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ENVIRONMENTAL MICROBIOLOGY

Some Like it High! Phylogenetic Diversity of High-Elevation Cyanobacterial Community from Biological Soil Crusts of Western Himalaya

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Abstract The environment of high-altitudinal cold deserts of Western Himalaya is characterized by extensive development of biological soil crusts, with cyanobacteria as dominant component. The knowledge of their taxonomic composition and dependency on soil chemistry and elevation is still fragmentary. We studied the abundance and the phylogenetic diversity of the culturable cyanobacteria and eukaryotic microalgae in soil crusts along altitudinal gradients (4600–5900 m) at two sites in the dry mountains of Ladakh (SW Tibetan Plateau and Eastern Karakoram), using both microscopic and molecular approaches. The effects of environmental factors (altitude, mountain range, and soil physico-chemical parameters) on the composition and biovolume of phototrophs were tested by multivariate redundancy analysis and variance partitioning. Both phylogenetic diversity and composition of morphotypes were similar between Karakorum and Tibetan Plateau. Phylogenetic analysis of 16S rRNA gene revealed strains belonging to at least five genera. Besides clusters of common soil genera, e.g., *Microcoleus*, *Nodosilinea*, or *Nostoc*, two distinct clades of simple trichal taxa were newly discovered. The most abundant cyanobacterial orders were Oscillatoriales and Nostacales, whose biovolume increased with increasing elevation, while that of Chroococcales decreased. Cyanobacterial species richness was low in that only 15 morphotypes were detected. The environmental factors accounted for 52 % of the total variability in microbial data, 38.7 % of which was explained solely by soil chemical properties, 14.5 % by altitude, and 8.4 % by mountain range. The elevation, soil phosphate, and magnesium were the most important predictors of soil phototrophic communities in both mountain ranges despite their different bedrocks and origin. The present investigation represents a first record on phylogenetic diversity of the cyanobacterial community of biological soil crusts from Western Himalayas and first record from altitudes over 5000 m.

Keywords Soil crusts · Cyanobacterial diversity · Western Himalayas · High-elevation · Desert · Phosphorus

Introduction

The phototrophic microbial communities are important components of soils in arid and semi-arid ecosystems around the world. The knowledge of their taxonomic composition and dependency on soil chemistry and vegetation is, however, still unclear and requires further attention. Especially the remote mountain regions such as Western Himalaya are insufficiently explored in this regard. The dry mountains in the Western Himalaya are situated in the rain-shadow north of the Great Himalaya range. The harsh environment of this arid area is characterized by extreme diurnal temperature fluctuations, strong winds, and high UV radiation with sparse vegetation cover. These circumstances open the space for the extensive development of biological soil crusts (BSCs)

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and increase the importance of cyanobacterial communities, which are the dominant component in this area. BSCs carry out key processes in the development of soil [1, 2], biogeochemical cycling [3, 4], and plant colonization [5] in extreme environmental areas.

The diversity and abundance of soil cyanobacterial and microalgal communities as first colonizers of high-mountain soils may profoundly affect nutrient availability for pioneer vascular plants [6]. The biodiversity and functions of microbes in the mountain ecosystems have received increased attention, especially with respect to climate change, glacial retreat, and vascular plant distributional shift e.g., [7, 8]. These changes in biodiversity can alter ecosystem processes and the resilience and the resistance of ecosystems to environmental change [9, 10]. Without baseline data on soil diversity, however, we cannot track the effects of climate change, and without an understanding of the drivers of community composition, we cannot predict how climate change may affect these soil communities.

In our previous study, we investigated the abundance and the diversity of cyanobacteria in the BSCs along an altitudinal gradient (5300–5900 m a s l.) in a SW extension of Tibetan Plateau, Ladakh [11]. The diversity was determined only according to the morphology of cyanobacteria, bringing the basic picture about the cyanobacterial diversity of BSCs from this region.

The aim of this study is detailed assessment of cyanobacterial diversity based on molecular phylogenetic taxonomy, using the 16S rRNA gene sequencing of unialgal strains. DNA sequence data are therefore well placed to provide more insight into the cyanobacterial diversity of the BSCs from the high altitudes of the Western Himalayas. By using the molecular advance, we are able to compare the taxonomical composition with other regions to assess whether cyanobacteria of Ladakh BSCs are mainly cosmopolitan or they rather form isolated populations. We also incorporate the morphological and molecular data in an effort to provide accurate identifications and description of gained strains.

The study is extended by comparing the taxonomic composition of phototrophs, based on molecular phylogeny, in two mountain ranges in the Western Himalayas—southwestern spur of Tibetan Plateau with Eastern Karakoram. These mountain ranges have different bedrocks and origin, allowing us to compare the cyanobacterial diversity in the alpine and subnival soils in relation to local environmental conditions. The present investigation represents, to the best of our knowledge, a first record on phylogenetic diversity of the cyanobacterial community of BSCs from Western Himalayas and first record on phylogenetic diversity in altitudes over 5000 m elevation.

Methods

Sampling Sites and Sample Collection

The study area is in Ladakh, Jammu, and Kashmir States, India, and is characterized by an arid cold-desert environment because the precipitation seldom crosses the high crest of the main Himalayan range [12]. The fieldwork was conducted in August 2010 in two mountain areas of Ladakh: in the Eastern Karakoram Range in the Nubra Valley site (34°45′N, 77°35′E) and in the southwestern extension of the Tibetan Plateau on the western slope of Chamser Kangri peak above Tso Moriri lake (32°59′N, 78°24′E). The Chamser Kangri Plateau consists mainly of gneisses [13], while the Nubra Valley consists mainly of leucogranits [14]. The samples of BSCs were collected along two elevational transects at both mountain sites. The transect in E Karakoram was 9 km long, the sampling sites were in four altitudes 4600, 4800, 5000, and 5200 m. The transect at the Tibetan Plateau was 12 km long, with sampling sites in four altitudes between 5300, 5500, 5700, and 5900 m. The sampling points on each transect cover the different major vegetation types. The altitudinal zonation of vegetation included steppes and semi-deserts at lower elevations, and alpine meadows, screes, and the subnival zone close to glaciers. The cover of vascular plant vegetation ranged from 0 to 50 %. The data on vegetation type and cover at the same sampling points were documented by [15–18].

At each sampling point on each transect, five composite samples of BSCs were collected. In total, 20 samples of BSCs were collected from each mountain range (four elevations, five replicates). Soil was taken from an area of 10 m^2 and 2–4 cm deep (the thickness of BSC) with a sterile spatula. The soil was air-dried on aluminum plates for 10 h immediately after collection, because the field conditions do not allow other storage of collected material. The BSC and arid land soil are commonly exposed to the drying and freezing in the Himalayas. This method of preservation is recommended for arid land soils and BSC samples by Campbell et al. [19], because it prevents microbial activity in a naturally occurring manner, without the cell damage that may be associated with freezing and, particularly, thawing cycles. The samples were placed in sterile 100 ml polypropylene bags (Nasco Whirl-Pak®) and transported to the laboratory for analysis.

Physico-Chemical Characteristics of Soil

Subsamples of soil were used for the determination of pH, organic matter content, and texture as described by Kaštovská and colleagues [20]. For the determination of total nitrogen, methods by Zbíral and colleagues [21] were used. The technique described in Mehlich [22] was used for the extraction of phosphorus (P) from samples, and the concentration of P was measured by using ascorbic acid-molybdate and a

SHIMADZU UV-1650PC spectrophotometer. The macroelements (Ca, Mg, K, and Na) were extracted from the soil according to US EPA method 200.2 (HCl–HNO3) http://www.epa.gov/epaoswer/hazwaste/test/3050b.pdf) and determined spectrochemically using US EPA method 3050 [23]. Soil organic carbon was determined by wet oxidation with acidified dichromate [24]. Mid-season volumetric water content was measured at each soil sampling point immediately during the collection by a Hydrosense II Soil Moisture Measurement System (Campbell Scientific, Australia).

Abundance and Diversity of the Phototrophic Microbial Communities

The samples for abundance and diversity investigation were prepared as follows. One gram of mixed soil was diluted in 5 ml of distilled water. The slurry was disintegrated at the beginning manually with pestle and consequently with sonificator (Bandelin Sonorex) for 1 min. Twenty microliter of slurry was put under cover glass cover area 22×22 mm. Ten stripes (area of one stripe is 11 mm^2) were counted. Samples were observed under microscopy Olympus BX 60, magnification 400×.

Biovolume and the number of microalgal and cyanobacterial cells as well as the taxonomic composition of communities were determined using light and epifluorescence microscopy (Olympus BX 60). Green and blue excitations (MWB filter cube blue excitation 450–480, emission 515+ for eukaryotic algae; MWG filter cube green excitation 510–550, emission 590+ for cyanobacteria) were used [20]. The term "eukaryotic" alga is used in this paper for the taxa from classes Chlorophyceae and Tribophyceae. Cyanobacteria were classified into three orders according to their morphology: Chroococcales (single-celled organisms), Oscillatorialles (filamentous cyanobacteria without heterocytes and akinetes), and Nostocales (filamentous or colonial cyanobacteria with heterocytes and akinetes). In Oscillatoriales, the taxa were determined according to the width of the filaments, shape of the vegetative cells, and presence/absence of mucilaginous sheaths. In Nostocales, morphotypes were distinguished according to their life form (colonies or filaments) and the shape of the vegetative cells and heterocytes. For the order Chrooccocales, it was possible to recognize taxa according to the vegetative cells' shape and dimension and division of cells. For the dimension of single morphotypes, see Table 1.

Cultivation, Isolation, and Sequencing of Strains

The soil subsamples were placed on plates with solid BBM medium (Bold-Basal/Bristol Medium, the basic medium for cultivation of terrestrial algae), prepared as described in Bischoff and Bold 1963 [25]. Cyanobacterial strains were

Table 1 The biovolume of phototrophs in μm^3 mg^{-1}DW (means) in soil crusts at four elevation sites on the western slope of Saser Kangri, East Karakoram (4600, 4800, 5000, 5200 m) and between four elevation sites on the western slope of Chamser Kangri, Tibetan Plateau (5300, 5500, 5700, 5900 m a s l). An *upward* or *downward pointing arrow* indicates a positive or negative relationship between the dependent variable and altitude (↑ =increase in crust), and *ns* indicate that the relationship is not significant. The numbers in parenthesis are mean value of length and width of cyanobacterial strains in μm

	East Karakoram			Tibetan Plateau		
Morphotype	Mean	SDEV	Elevation	Mean	SDEV	Elevation
Nostoc sp. (5x5)	77.67	52.12	ns	137.23	89.23	↑ 0.000
Phormidium sp. (5x10)	261.69	162.58	↑ 0.046	297.89	193.05	↑ 0.006
Microcoleus vaginatus (5x10)	356.50	410.45	↑ 0.002	490.17	379.74	↑ 0.007
Microcoleus sp. (2,5x10)	3.09	8.35	ns	109.51	294.43	ns
Nodularia sp.(10x10)	30.09	31.01	↓ 0.007	40.42	56.64	↓ 0.000
Leptolyngbya sp.(2x10)	35.31	45.00	ns	28.57	18.93	ns
cf. *Calothrix* (5x10)	0.35	0.92	ns	0.84	3.24	ns
Cyanothece (5x10)	8.21	21.64	ns	5.95	9.81	ns
Cyanothece (10x15)	0.51	1.68	ns	1.35	4.08	↓ 0.047
Chroococcus 1 (2,5x5)	0.82	2.49	ns	1.47	3.73	ns
Chroococcus 2 (5x5)	6.58	12.54	↓ 0.038	0.04	0.09	ns
Chroococcus 3 (5x7,5)	0.18	0.79	ns	0.02	0.10	ns
Chroococcales (2,5x2,5) G1	0.20	0.45	↓ 0.048	0.59	0.63	ns
Chroococcales (5x5) G2	6.19	5.08	↓ 0.059	6.17	8.02	↓ 0.023
Chroococcales (10x10) G3	1.52	3.25	ns	0.77	2.17	ns
coccal microalgae (15x15) B1	0.56	1.86	↓ 0.069	2.92	13.04	ns
Chroccocales	24.21	26.76	↓ 0.002	16.36	13.28	↓ 0.021
Oscillatoriales	656.59	526.83	↑ 0.004	926.14	501.50	↑ 0.022
Nostocales	108.10	54.31	ns	178.49	79.28	ns
eukaryotic algae	0.56	1.86	↓ 0.049	2.92	13.04	ns
Total biovolume	789.46	540.86	↑ 0.013	1123.91	499.84	↑ 0.012

picked up from colonies that grew on plates and transferred onto new plates in order to obtain unialgal colonies. Cultures were maintained in ambient light at 15 °C. Strains were morphologically characterized in high-resolution Olympus photomicroscope BX 60. The DNA of isolates was extracted using the UltraClean™ Microbial DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) following manufacturer's protocols. The 16S rRNA gene and the internal transcribed spacer (ITS) region were amplified with primers 16S27F (5′- AGA GTT TGA TCC TGG CTC AG -3′) and 23S30R 23S30R (5′- CTT CGC CTC TGT GTG CCT AGG T -3′) [26]. Amplification was carried out as follows: one cycle of 5 min at 94 °C; 10 cycles of 45 s at 94 °C, 45 s at 57 °C, and 2 min at 72 °C; 25 cycles of 45 s at 94 °C, 45 s at 54 °C, and 2 min at 72 °C; and a final elongation step of 7 min at 72 °C. PCR product was used as a template for sequencing (Applied Biosystems 3130xl Genetic Analyzer) with primers 16S27F, 23S30R [26], primer CYA781F(a) (5′ AAT GGG ATT AGA TAC CCC AGT AGT A – 3′) [27], and the reverse complement of Primer 14(5′-TGT ACA CAC CGC CCG TC-3′) [28]. The length of the sequences was 1000 bps.

Phylogenetic Analyses

Obtained sequences were aligned using MAFFT v. 7 [29] with default settings together with sequences of other 66 operational taxonomic units (OTUs) representing main groups of simple trichal cyanobacteria and with sequences of other 67 OTUs representing main groups of heterocystous cyanobacteria. The alignment lengths are 1385 bp. Phylogenetic calculations involved Bayesian inferences performed in MrBayes 3.2.2 [30], maximum likelihood (ML) analysis in PhyML 3.0 [31], and maximum parsimony analysis in SeaView 4.5.1 [32].). For Bayesian inference, two runs of eight Markov chains were executed for 1 million generations with default parameters, sampling every 100 generations (the final average standard deviation of split frequencies was lower than 0.01). The first 25 % of sampled trees were discarded as burn-in. The ML tree was constructed applying the GTR+I+Γ model chosen according to Akaike information criterion provided by jModelTest 2 [33]. A total of 1000 bootstrap replicate searches were conducted to evaluate the relative support of branches. A maximum parsimony analysis involved 1000 replicate searches using the tree bisection-reconnection (TBR) branch swapping algorithm. Sequences obtained as part of this work were submitted to the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under accession numbers LN849921–LN849937 and LN877213.

Statistical Analysis

Differences in cyanobacterial and algal composition between elevation sites, two mountain areas (East Karakoram versus Tibetan Plateau), and due to variation in soil chemical properties were analyzed by canonical redundancy analysis (RDA), which is a constrained ordination method, in the Canoco 5 [34]. RDA was used because environmental variables were in the form of both categorical (dummy variables) and continuous predictors. Standardization by species (dependent variables) was used because the data analyzed were of various types and units. The variance partitioning procedure was performed using several RDAs with explanatory variables and covariables to remove their effects and to obtain the net effect of an individual factor. Using this approach, we constructed tests analogous to the testing of particular terms in ANOVA models but for multivariate data; for details, see Lepš and Šmilauer [35]. Three sets of analyses were carried out: (1) elevation x mountain (categorical variable, coded as a set of indicator variables) x Soil chemistry as the explanatory variables—the analysis accounts for all, the main effect of mountain range, elevation, soil chemistry, and their interactions, (2) mountain range being an environmental variable, and elevation and soil chemistry a covariable and vice versa—this accounts just for additive (net) effect of each environmental variable, (3) separate analyses for each mountain range, repeating the variance partitioning procedure of tests 1 and 2 with elevation and soil chemical properties to account for their net effects. The significance of these relationships was tested using the Monte Carlo permutation test (999 permutations, see Lepš and Šmilauer, [35]). The results of multivariate analyses were visualized in the form of a biplot ordination diagram.

We further used a generalized linear model with altitude as the explanatory variable to test for changes (increase, decrease, hump shape, and valley shape) in algal and cyanobacterial abundance. The significance of the linear model was tested first; if the form of linear dependence was not accepted, a second-order polynomial was fitted using the glm function in the R program [36]. To control for familywise error rate, the false discovery rate procedure was performed [37].

Results

Phylogenetic Diversity of Cyanobacterial Community

The molecular analyses showed that the phylogenetic diversity of cyanobacterial community from BSCs is highly similar between E Karakorum and Tibetan Plateau. Phylogenetic analysis of 16S rRNA gene revealed strains belonging to at least five genera. Besides clusters of common soil genera, e.g., *Microcoleus* or *Nodosilinea* two distinct clades of simple trichal taxa occurred (Fig. 1). Both represent probably new taxa and their life cycle,

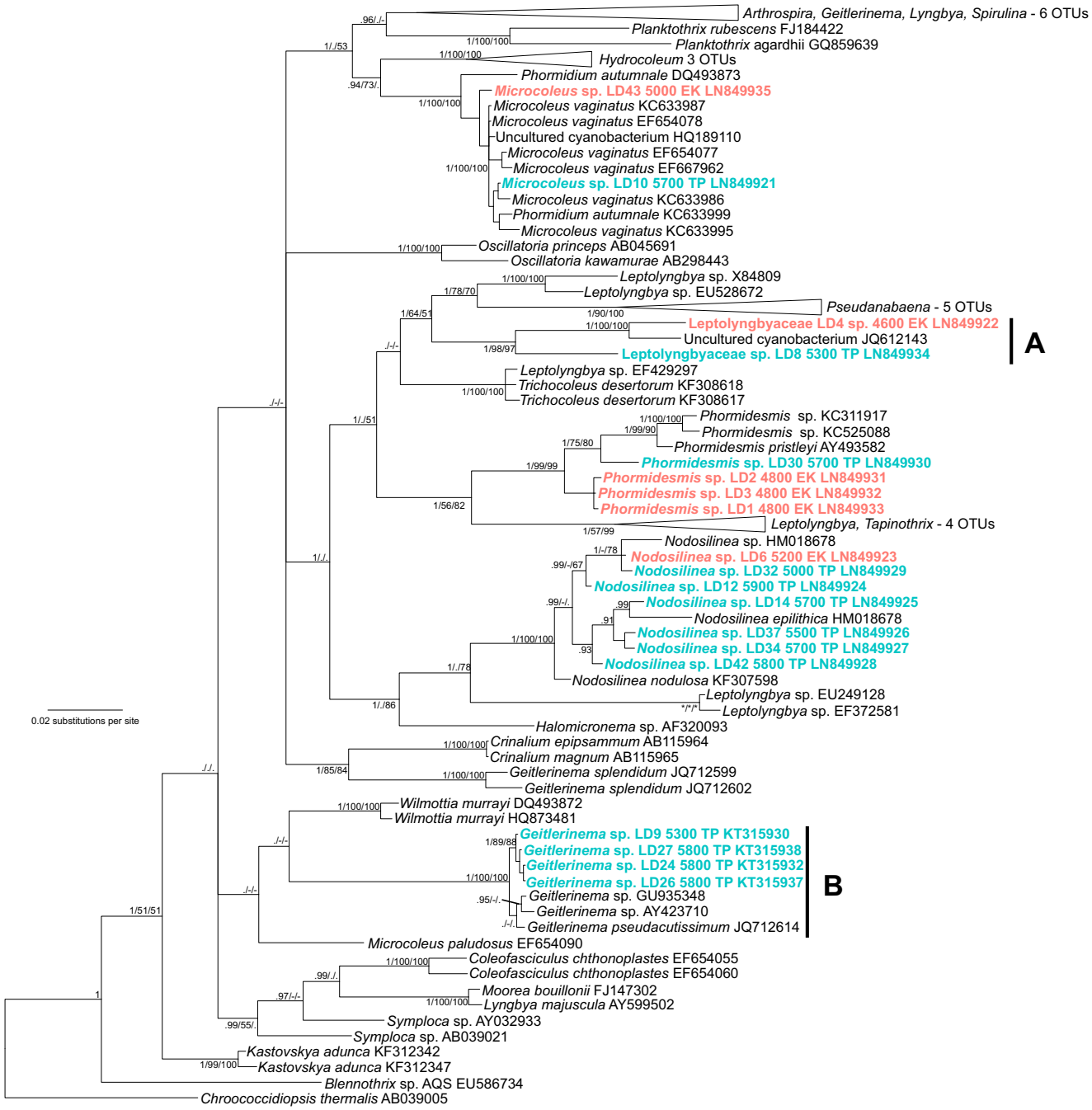

Fig. 1 Phylogenetic relationships of simple trichal cyanobacteria dominant in soil crusts in Ladakh using topology given by Bayesian analysis. *OTUs typed in orange* are from Eastern Karakoram, *OTUs typed in blue* are from Tibetan Plateau. *Clusters A* and *B* represent probably genera new to science. The given support values are given for Bayesian posterior probabilities >0.9 and for Maximum likelihood and Maximum parsimony ≥50 %

morphology, and ultrastructure need to be studied more in detail. First is marked as cluster A in Fig. 1. It is formed by unidentified members of family Leptolyngbyaceae. The two original sequences are from strains isolated from two different localities, third sequence in this cluster is from microbial mat in calcareous river in Spain. The second cluster marked as B in Fig. 1 contains sequences of strains similar to genus *Geitlerinema*. As notable from Fig. 1, these types belong to a different, yet undescribed genus, because sequences of type species of *Geitlerinema*, *G. splendidum* are in very distant cluster with members of *Crinalium*.

The number of heterocytous types is lower than simple trichal types, but there occur two facts notable in Fig. 2: 1) several strains of soil *Nodularia* types cluster with planktic *Nodularia*,(2) *Calothrix*-like strains isolated from Ladakh

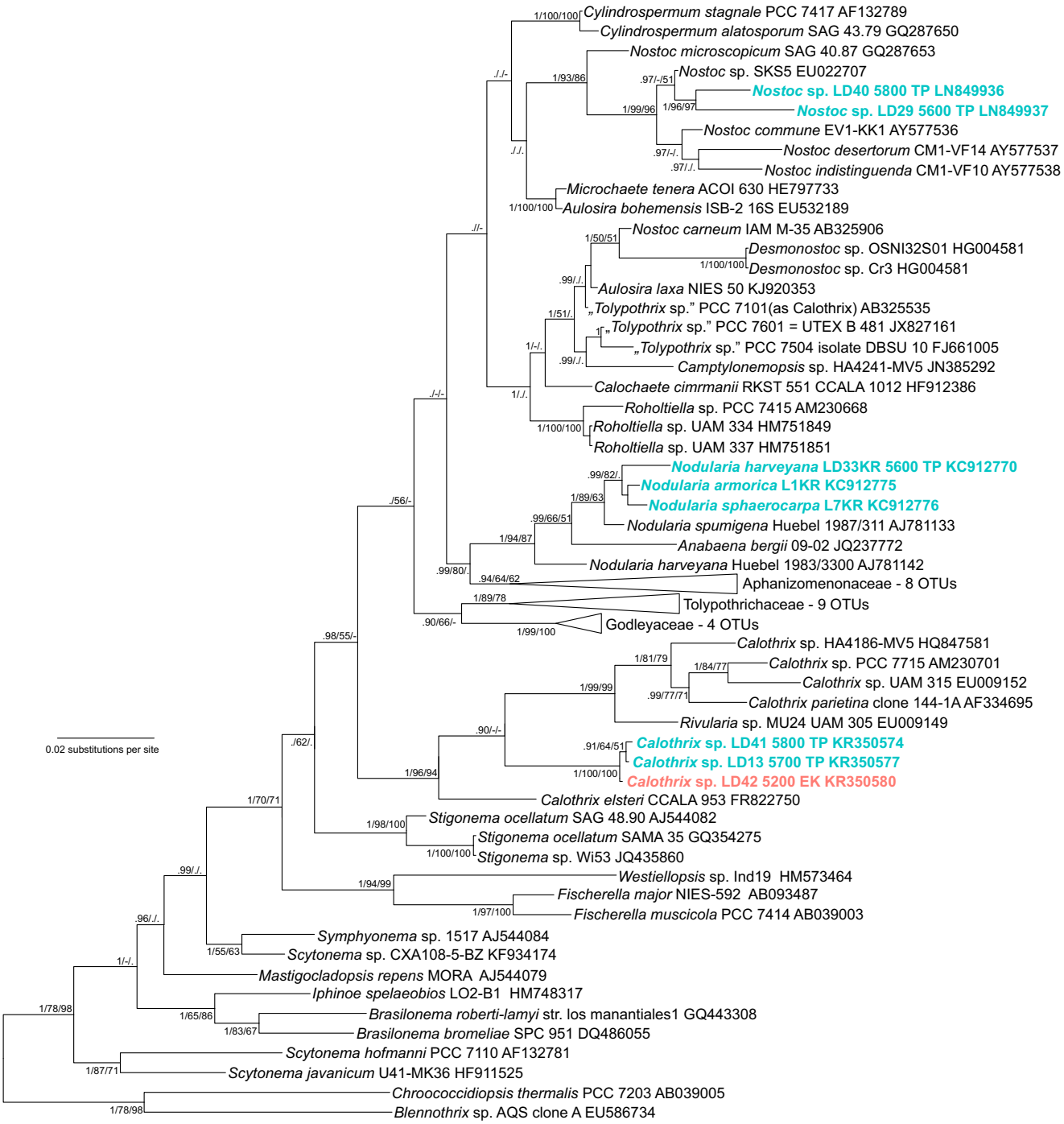

Fig. 2 Phylogenetic relationships of heterocytous cyanobacteria dominant in soil crusts in Ladakh using topology given by Bayesian analysis. *OTUs typed in orange* are from Eastern Karakoram, *OTUs typed in blue* are from Tibetan Plateau. The given support values are given for Bayesian posterior probabilities >0.9 and for Maximum likelihood and Maximum parsimony ≥50 %

form a cluster outside other Rivulariaceae with p-distance 0.051 or higher, which means, that they represent a new genus. The phylogenetic analysis confirmed the previous results about the cyanobacterial composition based only on morphological identification and considerably increased our knowledge about the cyanobacterial diversity of BSCs from W Himalayas.

Environmental Condition With Relationship to Phototrophic Diversity

Phototrophic microorganisms were found in all examined samples of BSCs, with cyanobacteria being the dominant component of the communities. Phototrophic communities contained 16 morphotypes: 15 for cyanobacteria, one for green algae. The

composition of morphotypes was identical for the E Karakoram and Tibetan Plateau transects (Table 1). The most abundant cyanobacterial order was Oscillatoriales, taxa *Microcoleus vaginatus* and *Phormidium* spp. accounted for most of the biomass. The total biovolume of phototrophs in BSCs at E Karakoram was lower than in crusts on Tibetan Plateau but increased significantly with elevation at both localities (Fig. 3). The biovolume of cyanobacterial order Oscillatoriales increased significantly with altitude at both mountain ranges, as well as the biovolume of *Nostoc* genera, while the biovolume of order Chroococcales and of the genera *Nodularia* decreased with elevation significantly (Fig. 3). This trend in the abundance of main cyanobacterial orders along altitudinal gradient was the same in E Karakoram as well as on Tibetan Plateau.

The combined effect of elevation, mountain range, and soil chemistry on the composition of soil phototrophic communities explained 52 % of the total data variation and was statistically significant (RDA: F=2.6, P=0.002). Variance partitioning revealed that 8.4 % was explained solely by mountain range (F=3.6, P=0.004), 14.5 % by elevation (F=6.5, P=0.002), and 38.7 % by soil chemical properties (F=1.6, P=0.002), mostly by organic carbon (10.2 %), potassium (8.7 %), nitrate (8 %), magnesium (7 %), and phosphate (4.2 %). Forward selection of variables showed that phosphate and magnesium are the most important predictors of soil phototrophic communities in the East Karakoram transect (4600-5200 m, Fig. 4a). The soil phosphate concentration is higher at lower elevation (Fig. 5), and is associated with the main compositional changes along the first RDA ordination axis (Fig. 4a), clearly separating the soil crust communities at lower elevation with higher biovolume of Chroococcales (Fig. 3), from those at higher elevation with low phosphate content and higher biovolume of Oscillatoriales, Nostocales, and in particular *Microcoleus vaginatus* and *Phormidium*. Higher elevation soils in the East Karakoram transect had more organic carbon, total nitrogen, and calcium, and higher pH compared to lower elevation sites (Fig. 5).

The same altitudinal pattern of compositional changes in soil phototrophic communities and soil chemistry as in the East Karakoram was found along the Tibetan Plateau transect (5300–5900 m, Fig. 4b). The combined effect of all environmental variables explained 29.2 % variation in the soil phototrophic composition (RDA: F=1.7, P=0.006). Forward selection of variables showed that elevation and soil phosphate are the most important predictors of soil phototrophic communities in the Tibetan Plateau transect (5300–5900 m), accounting for 21.7 % variability. In the RDA ordination diagram (Fig. 4b), the main compositional changes along the first ordination axis are associated with elevation and soil phosphate content, clearly separating cyanobacteria from the orders Oscillatoriales and Nostocales predominating at the higher elevation sites (5700 and 5900 m), from Chroococcales that prevail at the lower elevation sites (5300 and 5500 m). Higher elevation sites had more soil organic matter due to higher soil moisture and lower phosphate and nitrate content and higher total phototrophic biovolume. The second RDA axis corresponds to the gradient of soil reaction and total nitrogen content, with higher biovolume of *Cyanothece* in soil with higher pH and lower soil Ca, Na, and total N content.

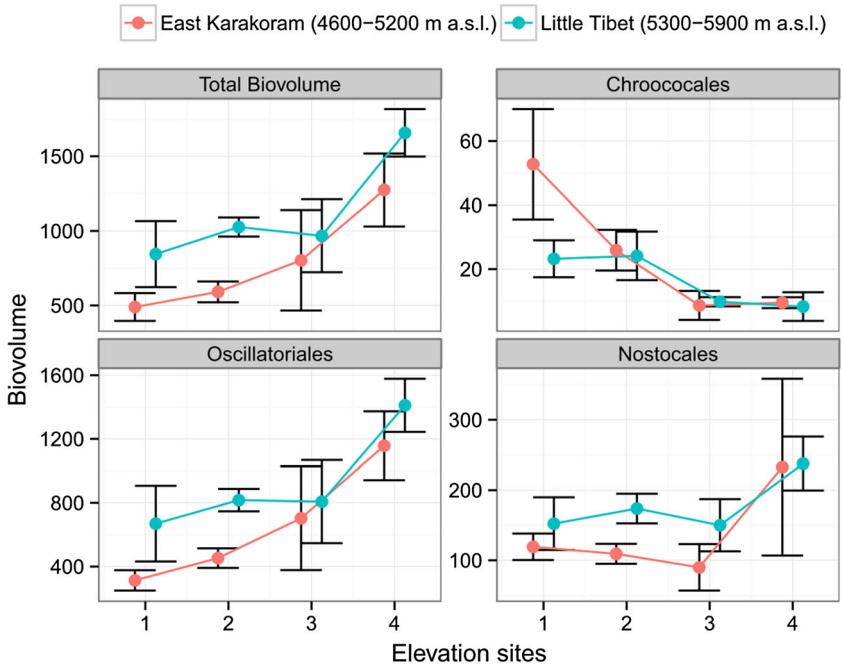

Fig. 3 Comparison of soil phototrophic biovolume (µm3 mg^{-1} dry soil) between four elevation sites at E Karakoram (reddots, 4600, 4800, 5000, 5200 m) and between four elevation sites at Tibetan Plateau (*green dots*, 5300, 5500, 5700, 5900 m a.s.l.), Ladakh, NW India

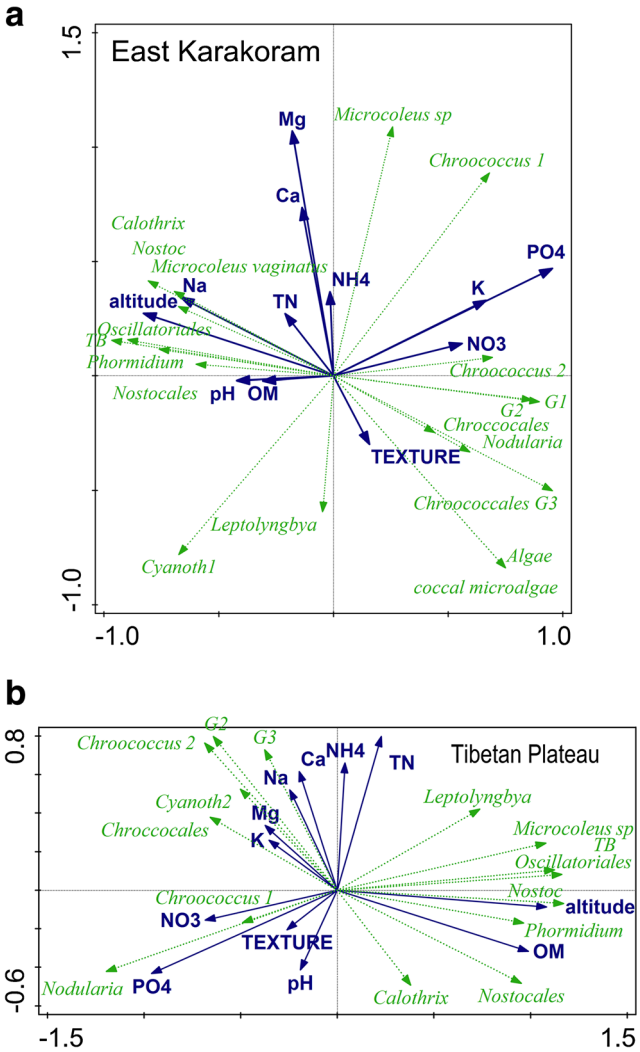

Fig. 4 Two redundancy analysis biplots (RDA) of soil phototrophs (response variables) in relation to elevation and soil nutrients at E Karakoram (4**a**) and at Tibetan Plateau (4**b**). Response variables are represented by *vectors* (*green arrows*) and are related to elevation physico-chemical characteristics of soil (*blue arrows*). The *angles between arrows* indicate correlations between variables. Legend: "OM"—organic matter, "TB" total biovolume

Discussion

For the first time, the soil cyanobacterial diversity from elevations 5000–6000 m was investigated using microscopic and molecular approaches together. The harsh environment of this arid area is characterized by extensive development of BSCs, which form the crucial component of studied habitats. BSCs are dominated by cyanobacterial communities making them one of the most important ecosystem engineers here. Our previous studies reported the presence of phototrophs in subnival zones of Western Himalaya, but the species composition was investigated by traditional light microscopy only to cover generic identity of dominant types [11, 38]. In the present study, our research of cyanobacterial diversity was improved by the 16S rRNA gene sequencing of isolated uni-algal strains to precise their determination. It allows us to see cyanobacterial diversity in broader ecosystem perspective.

We used the phylogenetic analyses of individual-cultivated strains because the proper placement of cyanobacteria into a taxonomic construct requires combination of morphology and sequence information. This is needed especially with respect to the aspects of ecosystem function, as the value of sequence data lacking morphological and ecological background is strongly limited.

Despite recent increase of interest for soil environments, the link between local diversity of microorganisms and environmental factors is still not well understood. It is supposed that microbial biogeographical patterns are shaped by environmental factors [39, 40]. Our studied mountains ranges differ in geological origin and bedrock, as well as their local environmental conditions, such as temperature and humidity [41]. However, we found out that the overall diversity of cyanobacterial communities is highly similar at the Eastern Karakoram and Tibetan Plateau transects. The molecular analyses showed that also the phylogenic diversity of cyanobacteria from BSCs is highly similar between both localities.

Phototrophic communities are composed mainly by orders Oscillatoriales, Nostocales, and Chroococcales, represented by common soil genera, e.g., *Microcoleus*, *Nodosilinea*, or *Nostoc*. These genera have extremely broad ecological valence, they occur in a wide range of habitats all over the world including extreme ecosystems. Large filamentous cyanobacteria, such as *Microcoleus* spp., *Nodosilinea* spp., or *Nodularia* spp. are responsible for an initial process in the origin of BSC [6]. After the filaments bound the soil particles together, the smaller taxa such as *Nostoc* spp. or *Calothrix* spp. followed them. These genera are crucial for origin of BSCs, so they are mentioned as a part of BSCs from all over the world [6] [20] [42]. The high similarity of cyanobacterial community of BSCs of E Karakoram and Tibetan Plateau is principally given by the inherent process of BSC formation, which is species-dependent rather than environmental conditions-dependent.

Our study confirmed the previous results concerning the cyanobacterial community structure with molecular data and increased our knowledge about cyanobacterial types present in BSCs. Phylogenetic analysis of 16S rRNA gene revealed strains belonging to at least five genera. Besides clusters of common soil genera, e.g., *Microcoleus*, *Nodosilinea*, or *Nostoc*, two distinct clades of simple trichal taxa occurred. Even though the cold deserts of W Himalayas are extremely arid areas, several close relatives have been found from aquatic habitats. One of them is the clade formed by unidentified members of family Leptolyngbyaceae (Fig. 1, clade A). The two original sequences are strains isolated from two different localities, third sequence in this cluster comes from microbial mat of calcareous river in Spain. The Leptolyngbyaceae has

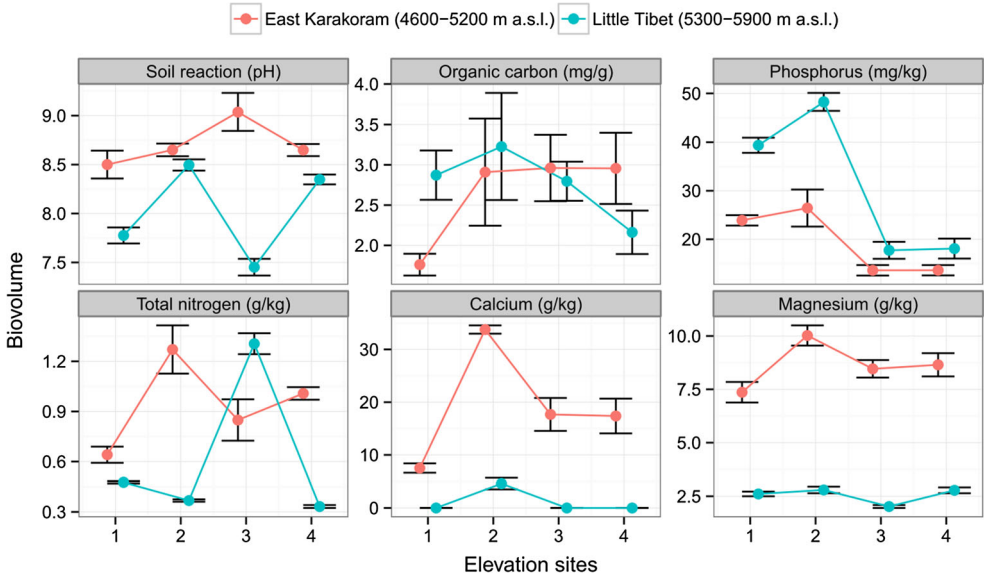

Fig. 5 Comparison of concentration of selected nutrients between four elevation sites on the western slope of Saser Kangri, East Karakoram (*red dots*, 4600, 4800, 5000, 5200 m) and between four elevation sites on the western slope of Chamser Kangri, Tibetan Plateau (*green dots*, 5300, 5500, 5700, 5900 m a.s.l.)

been found to be a very genetically diverse group [3]. Our clade represents probably a new taxon. Their life cycle, morphology, and ultrastructure will be studied in more detail in near future. The second cluster with aquatic relatives is formed by strains of soil *Nodularia* types which match together with planktonic types. Furthermore, according to Komárek [43] no *Nodularia* from arid soils has been recorded by now. The phylogenetic analysis revealed also non-relatedness of *Geitlerinema* types. As noticeable in Fig. 1, the type of the genus, i.e., *Geitlerinema splendidum* is in a distant cluster to *G. pseudacutissimum* and other sequences including our original ones (Fig. 1, cluster B), which probably means, that these types represent a new genus. However, this must be proven by further analyses.

The number of heterocytous types is lower than simple trichal types mentioned above. However, there are more important facts regarding heterocytous types. The *Calothrix*-like strains isolated from Ladakh form a separate cluster outside other Rivulariaceae with p-distance 0.051 or higher, which means, that they represent a new genus according to limits published by Stackebrand & Goebel [44]. Recently, the taxonomical revision of this group was finished by Berrenedro et al. 2015 [45]. Although members of the genus *Nostoc* are present in every desert soil all over the world [3, 46], and the abundance of *Nostoc* in our samples is relatively high, we managed to obtain only few *Nostoc* strains sequences. This is caused by problematic DNA extraction due to thick mucilage and relatively problematic strains cultivation.

Detailed view on the cyanobacterial diversity distribution among elevational gradient shows very similar trends at both studied localities. The total biovolume of phototrophs in BSCs at E Karakoram were lower than in crusts on Tibetan plateau but increased significantly with elevation at both localities. This increase of cyanobacterial biomass within soil crust with increasing elevation is in sharp contrast to the commonly observed trend of a decreasing biomass of organisms with more severe environmental conditions [47, 48]. The reason of different abundance of cyanobacterial biomass between E Karakoram and Tibetian Plateau can be caused by various covers of vascular plant vegetation. The vegetation cover along the lower altitudinal gradient at E Karakorum (4600–5200 m) varied between 10–40 %, while the plant cover along the transect on Tibetan Plateau in higher altitudes (5300–5900) was considerably lower (5–15 %) [17, 18]. Less competition with vascular plants for resources such as nutrients, water, and light open the gap for extensive development of phototrophic communities in the BSCs. The temperature profile during the vegetation season provides better growing conditions for microorganisms than for higher plants. Short growing season and water shortage are conditions, which microorganisms are able to resist [49]. Phototrophic microorganisms have a much faster metabolic rate and a shorter generation time than vascular plants have. Furthermore, discovered genera of cyanobacteria are highly adapted to extreme conditions. They possess thick mucilaginous sheets with pigments, which are resistant to water shortage, high UV radiation, and to the high temperature oscillations. All these circumstances allow occurrence of abundant phototrophic microbial biomass in areas with sparse vegetation cover.

The same explanation holds for the trend of increasing biomass with elevation recorded at both localities. The higher elevations at both sites have lower vegetation cover than lower elevations. The highest elevation of Tibetan Plateau area has negligible plant cover of less than 10 % [17] in contrast to BSCs, which cover more than 40 % of ground.

Comparison of the overall taxonomic diversity of phototrophs between two mountain ranges showed that on the both altitudinal transects Oscillatoriales and Nostocales prevailed in subnival

soils, while Chroococcales was dominant in the soil crusts of alpine steppes and screes at lower elevations. By testing the effect of soil physicochemical parameters together with altitude on the composition and biovolume of cyanobacterial communities, we found out that despite of different bedrocks and origin of studied mountain ranges, the same altitudinal pattern of compositional changes in soil phototrophic communities and soil chemistry was found along the Tibetan Plateau transect as well as along the East Karakoram transect. Results showed that altitude and soil phosphate concentration are the most important predictors of soil phototrophic communities at both sites. Both factors are very likely connected together. Phosphate concentration decreases with increasing altitude as a soil temperature and moisture regime change. The main source of phosphate for soil comes from bedrock weathering. Weathering would be slower at high cold altitudes and faster at lower altitudes, where can be furthermore accelerated by organic acids production by plants or soil microbes or coming from OM decomposition. Natural phosphate gradient along altitudinal transect brought the main constrain especially for phototrophic microorganisms in BSCs. Phototrophic organisms gain carbon and nitrogen from atmosphere, where both elements are abundant. However, their gain via photosynthesis and N-fixation is energetically demanding and manufacture demanding. In microbial cells, two most P-rich compounds are ATP and ribosomal RNA. First, one provides energy, whereas, second one provides machinery for protein synthesis. Both compounds make most of the P of whole microbial cell. Therefore, phosphate availability in soil is crucial for cyanobacterial C and N uptake and thus growth. In our study, we found increasing dominance of filamentous cyanobacteria within BSCs with decreasing phosphate concentration. Filamentous microorganisms are known to have greater affinity to nutrients. They are able to take up nutrients that are at very low concentrations [6]. This ability gave filamentous organisms competitive benefits above the others and thus they dominate in higher altitudes, where phosphate concentration is low. Even though, Ca and Mg concentrations differ largely among localities, they were not shown to be a significant predictor of cyanobacterial diversity. The reason for that is that both elements are considered to be micronutrients. That means, their demand by cyanobacteria is small and hence their unlimited amount is reached at all localities.

The investigation of cyanobacteria in BSCs around the world revealed relatively small and similar taxonomical diversity of phototrophs in the studied BSCs. It is very likely that this fact is connected to unique and extreme environmental conditions, where BSCs usually occur. These conditions are inherently highly selective for the best-adapted species as the conditions of the high-mountain mineral soils are supposed to be more limiting to life than other conditions on the surface of the Earth [50]. Obviously, there are only few species able to survive high-mountain conditions and in opposite there are much more species unable to survive them. Therefore, open and almost competition-free niche for few well-adapted species is available in high mountains. Cyanobacteria could prosper here in unexpected extent.

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References

1. Kubečková K, Johansen JR, Warren SD, Sparks RL (2003) Development of immobilized cyanobacterial amendments for reclamation of microbiotic soil crusts. Algol Stud 109:341–362
2. Tirkey J, Adhikary SP (2005) Cyanobacteria in biological soil crusts of India. Curr Sci 89:515–521
3. Johansen JR (1993) Cryptogamic crusts of semiarid and arid lands of North America. J Phycol 29:140–147. doi:10.1111/j.0022-3646.1993.00140.x
4. Heckman KA, Anderson WB, Wait DA (2006) Distribution and activity of hypolithic soil crusts in a hyperarid desert (Baja California, Mexico). Biol Fertil Soils 43:263–266. doi:10.1007/s00374-006-0104-7
5. Belnap J, Harper KT (1995) Influence of cryptobitic soil crusts on lemental content of tissue of 2 desert seed plants. Arid Soil Res Rehabil 9:107–115
6. Belnap J, Lange O (2001) Biological soil crusts: structure, function and management. Springer Verlag, Berlin
7. Schmidt SK, Reed SC, Nemergut DR, Grandy AS, Cleveland CC, Weintraub MN, Hill AW, Costello EK, Meyer AF, Neff JC, Martin AM (2008) The earliest stages of ecosystem succession in high-elevation (5000 metres above sea level), recently deglaciated soils. Proc R Soc B Biol Sci 275:2793–2802. doi:10.1098/rspb.2008.0808
8. Lamb EG, Han S, Lanoil BD, Henry GHR, Brummell ME, Banerjee S, Siciliano SD (2011) A high arctic soil ecosystem resists long-term environmental manipulations. Glob Chang Biol 17:3187–3194. doi:10.1111/j.1365-2486.2011.02431.x
9. Ives AR, Cardinale BJ (2004) Food-web interactions govern the resistance of communities after non-random extinctions. Nature 429:174–177. doi:10.1038/nature02515
10. Ives AR, Carpenter SR (2007) Stability and diversity of ecosystems. Science 317:58–62. doi:10.1126/science.1133258
11. Janatkova K, Rehakova K, Dolezal J, Simek M, Chlumska Z, Dvorsky M, Kopecky M (2013) Community structure of soil phototrophs along environmental gradients in arid Himalaya. Environ Microbiol 15:2505–2516. doi:10.1111/1462-2920.12132
12. Bhattacharyya A (1989) Vegetation and climate during the last 30,000 years in Ladakh. Palaeogeogr Palaeoclimatol Palaeoecol 73:25–38. doi:10.1016/0031-0182(89)90042-4
13. Epard J-L, Steck A (2008) Structural development of the Tso Morari ultra-high pressure nappe of the Ladakh Himalaya. Tectonophysics 451:242–264. doi:10.1016/j.tecto.2007.11.050
14. Phillips RJ (2008) Geological map of the Karakoram fault zone, Eastern Karakoram, Ladakh, NW Himalaya. Journal of Maps: 21-37

15. Klimes L (2003) Life-forms and clonality of vascular plants along an altitudinal gradient in E ladakh (NW Himalayas). Basic Appl Ecol 4:317–328. doi:10.1078/1439-1791-00163
16. Klimes L, Dolezal J (2010) An experimental assessment of the upper elevational limit of flowering plants in the western Himalayas. Ecography 33:590–596. doi:10.1111/j.1600-0587.2009.05967.x
17. Dvorsky M, Dolezal J, de Bello F, Klimesova J, Klimes L (2011) Vegetation types of East Ladakh: species and growth form composition along main environmental gradients. Appl Veg Sci 14:132–147. doi:10.1111/j.1654-109X.2010.01103.x
18. Klimesova J, Dolezal J, Dvorsky M, de Bello F, Klimes L (2011) Clonal growth forms in Eastern Ladakh, Western Himalayas: classification and habitat preferences. Folia Geobotanica 46:191–217. doi:10.1007/s12224-010-9076-3
19. Campbell BJ, Polson SW, Hanson TE, Mack MC, Schuur EAG (2010) The effect of nutrient deposition on bacterial communities in arctic tundra soil. Environ Microbiol 12:1842–1854. doi:10.1111/j.1462-2920.2010.02189.x
20. Kastovska K, Elster J, Stibal M, Santruckova H (2005) Microbial assemblages in soil microbial succession after glacial retreat in Svalbard (high arctic). Microb Ecol 50:396–407. doi:10.1007/s00248-005-0246-4
21. Zbíral J, Honsa I, Malý S (1997) Analýza půd III—jednotné pracovní postupy. ÚKZÚZ, Brno
22. Mehlich A (1978) New extraction for soil test evaluation of phosphorus, potassium, magnesium, sodium, manganese and zinc. Commun Soil Sci Plant Anal 9:477–492. doi:10.1080/00103627809366824
23. Kimbrough DE, Wakakuwa J (1991) Report of an interlaboratory study comparing EPA SW-846 method 3050(1) and an alternative method from the California Department of Health Services. queryWaste Testing and Quality Assurance : Third Volume 1075: 231–244. doi:10.1520/stp25480s
24. Rowel LD (1994) Soil science: methods and applications. Longman Scientific & Technical, Burnt Mill, Harlow
25. Bischoff HW, Bold H (1963) Some soil algae from enchanted rock and related algal species. Univ Texas Publ, Phycological studies IV
26. Taton A, Grubisic S, Brambilla E, De Wit R, Wilmotte A (2003) Cyanobacterial diversity in natural and artificial microbial mats of lake fryxell (McMurdo dry valleys, antarctica): a morphological and molecular approach. Appl Environ Microbiol 69:5157–5169. doi:10.1128/aem.69.9.5157-5169.2003
27. Nubel U, GarciaPichel F, Muyzer G (1997) PCR primers to amplify 16S rRNA genes from cyanobacteria. Appl Environ Microbiol 63: 3327–3332
28. Wilmotte A, Vanderauwera G, Dewachter R (1993) Structure of the 16-S ribosomal-RNA of the thermophilic cyanobacterium chlorogleopsis (mastigocladus-laminosus HTF) strain PCC7518, and phylogenetic analyses. FEBS Lett 317:96–100. doi:10.1016/0014-5793(93)81499-p
29. Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. doi:10.1093/molbev/mst010
30. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. doi:10.1093/sysbio/sys029
31. Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321. doi:10.1093/sysbio/syq010
32. Gouy M, Guindon S, Gascuel O (2010) SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol 27:221–224. doi:10.1093/molbev/msp259
33. Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772. doi:10.1038/nmeth.2109
34. ter Braak CJF, Smilauer P (2012) CANOCO reference manual and user's guide to CANOCO for windows. Centre for Biometry, Wageningen
35. Jan L, Petr Š (2003) Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge
36. Team RDC (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
37. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate—a practical and powerful approach to multiple testing. J R Stat Soc Ser B Methodol 57:289–300
38. Rehakova K, Chlumska Z, Dolezal J (2011) Soil cyanobacterial and microalgal diversity in Dry mountains of ladakh, NW Himalaya, as related to site, altitude, and vegetation. Microb Ecol 62:337–346. doi:10.1007/s00248-011-9878-8
39. Mitchell RJ, Campbell CD, Chapman SJ, Cameron CM (2010) The ecological engineering impact of a single tree species on the soil microbial community. J Ecol 98:50–61. doi:10.1111/j.1365-2745.2009.01601.x
40. Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci U S A 103:626–631. doi:10.1073/pnas.0507535103
41. Dvorsky M, Dolezal J, Kopecky M, Chlumska Z, Janatkova K, Altman J, de Bello F, Rehakova K (2013) Testing the stress-gradient hypothesis at the roof of the world: effects of the cushion plant thylacospermum caespitosum on species assemblages. Plos One 8(1):e53514. doi:10.1371/journal.pone.0053514
42. Dor I, Danin A (2001) Life strategies of Microcoleus vaginatus: A crust-forming cyanophyte on desert soils. Nova Hedwigia Beiheft Nova Hedwigia Beiheft Algae and extreme environments Ecology and Physiology Proceedings of the International Conference 11-16 September, Trebon, Czech Republic 123: 317-339
43. Komárek J (2013) Cyanoprokaryota 3: Heterocytous genera. In: Budel B, Gartner G, Krienitz L, Schagerl M (eds), Süßwasserflora von Mitteleuropa (vol 19/3). Spektrum Akademischer Verlag, pp 1130
44. Stackebrandt E, Goebel BM (1994) A place for DNA-DNA reassociation and 16S ribosomal-RNA-sequence analyses in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849
45. Berrendero GE, Johansen J, Kaštovský J,Bohunická M, Čapková K, (2015) Macrochaete gen. nov. (Cyanobacteria): The first step in solving the extensively polyphyletic genus Calothrix. J Phycol (*under review*)
46. Evans RD, Johansen JR (1999) Microbiotic crusts and ecosystem processes. Crit Rev Plant Sci 18:183–225. doi:10.1080/07352689991309199
47. Ma XJ, Chen T, Zhang GS, Wang R (2004) Microbial community structure along an altitude gradient in three different localities. Folia Microbiol 49:105–111. doi:10.1007/bf02931382
48. Balser TC, Gutknecht JLM, Liang C (2010) How will climate change impact soil microbial communities? In: Dixon GR, Emma T (eds) Soil microbiology and sustainable crop production. University of Reading Press, Reading, pp 373–397
49. Zelikova TJ, Housman DC, Grote EE, Neher DA, Belnap J (2012) Warming and increased precipitation frequency on the Colorado plateau: implications for biological soil crusts and soil processes. Plant Soil 355:265–282. doi:10.1007/s11104-011-1097-z
50. Lynch RC, King AJ, Farias ME, Sowell P, Vitry C, Schmidt SK (2012) The potential for microbial life in the highest-elevation (>6000 m.a.s.l.) mineral soils of the Atacama region. J Geophys Res Biogeosci 117. doi: 10.1029/2012jg001961