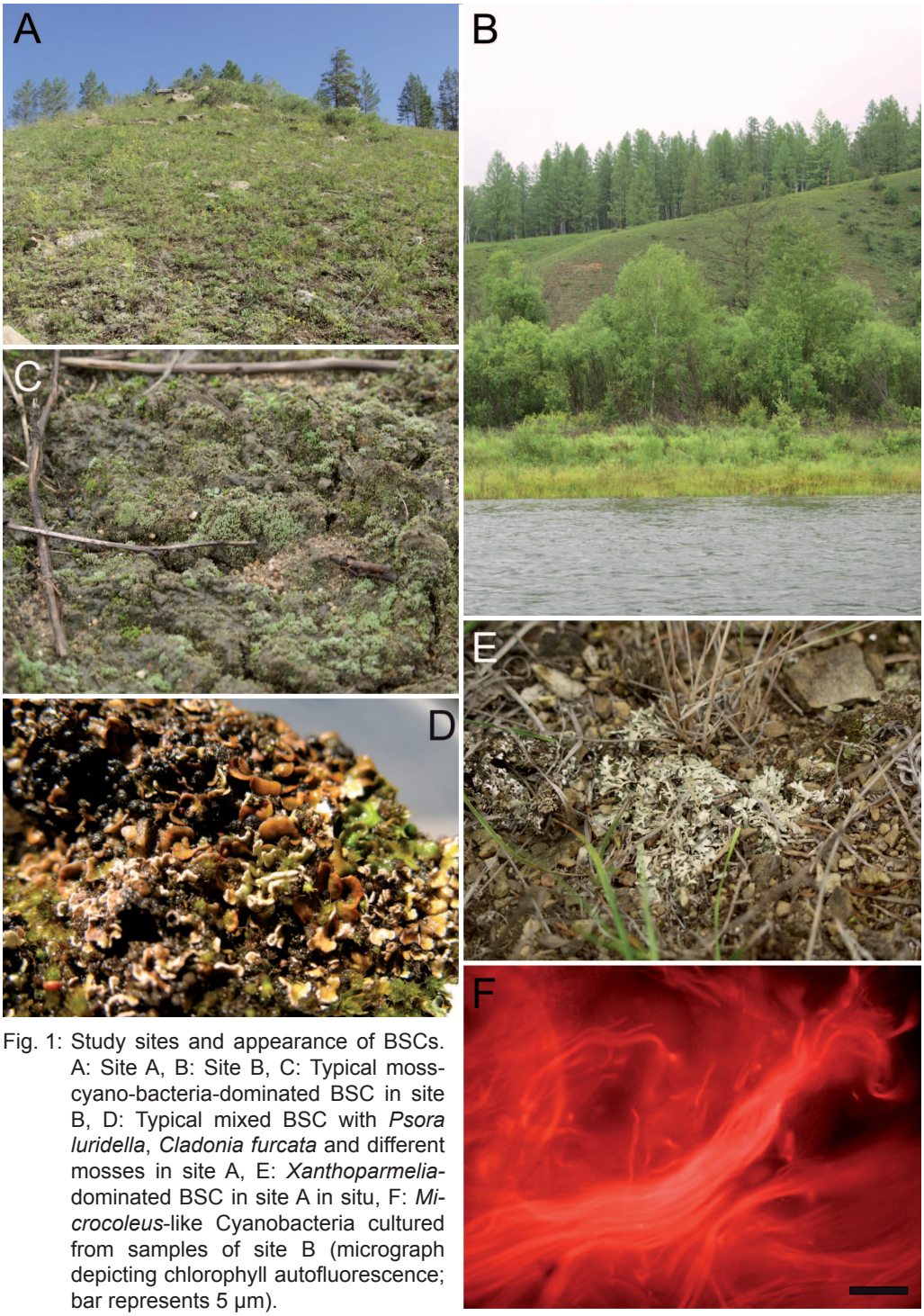

Fig. 1: Study sites and appearance of BSCs. A: Site A, B: Site B, C: Typical moss-cyano-bacteria-dominated BSC in site B, D: Typical mixed BSC with *Psora luridella*, *Cladonia furcata* and different mosses in site A, E: *Xanthoparmelia*-dominated BSC in site A in situ, F: *Microcoleus*-like Cyanobacteria cultured from samples of site B (micrograph depicting chlorophyll autofluorescence; bar represents 5 µm).

At slope A there is a wide range of different expositions, (SE - SW). The exposition of slope B is orientated south to southwest; this slope is, on average, steeper.

Table 2: Characteristics of the sites

characteristics	**slope A**	**slope B**
disturbance	low	high
location	UTM: 48U0667396 5438800	UTM: 48U0666979 5440021
height	1002 m above sea-level	954 m above sea-level
exposition	135 – 240°	180 – 220°
inclination	25 – 40°	30 – 50°
position in the landscape	wooded at the foot of the hill, but no shading in the BSC field	unforested, oriented towards the river valley
number of characteristic species for		
Artemisia frigida - MDS	4	2
Carex amgunensis - MDS	-	-
Festuca lenensis - MDS	1	1
Pulsatilla ambigua - MDS	-	-
Ulmus pumila - OWL	2	2
Plant cover within the BSC field	60 – 95	10 – 40
BSC type dominating	*Xanthoparmelia* spec., *Cladonia* spec., *Psora luridella, Catapyrenium lachneum*	*Catapyrenium lachneum, Collema* spec., mosses, initial crusts with algae
BSC coverage	10 – 70 %	0 –10 %
Number of cryptogamic species	18	6
pH	6.74	7.67
Δ pH (with KCl addition)	-0,39	-0.17
Quantities of heat % inverted (100 % = completely shaded)	40.28 (34.69-50.68)	49.15
WHC_{max} (g water/g soil)	0.6	0.6
C_{total} (mmol/g)	2.93	2.2
N_{total}(mmol/g)	0.26	0.19
C_{org} (mmol/g)	2.91	2.09
C_{org}/N_{total} (mol/mol)	11.43	10.98

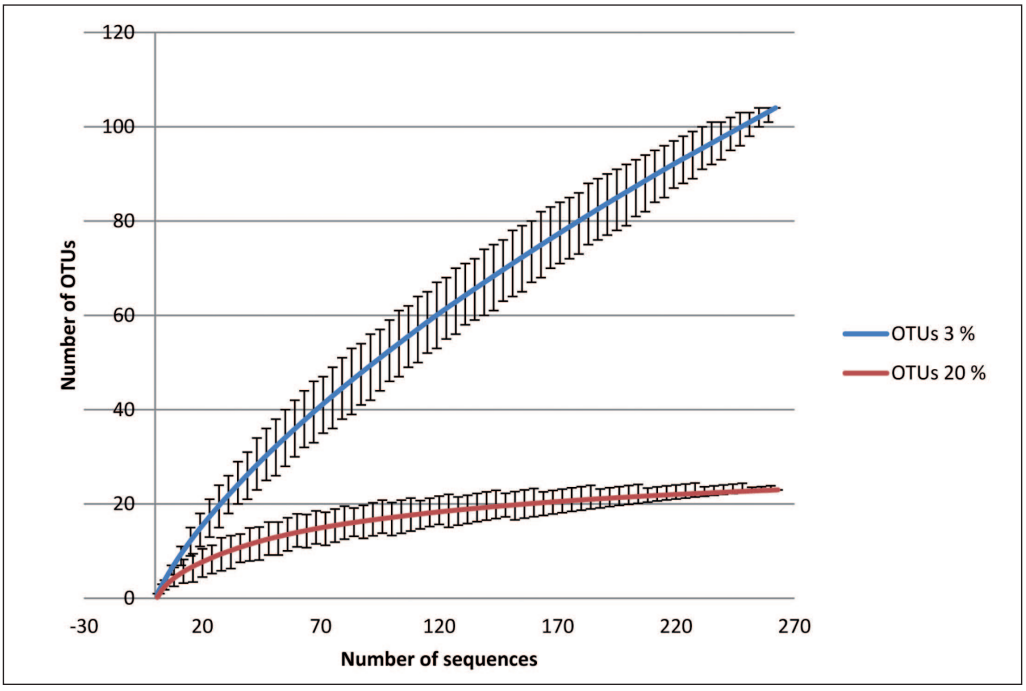

Fig. 2a: Rarefaction analyses by the program DOTUR for site A.

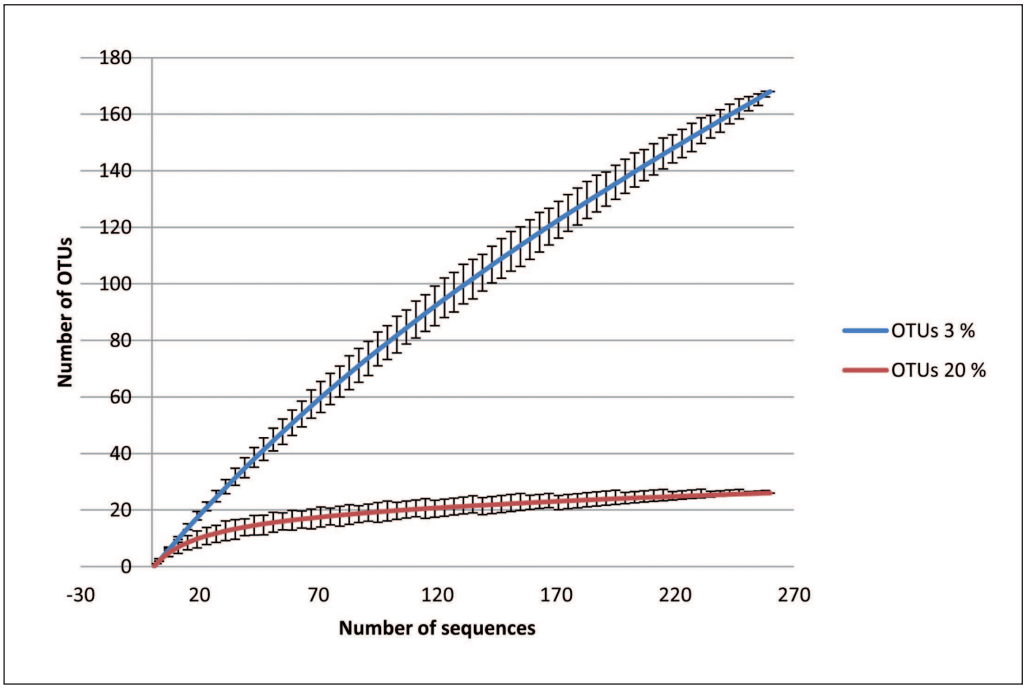

Fig. 2b: Rarefaction analyses by the program DOTUR for site B.

Site A however is situated behind a forested hill and less affected by grazers than site B, as estimated by the amount of hoofprint and feces. Lichens of the genera *Psora* and *Cladonia* were dominant, *Xanthoparmelia* and *Collema* were frequent. The cover with phanerogams was considerably higher than at site B.

For phylogenetic analysis, a total of 259 (site A) and 258 (site B) high-quality 16S rRNA gene sequences were retrieved after Chimera check. Rarefaction curves are shown in fig. 2 a, b. The error bars are confidence intervals of the estimate values. The results show for both sites that the bacterial diversity at phylum level is near saturation, whereas the bacterial diversity on species level is even after the evaluation of 260 sequences far away from saturation.

Table 3: Detected OTUs (operational taxonomical units) compared with estimated values

site	distance	number of OTUs	ACE*	Chao1 **	Shannon-Weaver***
A	0.03	104	304	282	3.93
	0.2	23	26	25	2.12
B	0.03	168	483	494	4.93
	0.2	26	33	30	2.64

* abundance based coverage estimator (ACE), not parametric abundance estimator based on abundance (> 10) and rareness (< 10) of OTUs; ** not parametric abundance estimator based on one- and two-elemental masses; *** Shannon-Weaver-index of diversity; as higher as more diverse

The amount of OTUs detected via *DOTUR* analysis at a genetic distance of 0.03 (at least 97 % identity of the sequences) and 0.2 (80 % identity) is shown in fig. 6, as well as the values calculated via the coverage estimators (*ACE*) and of *Chao1*. For site A, at a distance of 0.03 (species level) coverage of approximately 35 % was achieved. For a genetic distance of 0.2 (phylum level) 25 to 26 OTUs were expected by the richness estimators and 23 were in fact detected. This corresponds to a coverage of approximately 90 %. For the site B, similar values were determined (35 % at species level, 80 % to 85 % at phylum level). Differing values result from the two different estimation methods *ACE* and *Chao1*. It has to be pointed out that for genetic distances below 5 % the rarefaction analyses rather underestimates bacterial diversity whereas *Chao1* rather overestimates it (ROESCH et al. 2007). The Shannon-Weaver index, describing the biodiversity in a specific sample on either species or phylum level, is within the expected range: for the bacterial diversity of BSCs, values between 2 and 5 were frequently found (e.g. NAGY et al. 2005, FIERER & JACKSON 2006, BACHAR et al. 2010, ABED et. al. 2010), although the different methods used by various authors have to be taken into account. Furthermore, the Shannon-Weaver index depends on the amount of samples; for direct comparison, this amount should be approximately equal. Concerning our own study, it is obvious that the bacterial diversity on site B is higher than on site A. This was not expected, as at site A an older and higher developed crust cover was observed. Generally, disturbance reduces biodiversity in BSCs with respect to higher cryptogams (mosses, lichens) and vascular plants (ELDRIDGE & KOEN 1998). KUSKE et al. (2012) also observed after 10 years of human foot trampling a decline in the number of individuals of the Cyanobacterium *Microcoleus vaginatus*, besides the decrease in higher cryptogams (see also below). In contrast, the intermediate disturbance hypothesis (CONNELL 1978) points out that site with intermediate rates of disturbance will show highest species diversities. Supporting observations were made in various studies (HUANG et al. 2011, MARILLEY & ARAGNO 1999). According to our example, disturbance may lead to an increase in diversity of bacterial communities.

The Classifier tool of the Ribosomal Database Project (MAIDAK et al. 1994) was used to assign analyzed sequences to specific phyla. See BRENNER et al. (2004) for a detailed description of the bacterial phyla. Site B exhibits more phyla than site A, but this finding should not be overinterpreted. Low abundant phyla (Planctomycetes, Firmicutes) may have escaped from analysis or may be underestimated due to technical limitations during DNA extraction. Five phyla (Bacteriodetes, Actinobacteria, Cyanobacteria, Acidobacteria and Proteobacteria) are highly abundant in both sites, but at different proportions. Bacteroidetes represent at site B 18.6 % of all sequences, while they are less abundant at site A (9.6 %). The abundances of Actinobacteria (6.6 % at B and 8.7 % at A), and the largely unknown Acidobacteria (14.4 % in B, 10.6 % in A) are similar at both sides. Proteobacteria are a phylogenetically diverse group of Gram negative bacteria, among them typical soil inhabitants, representing 25.3 % of all sequences at site B and 33 % of all sequences at site A. It has to be noted that the subgroup of Deltaproteobacteria within the Proteobacteria is completely missing at site A. For Cyanobacteria the differences in abundance were highest among all groups (9 % in B, 24 % in A).

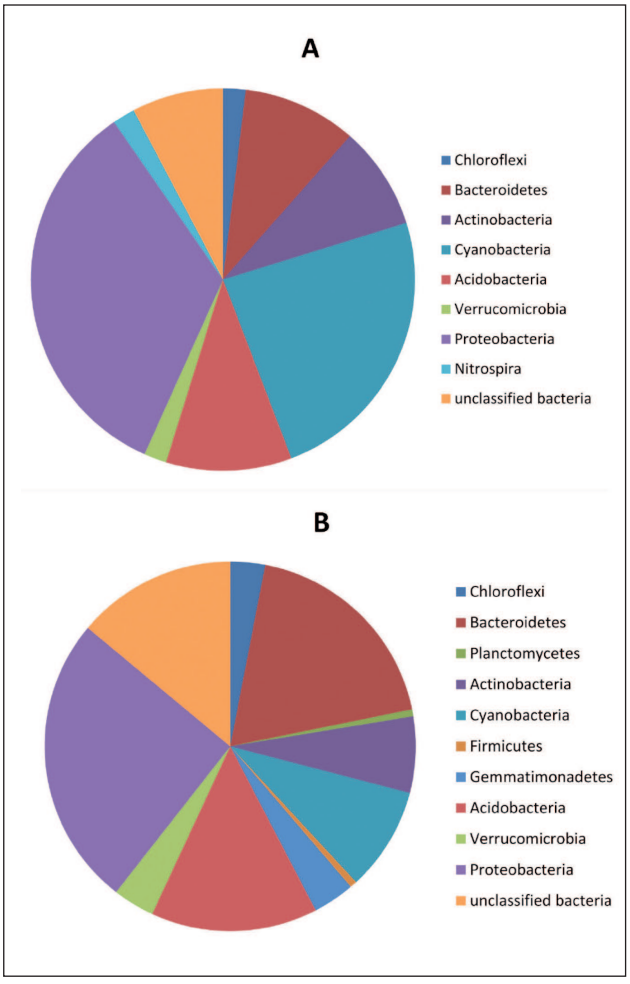

Fig. 3: Phyla distribution of the 16S rRNA gene sequences at sites A and B, based on RDP classification (MAIDAK et al. 1994).

It is obvious that the sites differ in diversity of the bacterial communities, but also in the proportions of abundant groups This is particularly the case for Cyanobacteria. Though the dominance of Cyanobacteria in BSC's based on microscopic identification and/or spectrometry is widely known (see above), analysis based on environmental rRNA gene sequences are still rare. ABED et al. (2010) described bacterial diversity of desert BSCs from the Sultanate Oman where Cyanobacteria were found to be dominant, representing 80 % of all sequences, and Proteobacteria are the second largest group. BACHAR et al. (2010) showed that the abundance of Cyanobacteria increased with aridity of sites in the Negev desert (Israel). However, in their study, Actinobacteria were dominant, followed by Proteobacteria and Acidobacteria.

Many authors, who examined factors influencing the bacterial community in soil found the pH to be important, sometimes it was described as most important factor (LAUBER et al. 2009, JONES et al. 2009). NACKE et al. (2011) detected a positive correlation between the phyla Bacteroidetes and Actinobacteria, and soil pH, but at a much broader pH range as on the sites described here.

Other factors influencing the bacterial community structure may be the soil type (GIRVAN et al. 2003) or texture (SESSITSCH et al. 2001), nitrogen availability (FREY et al. 2004) or surrounding plant species (WIELAND et al. 2001). The influence of root exudates on BSCs is so far unknown. ZUL et al. (2007) observed that the presence of higher plants may affect the species composition of bacteria even outside the rhizosphere.

KUSKE et al. (2012) reported a reduction of cyanobacterial species and an increase in other bacterial phyla in Colorado Plateau BSCs after disturbance by trampling, which is in accordance with our observations. Under stress conditions other phyla may then replace Cyanobacteria in the crust cover and diversity may increase. Thus, the abundances of phylogenic groups may be a significant parameter for disturbance with respect to BSCs.

Acknowledgements

We would like to thank the Field Research Station “Khonin Nuga” from the Universities Ulaanbaatar and Göttingen for supplying infrastructure during the field periods and all of our Russian, Mongolian and German colleagues there for their support especially Prof. Dr. Samjaa and Prof. Dr. Mühlenberg as leaders of Khonin Nuga. More thanks to the DAAD for funding and to the colleagues of the AG Daniel and the Göttingen Genomics Laboratory for their support, to AG Leuschner (Plant Ecology and Ecosystem Research, Göttingen) for soil analyses and a special thank to Einar Timdal (Oslo) for the identification of *Psora luridella*.

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