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ENVIRONMENTAL MICROBIOLOGY

Soil Cyanobacterial and Microalgal Diversity in Dry Mountains of Ladakh, NW Himalaya, as Related to Site, Altitude, and Vegetation

Klára Řeháková · Zuzana Chlumská · Jiří Doležal

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Abstract Although 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 still fragmentary. We studied the abundance and the diversity of cyanobacteria and eukaryotic microalgae along altitudinal gradients (3,700–5,970 m) at four sites in the dry mountains of Ladakh (Little Tibet, Zanskar Mountains, and Eastern Karakoram), using epifluorescence. The effects of environmental factors (altitude, mountain range, and vegetation type) on soil physicochemical parameters (pH; texture; organic matter, nitrogen, ammonia, and phosphorus contents; and concentration of chlorophylls and carotenoids) and on the composition and biovolume of phototrophs were tested by multivariate redundancy analysis and variance partitioning. Phototrophs were identified in all collected samples, and phototroph biovolume ranged from 0.08 to 0.32 $mm^3\ g^{-1}$ dry weight. The dominant component was cyanobacteria, which represented 70.9% to 98.6% of the biovolume. Cyanobacterial species richness was low in that only 28 morphotypes were detected. The biovolume of Oscillatoriales consisted mainly of *Phormidium* spp. and *Microcoleus vaginatus*. The environmental factors accounted for 43.8% of the total variability in microbial and soil data, 20.6% of which was explained solely by mountain range, 7.0% by altitude, and 8.4% by vegetation type. Oscillatoriales prevailed in alpine meadows (which had relatively high organic matter and fine soil texture), while Nostocales dominated in the subnival zone and screes. Eukaryotic microalgae together with cyanobacteria in the order Chroococcales were mostly present in the subnival zone. We conclude that the high elevation, semiarid, and arid soils in Ladakh are suitable habitats for microbial phototrophic communities and that the differences in these communities are associated with site, altitude, and vegetation type.

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K. Řeháková (✉) · Z. Chlumská · J. Doležal
Institute of Botany, Academy of Sciences of the Czech Republic,
Dukelská 135,
37982, Třeboň, Czech Republic
e-mail: Krehakova@email.cz

Z. Chlumská · J. Doležal
Faculty of Science, University of South Bohemia,
Branišovská 31,
370 05, České Budějovice, Czech Republic

Introduction

Biodiversity of plants in high mountain ecosystems and their geographical distribution have received considerable attention, especially with respect to climate change. The mean surface temperature of the globe has increased by around 1°C over the last century, and most scenarios predict that these changes will accelerate in the coming decades [23, 55]. Changes in the distribution of plant species associated with increased temperature have already been recorded in alpine areas in Europe, North America, and New Zealand [20, 47, 56]. Our knowledge of cold alpine environments and their biota, however, are still fragmentary, particularly with respect to phototrophic microbial communities in alpine soils.

Soil environments in high-altitude and high-latitude ecosystems provide habitat for numerous microorganisms, despite being subject to extremes of environmental stress, principally freezing and desiccation [e.g., 16, 30, 31, 45]. Phototrophic microorganisms—cyanobacteria and eukary-

otic microalgae—are important components of these soil environments, especially in arid and semiarid regions around the world. They improve soil structure, stabilizing the soil against erosion [39] and enhancing plant seedling establishment [5], especially when they create biological soil crusts. They also may increase infiltration of rainwater into soil [12]. Soil phototrophs fix inorganic carbon from the atmosphere via photosynthesis [21, 53], and some are able to fix atmospheric nitrogen [5, 27].

Despite the importance of microbes in alpine soils, the biodiversity and the functions of phototrophic microbes in alpine soils are still unclear and require further attention, especially in remote mountain regions such as the Himalayas. This high-altitude region is being strongly impacted by climate change and is experiencing rapid changes in biodiversity [20]. These changes in biodiversity can alter ecosystem processes and the resilience and the resistance of ecosystems to environmental change [24, 25]. 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.

Our knowledge about biodiversity of phototrophs in high-altitude regions of Himalayas could be improved by comparing the taxonomic composition of phototrophs in the alpine and subnival soils in relation to local environmental conditions. In this paper, we assessed whether abiotic factors (altitude and soil physico-chemical composition) and biotic factors (vegetation types) predict the taxonomic composition and biovolume of phototrophic microbial communities in the dry mountains of Ladakh (Tibetan Plateau, Zanskar Mountains, and Eastern Karakoram). The present investigation is, to the best of our knowledge, the first study of phototrophic communities in the remote region of NW Himalayas.

Materials and Methods

Study Area

The study area is in Ladakh, Jammu and Kashmir States, India, and is characterized by an arid environment rarely affected by monsoonal precipitation because such precipitation seldom crosses the high crest of the main Himalayan range [6]. At lower and middle elevations, evaporation exceeds precipitation. Along the Indus Valley, precipitation decreases from 115 mm at Leh (3,514 m, 34°09′N, 77°34′E, about 50 km NW of the study region) to 54 mm at Gar in SW Tibet (4,232 m, 32°07′N, 80°04′E, c. 160 km SE of the study region). Elevation in the study region ranges from 3,550 m at the bottom of the Indus Valley to 7,672 m above sea level (m a.s.l.) at Saser Kangri peak in the easternmost subrange of the Karakoram Range in India [29].

The fieldwork was conducted in August 2008 at four sites in three mountain areas of Ladakh: the Nubra Valley site (34°45′N, 77°35′E) is located in the Eastern Karakoram range; the Chamser Kangri site (32°59′N, 78°24′E) and the Mentok Kangri site (32°59′N, 78°24′E) are located in the southwestern extension of the Tibetan Plateau; and the Stock Kangri site (33°58′N, 77°28′E) is located in the Zanskar Mountains (Fig. 1). The Tibetan Plateau consists mainly of siliceous rocks (Precambrian granites, Tso Morari gneiss) [13], the Nubra Valley consists mainly of granitoids [48], and Stock Kangri consists mainly of multicolor sediments such as metapelites and metapsammites.

In total, 35 soil samples were collected along four altitudinal transects at the four mountain sites. The sampling points were located along a single altitudinal transect. The transect at the Nubra Valley site was 8.8 km long, 4,132–5,179 m a.s.l., and included 11 sampling points. The transect at the Chamser Kangri site was 11.7 km long, 4,600–5,965 m a.s.l., and included 11 sampling points. The transect at Mentok Kangri was 4.1 km long, 4,761–5,694 m a.s.l., and included nine sampling points. The transect at the Stock Kangri site was 7.3 km long, 3,675–4,900 m a.s.l., and included four sampling points. The sampling points on each transect were 300–400 m apart so as to cover the physiognomically different major vegetation types while avoiding places devoid of soil and vascular plants (very steep unstable slopes, glaciers, lakes, big boulder moraines, and elevations >6,100 m a.s.l.). The altitudinal zonation of vegetation included steppes and semi-deserts at lower elevations (collectively referred to as steppes hereafter), and alpine meadows, screes, and the subnival zone close to glaciers. Screes represent a habitat that partly overlaps in altitudinal range with alpine meadows and the subnival zone. All vegetation types occurred along each gradient except that the gradient at the Stock Kangri site lacked the subnival zone. 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 Klimeš [34], Klimeš and Doležal [35], Dvorský et al. [10], and Klimešová et al. [36].

Soil Sampling

At each sampling point on each gradient, one composite sample was taken from the bare surface layer of soil, 10 cm^2 in area and 1–3 cm deep, with a sterile spatula. The soil was air-dried on aluminum plates for 10 h. The samples were placed in sterile 100 ml polypropylene bags (Nasco Whirl-Pak®) and transported to the laboratory for analyses.

Physico-chemical Characteristics of Soil

The subsamples of soil were used for the determination of pH, organic matter content, and texture as described in

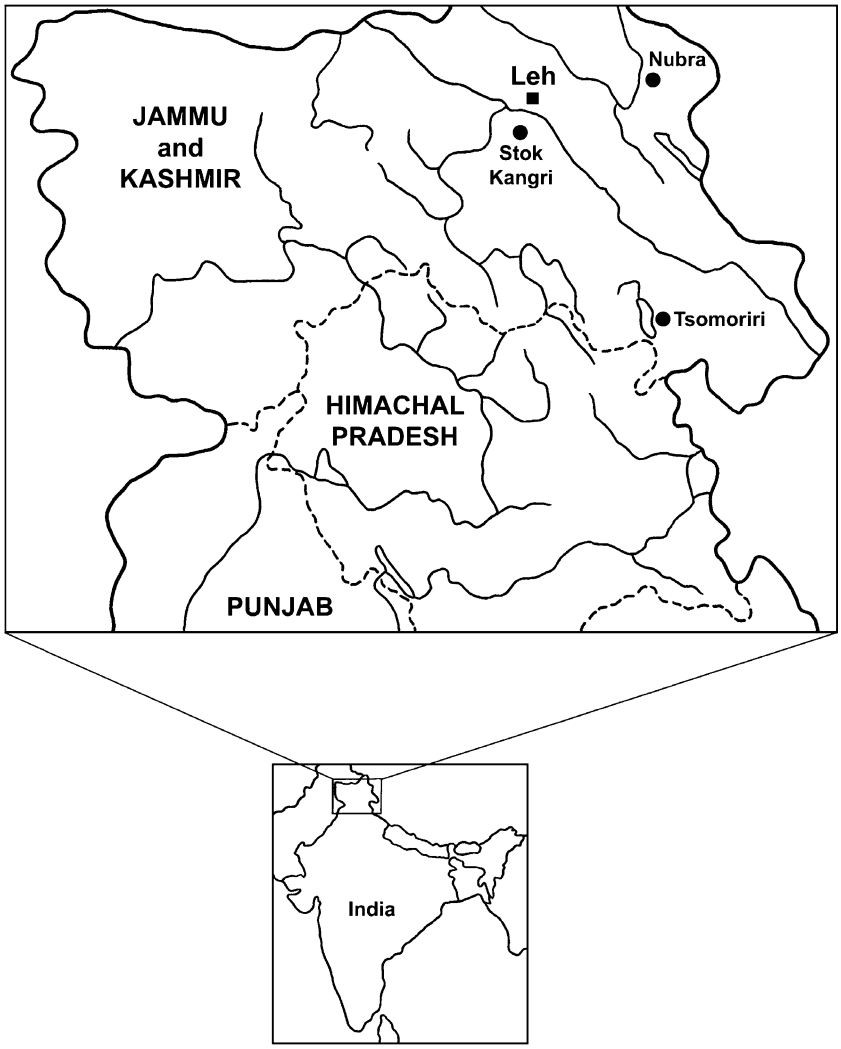

Figure 1 Map of the localities in the Ladakh. The *filled circles* indicate the locations of three mountain ranges where the study was performed. The Stok site is located in the Zanskar Mountains, the Nubra site is located in Eastern Karakoram, and the Mentok and Chamser sites are located near Tsomoriri Lake on the Tibetan Plateau

Kaštovská et al. [30]. The methods by Zbíral et al. [61], Kopáček and Hejzlar [38], and Wolf [58] were used for determinations of the total nitrogen, NH_4^+, and NO_3^-. Extractable phosphorus (P) in soil was extracted using a technique described in Mehlich [43], and the concentration of P was detected by using ascorbic acid–molybdate and a SHIMADZU UV-1650PC spectrophotometer. The macroelements (Ca, Mg, K, and Na) were extracted from soil according to US EPA method 200.2 ($HCl–HNO_3$; http://www.epa.gov/epaoswer/hazwaste/test/3050b.pdf) and determined spectrochemically using US EPA method 3050 [32]. The concentrations of chlorophylls were determined using methods by Kirkwood and Henley [33].

Algal and Cyanobacterial Abundance and Diversity

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 [30]. A term "eukaryotic alga" in this paper means taxa from the classes Chlorophyceae, Tribophyceae, and Bacillariophyceae. The green coccoid and filamentous algae included species from the classes Chlorophyceae and Tribophyceae; these were impossible to distinguish under the epifluorescence microscope. The Bacillariophyceae were combined with the green algae in the larger "eukaryotic algae" clade because their biovolume was very small to keep them as a separate group.

Cyanobacteria were classified into three orders according to their morphology: Chroococcales (single-celled organisms), Oscillatoriales (filamentous cyanobacteria without heterocytes and akinetes), and Nostocales (filamentous or colonial cyanobacteria with heterocytes and akinetes). The orders Oscillatoriales and Nostocales were

characterized in detail because they were more abundant than Chroococcales. In Oscillatoriales, the taxa *Phormidium* spp., *Leptolyngbya* spp., *Microcoleus vaginatus*, and other Oscillatoriales such as *Lyngbya*-like microorganisms were determined according to the width of filaments, the shape of vegetative cells, and the presence of mucilaginous sheaths. In Nostocales, two taxonomic groups, *Nostoc* spp. and other Nostocales, were separated according to their life form (colonies or filaments).

Statistical Analyses

Differences in cyanobacterial and algal abundance and physico-chemical characteristics of soil between four sites and four vegetation types were analyzed by canonical redundancy analysis (RDA), which is a constrained ordination method, in the CANOCO 4.5 program [52]. RDA was used because environmental variables—mountain range and vegetation type—were in the form of categorical predictors (dummy variables). Standardization by species (dependent variables) was used because the data analyzed were of various types and units. We performed several RDAs to assess the relative effects of different explanatory variables on microbial composition and soil physico-chemical properties. The variance partitioning procedure was performed with explanatory variables and covariables to remove their effects and to obtain a 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 [40]. Furthermore, a Monte Carlo randomization test with 999 unrestricted permutations was used to determine whether the environmental predictors had a significant effect on all analyzed data. The results of multivariate analyses were visualized in the form of a biplot ordination diagram constructed by the CanoDraw program (http://www.canodraw.com/).

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 and soil physico-chemical characteristics [42]. 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 R, version 2.10.1 (R Development Core Team 2009). Whether algal and cyanobacterial abundance and physico-chemical characteristics of soil differed between the four sites and four vegetation types were tested by analysis of variance. To satisfy the model assumptions, we log-transformed some of the variables. This did not only improve data normality and homoscedasticity, but also changed the effects from multiplicative to additive.

Results

Multivariate Analyses

The combined effect of altitude, mountain range, and vegetation type on all data analyzed (soil microbial communities and physico-chemical parameters) explained 43.8% of the total data variation and was highly significant (RDA: F=3.006, P=0.001). Concerning marginal effects of each explanatory variable (analyses with no covariables), RDA showed that the contribution to data variation was 27.6% for mountain range (F=3.945, P=0.001), 14.5% for altitude (F=5.589, P=0.001), and 13.7% for vegetation type (F=1.636, P=0.026). Variance partitioning revealed that 20.6% was explained solely by mountain range (F=3.291, P=0.001), 7.0% by altitude (F=3.351, P=0.006), and 8.4% by vegetation type (F=1.337, P=0.124). Because the three variables are intercorrelated, it is impossible to discern which of the environmental variables is responsible for the remaining 7.8% of variability.

In the RDA ordination diagrams, the main compositional changes along the first ordination axis are associated with altitude, i.e., the first axis clearly separates the two higher-altitude sites (Chamser and Mentok) from the two lower altitude sites (Nubra and Stok; Fig. 2a). This division corresponds with position of the four major habitats (Fig. 2b). The higher-altitude sites have large areas covered by alpine screes and the subnival zone (from 5,400 to 6,000 m a.s.l.), while the lower altitude sites have extensive steppes (3,700 to 4,700 m a.s.l.) and alpine meadows (from 4,800 to 5,300 m a.s.l.).

Differences in Soil Physico-chemical Parameters

In general, collected soils were highly variable in their physico-chemical properties except for pH, which only ranged from 8.1 to 8.9 (Table 1). The differences among mountain ranges and vegetation types were significant for most soil characteristics (Table 1 and Suppl. Table 1). RDA diagrams show that the higher-altitude sites (Chamser and Mentok), where alpine screes and the subnival zone prevail, had higher concentrations of nitrate, ammonia, and phosphorus; whereas, the lower altitude site (Nubra), where alpine meadows prevail, was characterized by a higher pH and higher concentration of base cations (Fig. 2). The concentrations of calcium and magnesium and the pH decreased significantly with increasing altitude (Table 1). Alpine meadows had the highest concentrations of calcium (22.4 $g kg^{-1}$) and magnesium (12.9 $g kg^{-1}$), while the subnival zone had the lowest calcium concentration (2.2 $g kg^{-1}$) and the screes had the lowest magnesium concentration (4.9 g kg^{-1}).

The concentrations of nitrogen, ammonia, phosphorus, and calcium and soil texture differed significantly among

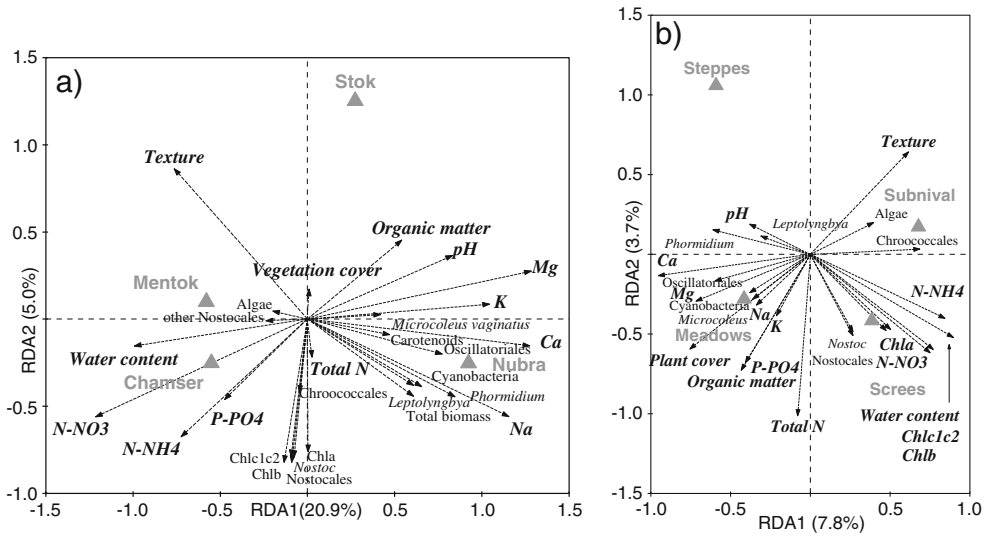

Figure 2 Redundancy analysis biplots of phototrophs and physico-chemical characteristics of soil (response variables) in relation to mountain ranges (**a**) and vegetation types (**b**). Response variables, represented by vectors (*arrows* for quantitative variables), are grouped into variables related to biovolume of phototrophs (*bold italic*), and those related to physico-chemical characteristics of soil (*regular font*). Environmental variables are represented by centroids. The *angles between arrows* indicate correlations between variables

vegetation types (Suppl. Table 1). The concentration of total nitrogen ranged from 338.7 to 1,154.4 mg kg^{-1}; the lowest concentration was detected in steppes and the highest in alpine meadows. The concentration of total nitrogen had a hump-shaped altitudinal pattern in two sites (Chamser and Stok, Fig. 3a); the peak concentrations corresponded to alpine meadows prevailing at middle elevations. Ammonia concentrations were threefold higher in the subnival zone and in screes than in meadows and steppes (Fig. 2b, Suppl. Table 1). Organic matter content was highest in soil from alpine meadows (up to 3.2%) and lowest in steppes (1.3%; Suppl. Table 1). The coarsest soil was found in subnival zone, where the fraction >0.5 mm represented up to 63.1% of the soil particles; the soil with highest proportion of fine particles was from alpine meadows (Suppl. Table 1; Fig. 2b). Hence, the texture of investigated soil tended to increase with altitude (P=0.098; Table 1, Fig. 3b). The concentrations of ammonia, nitrate, and chlorophyll *a* (Fig. 3c), *b*, and c_1 + c_2 significantly increased with altitude (Table 1).

Table 1 Soil physico-chemical characteristics (means and standard deviations) for the four sites

Dependent variable	Mountain site				ANOVA		Regression	
	Chamser	Mentok	Nubra	Stok	*F*	*P*	R^2	*P*
Plant cover (%)	31.4±17.7	35.6±24.1	29.4±22.4	33.5±4.4	0.16	0.924	↓ 0.08	0.097
TN (mg/kg)	871.9±776.5	967.2±617.2	981.2±854.2	625.6±380.6	0.25	0.857		ns
$N–NH^{4+}$ (mg/kg)	27.4±30.1	9.8±8.5	3.2±2.6	1.2±0.3	4.11	0.014	↑ 0.37	0.001
$N–NO^{3-}$ (mg/kg)	52.8±35.0	23.4±12.7	2.1±1.5	2.3±1.6	12.05	0.000	↑ 0.18	0.011
$P–PO_4^{3-}$ (mg/kg)	0.8±0.6	0.4±0.3	0.3±0.3	0.3±0.1	3.74	0.021	∩ 0.09	0.076
Ca (g/kg)	2.1±1.6	0.8±0.3	33.8±27.8	7.3±4.7	9.91	0.000	↓ 0.21	0.000
Mg (g/kg)	3.7±1.9	3.5±1.5	17.8±9.3	11.8±0.4	16.15	0.000	↓ 0.29	0.000
Na (g/kg)	0.1±0.0	0.1±0.0	0.3±0.2	0.1±0.0	17.51	0.000		ns
K (g/kg)	1.7±0.6	1.7±0.7	3.7±1.7	2.8±0.8	7.81	0.000		ns
pH	8.3±0.4	8.1±0.5	8.8±0.4	8.9±0.2	6.37	0.001	↓ 0.161	0.016
OM (%)	1.7±1.3	1.9±1.2	3.1±2.6	3.3±0.7	17.27	0.181	↓ 0.068	0.128
Soil particles >0.5 mm (%)	50.1±16.2	65.4±10.5	30.4±14.1	67.7±14.8	12.03	0.000	↑ 0.08	0.098
Chla (mg/g)	7.9±5.9	5.4±4.5	7.8±5.8	1.6±1.1	1.78	0.171	↑0.225	0.003
Chlb (mg/g)	9.0±7.7	5.5±6.0	7.1±7.1	0.8±0.9	1.60	0.210	↑0.321	0.000
$Chlc^1c^2$ (mg/g)	12.0±11.1	7.4±8.3	8.3±8.7	0.7±0.9	1.58	0.213	↑0.334	0.000
Car (mg/g)	0.2±0.4	0.6±0.9	0.9±1.0	0.3±0.4	2.01	0.133		ns

Also shown are *F* and *P* values (for type I error estimate) from ANOVA analyses comparing sites for each dependent variable, and R^2 value and the corresponding type I error estimate for the regression of the dependent variable on altitude for all sites together. An upward or downward pointing arrow indicates a positive or negative relationship between the dependent variable and altitude; the symbol ∩ indicates a hump-shaped relationship; and ns indicate that the relationship is not significant

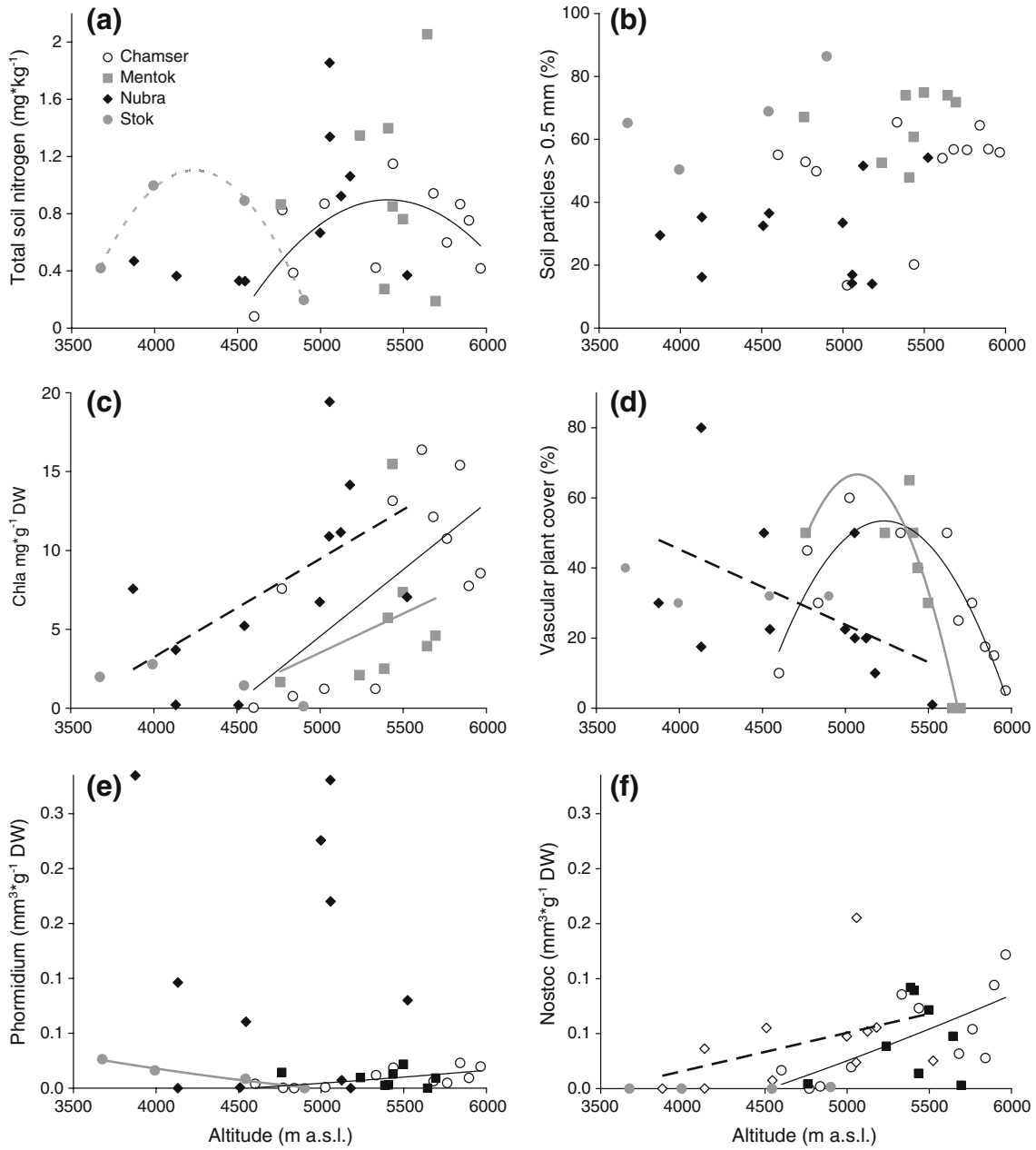

Figure 3 Dependence of selected parameters on the altitude in four study sites (Chamser, Mentok, Nubra, and Stok). **a** Total soil nitrogen. **b** Soil particles >0.5 mm in diameter (%). **c** Concentration of chlorophyll a. **d** Vascular plant cover (%). **e** Biovolume of *Phormidium* spp. **f** Biovolume of *Nostoc* spp.

The cover of vascular plants, which ranged from 18.2% to 51.3%, also significantly differed among vegetation types (Suppl. Table 1). The vascular plant cover was lowest in subnival zones and highest in screes (Fig. 2b). Hence, the hump-shaped trend with altitude was observed at higher-altitude sites—Chamser and Mentok (Fig. 3d). The concentrations of chlorophyll *a*, *b*, and $c_1 + c_2$ were highest in screes and lowest in steppes (Suppl. Table 1). Carotenoid concentration was lowest in screes and relatively similar among the other three vegetation types (Suppl. Table 1).

The Composition of Phototrophic Communities

Phototrophic microorganisms were found in all examined samples. The phototrophic communities contained 28 morphotypes: 14 for cyanobacteria, ten for green algae, and four for diatoms. Cell abundance ranged from 10^3 to 10^7 cells g^{-1}dry weight (DW), and the biovolume ranged from 0.00013 to 1.51 mm^3 g^{-1} DW. Cyanobacteria were the dominant component of the communities, contributing 70.9% to 98.6% of the biovolume (Table 2). Microalgae

Table 2 The biovolume of phototrophs (means and standard deviations) at the four study sites

Dependent variable	Mountain site				ANOVA		Regression	
	Chamser	Mentok	Nubra	Stok	*F*	*P*	R^2	*P*
TB (mm^3/1 g DW)	0.08±0.07	0.1±0.07	0.32±0.42	0.07±0.07	2.74	0.059	ns	
I. Cyanobacteria (%)	93.33	70.93	98.67	97.3	3.11	0.040	ns	
1. Oscillatoriales (%)	16.1	20.63	81.71	98.11	4.090	0.014	↓ 0.13	0.035
a. *Microcoleus vaginatus* (%)	1.47	0.00	47.74	77.22	0.815	0.495	↓ 0.14	0.03
b. *Phormidium* (%)	78.96	64.05	41.42	17.5	6.020	0.002	↓ 0.06	0.13
c. *Leptolyngbya* (%)	15.73	35.7	6.22	0.34	3.161	0.038	↑ 0.12	0.053
d. Other Oscillatoriales	3.84	0.26	4.61	4.92	0.893	0.450	ns	
2. Chroococcales	21.45	14.5	4.09	0.26	0.444	0.717	↑ 0.15	0.021
3. Nostocales	62.45	64.87	14.2	1.63	1.732	0.180	↑ 0.25	0.002
a. *Nostoc* (%)	97.12	97.85	99.55	29.82	1.741	0.179	↑ 0.24	0.003
b. Other Nostocales (%)	2.97	2.19	0.45	70.18	0.310	0.817	ns	
II. Algae (%)	7.19	29.08	1.40	2.92	0.961	0.423	U 0.08	0.099

Also shown are *F* and *P* values from ANOVA analyses (for type I error estimate) comparing sites, and R^2 value and the corresponding type I error estimate for the regression of the dependent variable on altitude for all sites together. An upward or downward pointing arrow indicates a positive or a negative relationship between the dependent variable and altitude, and ns indicates that the relationship is not significant. TB=total biovolume of algae and cyanobacteria per 1 g of dry soil. Percentage of cyanobacteria and algae is calculated from the total biovolume of phototrophic community. Percentage of cyanobacterial orders is calculated from the biovolume of cyanobacteria, where cyanobacterial biovolume represented 100%. The percentage of cyanobacterial genera is calculated from the biovolume of single orders, where 100% represents the biovolume of order, e.g., Oscillatoriales

(Chlorophyceae, Xanthophyceae, and diatoms) accounted for a small proportion of total phototrophic biovolume at Chamser, Nubra, and Stok sites (Table 2). At the Mentok site, however, microalgae were important components of the phototrophic communities of the subnival zone (Fig. 2a), representing 29% of the phototrophic biovolume (Table 2).

The RDA diagrams show that cyanobacteria from the order Nostocales predominated at the higher-altitude sites (Chamser and Mentok), while Oscillatoriales were a dominant part of the communities at the lower altitude sites (Nubra and Stock; Fig. 2a). Hence, Oscillatoriales prevailed in alpine meadows and steppe soils, while Nostocales were dominant in the alpine screes and subnival zone (Fig. 2b). Most of Nostocales biovolume was represented by the genus *Nostoc*, but the genus *Scytonema* prevailed at the Stock site (Table 2). Chroococcales contributed to the biovolume of phototrophs more at the sites where Nostocales dominated (Table 2). The relative abundance of Chroococcales was highest in the subnival zone at Chamser, where Chroococcales represented 27.8% of the total cyanobacterial biovolume (Table 3).

In the two lower altitude sites (Nubra and Stok), which had the highest biovolume of Oscillatoriales, *M. vaginatus* dominated together with *Phormidium* spp. (Fig. 2a). The biovolume of cyanobacteria *Phormidium* (Fig. 3e) and *M. vaginatus* decreased with increasing altitude, while the biovolume increased with altitude for *Nostoc* (Fig. 3f), *Leptolyngbya*, and the order Chroococcales (Table 2). The relationship between biovolume and altitude was statistically significant for *M. vaginatus*, *Leptolyngbya*, *Nostoc*, and Chroococcales ($P<0.05$), but not for *Phormidium* ($P=0.13$; Table 2). Hence, *M. vaginatus* represented a significant proportion of the phototrophic communities in alpine meadows (Table 3), and its biovolume was positively correlated with organic matter content (Fig. 2b). In alpine meadows, *Leptolyngbya* spp. and other Oscillatoriales were a minor part of the community (Table 3).

The percentage composition of Oscillatoriales was different in the higher-altitude sites, which were dominated by Nostocales, than in the lower altitude sites. In the higher-altitude sites, *M. vaginatus* was nearly absent, *Phormidium* dominated, and *Leptolyngbya* spp. was also abundant. Other Oscillatoriales were only a minor part of these higher-altitude communities (Table 2). The biovolumes of taxa from the order Oscillatoriales, especially *M. vaginatus*, were positively correlated with organic matter content (Fig. 2a). Also the concentration of cations was correlated with the biovolume of Oscillatoriales. *M. vaginatus* had larger biovolume in the soils with higher concentrations of potassium, while *Phormidium* had larger biovolume in the soils with higher concentration of sodium (Fig. 2a).

Discussion

Microbial biogeographical patterns are shaped by environmental factors [2, 14, 44]. Various environmental predictors

Table 3 The biovolume of phototrophs (means and standard deviations for densities and means alone for percentages) in relation to four vegetation types

Dependent variable	Vegetation type			
	Meadow	Subnival	Steppes	Screes
TB (mm^3/1 g DW)	0.22±0.36	0.11±0.08	0.12±0.18	0.07±0.05
I. Cyanobacteria (%)	97.90	79.97	94.86	100.00
1. Oscillatoriales (%)	77.04	21.96	87.49	13.46
a. *Microcoleus vaginatus* (%)	59.25	0.00	11.74	0.00
b. *Phormidium* (%)	35.45	75.00	78.12	73.53
c. *Leptolyngbya* (%)	5.30	25.00	10.15	26.47
d. Other Oscillatoriales	5.15	8.28	0.00	0.00
2. Chroococcales	2.79	27.83	0.91	16.29
3. Nostocales	20.17	50.21	11.60	70.25
a. *Nostoc* (%)	97.65	98.93	99.04	96.00
b. Other Nostocales	2.35	1.07	0.96	4.00
II. Algae (%)	2.10	20.03	5.14	0.00

TB=total biovolume of algae and cyanobacteria per 1 g of dry soil. The percentage of cyanobacteria and algae is calculated from total biovolume of phototrophic community. The percentage of cyanobacterial orders is calculated from the biovolume of cyanobacteria, where cyanobacterial biovolume represented 100%. The percentage of cyanobacterial genera is calculated from the biovolume of single orders, where 100% represents the biovolume of order, e.g., Oscillatoriales

for the macro- and micro-scale pattern of microbes in the soil have been found, and these include pH [15], vascular plant cover and temperature gradients [28, 59], precipitation pattern [2, 8], and land use [9]. Despite recent increased interest in soil environments, the links between local diversity of microorganisms and the factors that shape them are largely unexplored. In the current study, we described the phototrophic communities and determined which abiotic factors are associated with the composition of these communities in the high altitude of Ladakh. Our research revealed abundant phototrophic communities in all samples from four mountain ranges. We report the presence of phototrophs at 6000 m a.s.l. We assume that, like bacteria, cyanobacteria have no altitudinal limits as long the environment provides some organic matter and at least short periods with liquid water [50].

Cyanobacteria composed most of the biovolume of phototrophs in Himalayan soils. The prevalence of cyanobacteria is caused by a combination of factors, particularly high soil pH, undeveloped and unstable soil substrate, and high UV radiation. Numerous records for cyanobacteria in freshwater and soils indicate that their diversity and abundance are greatest at higher pH values, though the reasons for their success under these conditions are still unclear [57]. One factor could be CO_2-concentrating mechanisms; unlike microalgae, cyanobacteria are able to effectively use HCO_3^- as a source of carbon [19]. Another factor influencing the species composition is high UV radiation in the Himalayas. Many cyanobacteria tolerate high levels of UV radiation and produce a wide range of UV protectants (e.g., scytonemin, carotenoids, or mycosporine-like amino acids). Cyanobacteria originated in the Precambrian era when the ozone shield was absent; they presumably faced high fluxes of UV radiation, which likely acted as an evolutionary pressure leading to the selection for efficient mechanisms for protection against UV radiation [49]. Cyanobacterial tolerance of intense sunlight including UV radiation may have contributed to their success in colonizing high-altitude and high-latitude environments. The one exception to this trend was observed at Mentok, where a dense population of *Klebsormidium*-like alga caused microalgae to represent 29% of the total phototrophic biovolume (Table 2). This site likely had good microclimatic conditions for development of microalgae. The ability of cyanobacteria to grow on an unstable substrate of successionally young or undeveloped soils has been reported from deglaciated soils and subglacial sediments at Svalbard [30, 31], from soils without vegetation in Antarctic [45], and from arid and semiarid regions of the western USA [15].

Species composition was significantly influenced by altitude, particularly for the orders Nostocales and Chroococcales. Nostocales are usually thought to be able to colonize young undeveloped soils because of their ability to fix nitrogen [57], which may be the limiting nutrient in this type of soil. This statement, however, does not explain the current data because the concentrations of total nitrogen and ammonia increased significantly with altitude in Ladakh soils (Table 1, Fig. 3e). Another unidentified factor must have caused the increase of Nostocales biovolume

with altitude. Perhaps *Nostoc* is better adapted than many other phototrophs to cryoturbation and desiccation because it has a well-developed mucilaginous sheath, which protects against the cold and desiccation. *Nostoc* might also do well at high altitudes because its biovolume is independent on the concentration of organic matter, unlike *Microcoleus* (Fig. 2). The biovolume of Chroococcales may have increased with altitude because, as unicellular organisms with rapid growth rates [46], they do not require a stable substrate with a fine texture and high organic matter content [30]. In contrast, the biovolume of Oscillatoriales decreased with altitude. Oscillatoriales are more abundant in finer textured soils that contain relatively high concentrations of organic matter, as is typical for alpine meadows, which occurred in the lower altitudes in the current study. The requirement of Oscillatoriales for relatively high soil organic matter content was also observed at Svalbard [30].

An interesting relationship was observed between the proportion of biovolume represented by the oscillatorialen taxa *Phormidium* and *M. vaginatus* and the order Nostocales. *M. vaginatus* were absent or nearly absent if phototrophs in the order Nostocales dominated (Tables 2 and 3). In that case, oscillatorialen biovolume mostly consisted of *Phormidium*. Perhaps *Nostoc* spp. from Ladakh soils had an allelopathic effect on *M. vaginatus* but not on *Phormidium*. The release of allelopathic compounds by cyanobacteria and their effect on microalgae has been reported from water biotopes [11, 17] but not from soil environments. It is also possible that *M. vaginatus* and *Phormidium* are two morphotypes of one species, which change morphology according the environment. The trichomes and vegetative cells of *Phormidium* and *M. vaginatus* from the studied soils were morphologically identical (data not shown) with the exception of the presence/absence of a mucilaginous sheath. The dependency of cyanobacterial morphology on environmental parameters (temperature, concentration of phosphorus, presence of predator) has been reported from the water biotopes [26, 37, 60] but not from soil. Determining whether allelopathy or morphological response to environment can explain the relative abundances of *M. vaginatus* and *Phormidium* will require additional research.

Soil represents one of the most important reservoirs of biodiversity [18]. The biological diversity in soils is several orders of magnitude greater than that found above ground [22] and is seen as the last frontier for biodiversity on earth [51]. To our knowledge, data concerning the biodiversity of cyanobacteria and algae in India are still very fragmentary, especially in the soil biotope. Most papers have focused on freshwater algae, such as those from Lake Dal in the Jammu and Kashmir regions [4] or from rice field [e.g., 7]. One study is focused on the phototrophs at cultural monuments [1]. There are only few reports of soil cyanobacteria and algae from the desert soil [3, 41, 53, 54], and none from mountain soils. Our study from Ladakh, NW India, has shown that soil phototrophs form complex communities with specific environmental associations. Our results will be useful for designing future monitoring schemes and for assessing the effects of global change in these diverse but poorly studied regions. However, more intensive studies using environmental molecular techniques such as DGGE or microarrays are needed to reveal the molecular diversity of the whole microbial communities in the pristine soils of Ladakh.

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