School of Doctoral Studies in Biological Sciences
University of South Bohemia in České Budějovice
Faculty of Science

Ecology and diversity of microbial phototrophs in biological soil crusts of Polar Regions

Ph.D. Thesis

Ekaterina Pushkareva, M.Sc.

Supervisor: Doc. Ing. Josef Elster, Ph.D.

Centre for Polar Ecology, Faculty of Science, University of South Bohemia in České Budějovice

Centre for Phycology, Institute of Botany, AS CR, Třeboň, Czech Republic

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Annotation

Biological soil crusts (BSCs) are important components of semi-arid and arid environments and occupy a large area in Polar Regions. However, their ecological functions and the diversity of major organisms are still ambiguous. Given that rapid climate change is of particular significance and the current warming is already attributed to small variations on the Earth, it is important to obtain a more comprehensive picture about the environment to predict its changes. Moreover, climate change is faster and more severe in Polar Regions than in other parts of the world. In this context, the thesis is focused on the community structure of microbial phototrophs and their ecological functions in BSCs of the Arctic (Central Svalbard) and Antarctica (Dronning Maud Land). Combining molecular and morphological techniques we described cyanobacterial community composition in BSCs and its changes along the gradient of soil crust development. Moreover, we showed how the different stages of soil crust development (from poorly-developed to well-developed) influence photosynthetic and nitrogenase activities associated with the phototrophic community.

Declaration [in Czech]

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České Budějovice, 15.08.2017

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Ekaterina Pushkareva

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List of papers.

This thesis is based on the following papers.

I.

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Ekaterina Pushkareva was responsible for writing and revising the manuscript.

II

Pushkareva E, Elster J (2013) Biodiversity and ecological classification of cryptogamic soil crusts in the vicinity of Petunia Bay, Svalbard. Czech Polar Reports 3(1): 7-18.

Ekaterina Pushkareva collected soil crust samples, performed the analyses and wrote the manuscript.

III.

Pushkareva E, Pessi IS, Wilmotte A, Elster J (2015). Cyanobacterial community composition in Arctic soil crusts at different stages of development. FEMS Microbiology Ecology 91: fiv143

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IV.

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Ekaterina Pushkareva performed the field measurements and microscopy, participated in the data analyses and wrote the manuscript.

V.

Pessi IS, **Pushkareva E**, Lara Y, Borderie F, Wilmotte A, Elster J. Marked primary succession of cyanobacterial communities following glacier retreat in the High Arctic. *Manuscript.*

Ekaterina Pushkareva participated in designing the experiment, collected the soil crust samples and estimated cyanobacterial biovolume.

VI.

Pushkareva E, Pessi IS, Namsaraev Z, Mano M-J, Elster J, Wilmotte A. Cyanobacteria inhabiting soil crusts of a polar desert: Sør Rondane Mountains, Antarctica. *Manuscript.*

Ekaterina Pushkareva performed the pyrosequencing 454, participated in the data analyses and wrote the manuscript.

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1. Introduction

1.1. Definition of biological soil crusts

Soil crusts are thin continuous layers of the soil surface formed by raindrop impact or freeze-thaw processes. The importance of soil crusts in an ecosystem lies in the development of the soil crust and conversion of soil nutrition by ameliorating physicochemical and biological properties. Soil development is a product of both physical and biological processes and the details are poorly understood.

Generally, two main types of crust are distinguished: physical and biological (Belnap and Lange, 2003). Physical soil crusts are non-biotic, formed by the action of water and wind on soil particles on the surface of bare areas. Physical crusts can further develop into biological crusts.

Biological soil crusts (BSCs) are a community of organisms held together by soil aggregates and living on the upper part of the soil. They are composed of microbial phototrophs (eukaryotic microalgae and cyanobacteria), fungi, archaea, bacteria, mosses, lichens and liverworts (Belnap et al., 2001). Since microbial phototrophs require light for photosynthesis, most of their biomass is concentrated in the upper soil layers, while fungi, bacteria and archaea can be found in deeper soils (Bastida et al., 2014; Hu et al., 2012). Moreover, BSCs are highly stress-tolerant under extreme environmental conditions and therefore widespread in many ecosystems from hot deserts to polar regions (Büdel and Colesie, 2014; Hu et al., 2012; Karsten and Holzinger, 2014; Stewart et al., 2014).

BSCs are very important in the process of soil succession. In ecological theory, soil succession is defined as the predictable manner by which communities change over time during the colonization of a new environment (primary succession) or following a disturbance (secondary succession) (Begon et al., 2005). In primary succession, BSCs are often the first colonizers of a newly formed environment (Belnap and Lange, 2003). Further, the BSC community gradually develop through a number of different communities into a climax stage with a stable community where the types of vegetation or microbial phototrophs will no longer change unless another disruption occurs. In contrast, secondary succession, which occurs on a previously colonized, but disturbed (by fire, flood, hurricane and etc.) area, is a much more rapid process than primary succession because the soil and nutrients are already available. In addition,

under the large influence of abiotic factors, BSCs also play an important role in succession but tend to persist as a permanent component of undisturbed stable states (Bowker, 2007). However, their addition or loss may trigger transitions between steady states.

According to Belnap and Lange (2003), there are several types of BSCs depending on the dominant organisms: cyanobacterial, green algal, liverworts, moss and lichen crusts. The first two types of soil crusts consolidated by microbial phototrophs are the pioneer crusts, while the next three types are far more complex, as they are formed by a mixture of microbial phototrophs with lichens, moss and liverworts in different combinations and abundances (Büdel, 2005).

The crust type and its stage of development might modify soil properties to promote the growth of other BSC species, and the species composition can vary from one area to another (Li et al., 2010). Poorly-developed soil crusts usually have low nutrient contents and are consolidated mostly by microbiota. These BSCs are light in colour and, thus, are exposed to UV radiation. In the next step of development, mosses and lichens begin to appear and the soil crusts acquire a darker colour due to higher microalgal abundance. These BSCs usually have cyanobacteria on the surface, such as *Scytonema* sp. and *Nostoc* sp., which produce the dark colour (Housman et al., 2006). Moreover, dark-coloured BSCs retain heat which make the surface and the underlying soils relatively warmer on cold days, leading to more favourable temperatures for photosynthesis (Huang et al., 2014). In the last development stages, lichens or moss totally occupy the soil surface with moss and lichen soil crusts forming mosaic patterns with higher small-scale variation (Veste et al., 2011). In addition, nutrient concentrations also increase with soil crust development (Housman et al., 2006).

1.2. Distribution of biological soil crusts

BSCs can be found in almost any terrestrial environment where vegetation does not cover 100 % of the soil surface conditions (Sancho et al., 2014). In forests, BSCs colonizing plant interspaces increase nitrogen and phosphorus contents needed for plants growth (Baumann et al., 2017). They also act as an absorptive organ for water and therefore provide germination grounds for seeds. However, a negative effect of old BSCs on the emergence of vascular plant species has been reported as well (Langhans et al., 2009). Similarly, in grassland and tundra regions BSCs

colonize the soil between patchily distributed plants and lie primarily outside the influence of plant canopies and plant roots. In mature vascular plant communities, BSCs often cover up to 60-70% of the soil surface and the prokaryotic community differs from the root zone (soil containing live roots under a plant canopy) (Redfield et al., 2002). The bacterial communities of BSCs are enriched with cyanobacteria and anoxygenic phototrophs, whereas soils under plant canopies harbour larger populations of heterotrophic bacteria (Steven et al., 2012).

BSCs are very heterogeneous and several types of BSCs can be present in one area. For example, the Colorado Plateau of the western USA is very diverse in BSCs including cyanobacteria-, lichen- and moss-dominated crusts (e.g. Housman et al., 2006; Redfield et al., 2002). BSCs are very important in the areas where they occur and can be used for a wide range of purposes, like grazing and cropping. For example, box woodlands of eastern Australia, which have been considerably altered by European farming practices, support a rich suite of BSCs (Eldridge et al., 2006, 2000). They have tightly structured surfaces, primarily due to the binding of soil particles by polysaccharides and gels excreted by cyanobacteria. Recently, the use of cyanobacteria has been proposed as an approach to increase nutrient availability and soil stability for sustainable agriculture (Singh et al., 2011). EPS (extracellular polymeric substance)-producing cyanobacteria increase topsoil aggregation through the formation of stable sediment macro-aggregates and soil enrichment in organic matter (Rossi et al., 2017). Therefore, cyanobacterial crusts may accelerate the growth or establishment of vascular plants due to increased nitrogen and carbon supply. In addition, Bu et al. (2014) demonstrated that artificial rapid cultivation of BSCs dominated by cyanobacteria can provide a novel alternative to traditional biological methods (e.g. planting of trees, shrubs, and grasses) for controlling soil and water loss.

Mining-impacted BSCs have been intensively studied in the USA and Europe (Rojas et al., 2016). Soil substrates in post-mining areas contain little amounts of organic matter resulting in low water infiltration rates, high bulk densities, compaction, reduced water holding capacities and, therefore, higher susceptibility to wind and water erosion. The vegetation in this type of environment is limited and, thus, BSCs colonizing the soil surface under such extreme conditions without human support play a key role in soil development and niche formation for vascular plants. There are various development stages in post-mining areas, from initial

biological soil crusts composed of microbial phototrophs, to more developed soil crusts with mosses or/and lichens (Gypser et al., 2016).

1.3. Diversity of microbial phototrophs in biological soil crusts

A total of 46 genera of cyanobacteria and 70 genera of eukaryotic microalgae had been recorded in BSCs up to 2005 (Büdel, 2005). Since then, cyanobacterial diversity in BSCs has been studied in the deserts of the USA (e.g. Alwathnani and Johansen, 2011; Zhou et al., 2016), India (Kumar and Adhikary, 2015), Europe (Williams et al., 2016) and cold regions such as the Himalayas (Čapková et al., 2016; Janatková et al., 2013; Řeháková et al., 2011), the Tibetan Plateau (Liu et al., 2016), Svalbard (Kaštovská et al., 2007; Williams et al., 2017), Bolshevik Island in the Russian Arctic (Patova and Beljakova, 2006), Ellesmere Island in the Canadian Arctic (Elster et al., 1999) and Antarctica (Namsaraev et al., 2010; Obbels et al., 2016; Wood et al., 2008).

Culture-independent approaches, such as high-throughput sequencing (HTS), have become valuable tools to describe microbial communities using target genes that are PCR (polymerase chain reaction) amplified from a pool of soil DNA (Steven et al., 2012). This generates up to tens of millions of sequences on a single run, providing an in-depth inventory of the microbial diversity. HTS allows the investigation of rare, low-abundance taxa and thus provides better estimations of species richness and turnover in different environments compared to traditional techniques (Roesch et al., 2007). Surveys of soil bacteria by PCR amplification of 16S rRNA genes followed by HTS have shown that microbial communities in BSCs are extremely diverse and may contain abundant non-cultured representatives of novel, undescribed bacterial divisions (Hugenholtz et al., 1998). However, the use of universal primers can lead to under- or overrepresentation of specific bacterial groups. Therefore, HTS coupled with cyanobacteria-specific primers is a useful tool for a detail assessment of the cyanobacterial community in BSCs (Pessi et al., 2016). Furthermore, multiple approaches using both molecular and morphological techniques allow for obtaining a more accurate overview of BSC community structure.

In general, filamentous cyanobacteria (e.g. *Leptolyngbya* and *Microcoleus*) are the dominant cyanobacteria in BSCs around the world (Lan et al., 2012; Williams et al., 2017; Zhao et al., 2009). Their filaments are surrounded by extracellular sheaths which help to overpass soil layers.

When soil crusts become wet, the filaments glide out of their sheaths, and in a phototactic reaction, move up towards the soil surface (Belnap and Lange, 2003). In the time of drying, the filaments leave the surface, and the exposed filaments secrete new sheaths. They mostly stay beneath the soil surface where UV radiation is reduced, but photosynthetically active radiation (PAR) is still adequate. Nostocales (e.g. *Nostoc*, *Scytonema*, *Calothrix*) are common cyanobacteria in BSCs and are found both on and beneath the soil surface (Lan et al., 2012). These genera have limited mobility and therefore they produce pigments to protect themselves from excess radiation when they are exposed on the soil surface (Quesada et al., 1999). Moreover, these cyanobacteria are an important source of fixed nitrogen in soil ecosystems (Stewart et al., 2014). Unicellular cyanobacteria in soil crusts are represented by the coenobia-forming genus *Gloeocapsa*, and by single-celled Chroococcales (Belnap and Lange, 2003).

The eukaryotic microalgal community in BSCs is mainly represented by green algae (Chlorophyta), yellow-green algae (Xanthophyceae) and the filamentous genus *Klebsormidium* (Streptophyta) (Karsten and Holzinger, 2014; Lan et al., 2012; Zhao et al., 2009).

1.4. Ecological features of biological soil crusts

Photoautotrophs play an important role in soil crust ecosystems. BSC organisms fix atmospheric carbon (C) through photosynthesis and nitrogen (N) through nitrogen fixation and supply both C and N to underlying soil food webs (Elbert et al., 2012).

The C is mainly released in the respiration phase of photosynthesis. The optimum temperature for photosynthesis was detected to range from 10 to 28^{o}C (Serpe et al., 2013; Zhao et al., 2016), which explains the decreased rate of photosynthetic parameters in polar BSCs where temperatures reach a maximum of 15^{o}C in the summer (Sehnal et al., 2014). Photosynthesis is affected by several environmental factors. Extreme temperatures, both low and high, decrease photosynthetic efficiency. Likewise, excess and deficient light negatively influence photosynthetic activity of soil crust organisms. A good example is the well-known phenomenon of “midday depression” when an increase in photosynthetically active radiation (PAR) during the day inhibits photosynthesis in BSCs (Hui et al., 2015; Wu et al., 2013).

The photosynthesis rate depends on the species composition in BSCs. Soil photosynthetic organisms absorb light energy by photosynthetic pigments. Therefore, with soil crust development and the following increase of photosynthetic organisms in BSCs, overall Chl-a content increases as well (Lan et al., 2017). However, when lichens more pronouncedly contribute to biological soil crusts at later stages of development, the relation between biomass and chlorophyll content becomes less strong (Gypser et al., 2016).

Another important feature of BSCs is their ability to fix atmospheric nitrogen (N_2). Around 46% of biologically fixed nitrogen in the world is done by BSCs (Elbert et al., 2012). Cyanobacteria, mainly the order Nostocales, are one source of fixed nitrogen in soil crust ecosystems (Stewart et al., 2014). They are able to cleave atmospheric nitrogen (N_2) to produce nitrate (NO_3) and ammonium (NH_4), which can then be used by other organisms. These cyanobacteria have specialized nitrogen-fixing heterocysts that lack the oxygen-producing photosystem II (PSII). Heterocystous species, such as *Nostoc* spp. that also form free-living colonies on the soil surface, are perhaps the most important contributors to nitrogen fixation in both cold and hot deserts. Moreover, cyanobacterial symbiosis with mosses (Stewart et al., 2011) and fungi (= lichens) (Abed et al., 2013) increase the rate of nitrogen fixation especially in polar environments. Symbiosis in lichens helps to protect the cyanobacteria from low temperatures and water fluctuations while the bryophytes provide carbohydrates as well as protection against desiccation and UV-radiation. Other common heterocyst-forming cyanobacteria in soil crusts are *Scytonema* spp., *Calothrix* spp., *Tolypothrix* spp. and others. Several non-heterocystous cyanobacteria are also capable for fixing nitrogen, but only under anaerobic conditions.

The acetylene reduction assay (ARA), which uses the ability of the nitrogenase enzyme to reduces acetylene gas to ethylene, is a common way to measure nitrogen fixation in BSCs. Nitrogen fixation usually increases with temperature until reaching an optimal plateau (15-30^{o}C), after which it decreases (Barger et al., 2013; Zhou et al., 2016). Therefore, cold environments inhibit nitrogenase activity explaining the low ARA values in polar BSCs (Stewart et al., 2014). Moreover, dark BSCs have a higher acetylene reduction rate than the light one (Barger et al., 2013), most probably due to the higher abundance of nitrogen-fixing organisms. In addition, BSCs dominated by microbial phototrophs have higher ARA values than lichen and moss crusts (Su et al., 2011).

1.5. Polar soil crusts

The Arctic region, part of the Northern Hemisphere, lies north of the tree line and covers a land area of approximately 7.2 x 10^6 km^2 (Tamocai et al., 2009). It is characterized by short summers with 24 hours of daylight and long, extremely cold winters. Arctic vegetation is represented by continuous cover of shrub-tundra in the south, grading to a sparse cover of dwarf shrubs, herbs, mosses and lichens in the north. The transition zone from taiga to tundra is an open landscape with patches of trees that are short and have dense thickets of shrubs that, together with the trees, totally cover the ground surface (Callaghan et al., 2005). Sub-arctic tundra covers part of Alaska, a large area of the Canadian Arctic and Western Siberia. Mosses and lichens are common vegetation in this type of environment as well as several shrubs such as *Betula nana*, *Salix pulchra*, and *S. glauca*. Further to the north, the Arctic tundra is mostly covered by mosses and lichens, while shrubs such as *Dryas*, *Salix polaris*, and *S. reticulata* are present but rarer. The High Arctic, which is characterised as a polar desert and/or semi-desert, has low vegetation cover (less than 25%) composed of vascular plants such as *Salix polaris*, *Papaver radicatum*, *Draba subcapitata*, *Saxifraga cernua* etc.

The Arctic is characterized by the presence of continuous permafrost with exceptions in some areas (e.g. Kola Peninsula) (ACIA, 2005). The development of Arctic soils is dominated by cryogenic processes, which are driven by the formation of ice in the soils. Arctic soils have a wide range of textures, including clay, silty clay, loam, sandy loam and coarse gravelly sand, with the texture depending mainly on the mode of deposition of the parent material. Arctic soils have been classified by Tedrow (1966) into: polar desert soils, such as Arctic brown soils, and wet soils such as tundra and bog-marsh soils. Soils of the polar desert and the semi-desert soils are usually saturated by water during snow melt. During summers, the soils thaw quickly and soon appear dry. The harsh Arctic conditions, together with the poor soils, are generally unfavourable to plant life. The nitrogen content of Arctic soils is typically very low and considered to be more limiting factor for plant growth than phosphorus and potassium contents (Broll et al., 1999). Plant growth is also limited by low soil temperatures, high stone content and, in some cases, high carbonate content and the occurrence of salts (Bölter et al., 2006). Moreover, wind inhibits plant growth because of the cooling and drying

effect on plants in summer or winter and also because of the mechanical disturbance of drifting sand or snow crystals (Tedrow, 1966).

Contrary to the Arctic, Antarctica is a continent surrounded by the Southern Ocean. Most of the land is covered with ice and snow and ice-free areas are present mainly on the margins of the continent (Campbell and Claridge, 2009). Very low temperatures and negligible effective precipitation severely affect soil formation throughout the continent. Antarctic soils are coarse-textured, with particles more than 2 mm in size. The soil surface is usually a stony pavement including loose material derived from the fragmentation of surface clasts. Furthermore, because the climate is extremely arid, salts accumulate in the soil surface and strongly influence soil chemistry. Antarctica is more limited than the Arctic in respect to biodiversity due to the more severe conditions and geographical isolation. There are only two indigenous vascular plants, *Deschampsia antarctica* and *Colobanthus quitensis*, although the BSC components, such as mosses, lichens, liverworts and microalgae, are more diverse (Green and Broady, 2003). Moreover, microorganisms including cyanobacteria are dominant, both in terms of biomass and species number (Hughes and Convey, 2010).

BSCs in the Polar Regions develop similarly as in hot and warm environments (Belnap and Lange, 2003). First, pioneer organisms, mainly cyanobacteria, colonize the soil surface. Then algae, moss and lichens gradually appear resulting in development of the BSC with rich nutrient concentrations.

An interesting example of soil crust development and ecological succession is succession following glacial retreat, which is very common in polar and alpine environment (Bradley et al., 2014). Retreating of glaciers expose unique terrestrial environments, which have been previously hidden under the ice for thousands of years, and provide an interesting insight of primary colonization by simple cellular life. Autotrophic microorganisms, including photoautotrophs and chemoautotrophs, are pioneering colonizers (Kaštovská et al., 2005). Early-colonizing fungi and cyanobacteria stabilize the soil and facilitate the establishment of later colonizing cyanobacteria (Bradley et al., 2014). Generally, lichens and mosses colonize later because they require physically stable surfaces (Inoue et al., 2014). Furthermore, composition of the microfloral communities can distinguish BSCs along a gradient from early-successional, cyanobacteria-dominated crusts to late-successional, lichen- and/or moss-dominated crusts (Knelman et al., 2012;

Yoshitake et al., 2011). Several studies have focused on the microbial communities after glacial retreats in the High Arctic (Kwon et al., 2015), Antarctica (Bajerski and Wagner, 2013), the Alps (Frey et al., 2013; Rime et al., 2015; Zumsteg et al., 2012) and other regions (Knelman et al., 2012; Nemergut et al., 2007; Schmidt et al., 2008). Using molecular methods, they provide a description of the whole microbial community, of which cyanobacteria, however, only make up a small fraction.

2. Objectives of the thesis

BSCs are major players in the global carbon and nitrogen biogeochemical cycles and nowadays are incorporated in climate and Earth system models (Elbert et al., 2012). However, climate change together with increasing human activity in the Polar Regions results in the invasions by non-indigenous species which is one of the greatest threats to global biodiversity. Moreover, considerable levels of endemism within the Polar Regions (especially Antarctica) means that these species are vulnerable to the introduction of species from elsewhere in the Polar Regions and/or from outside the region. Thus, it is a matter of urgency to obtain the current picture on polar biodiversity and predict its changes.

BSCs have been extensively studied in warm and hot deserts. However, the differences between cold and warm/hot regions (e.g. climate, soil chemistry, vascular plant composition and etc.) likely result in different soil crust community composition and associated ecological processes (Makhalanyane et al., 2015).

Morphological characters traditionally used in cyanobacterial taxonomy (e.g. cell division pattern, colony formation, length/width measurement of cells and presence of extracellular envelopes and sheaths) are subject to plasticity, making it often difficult or impossible to distinguish between morphologically similar taxa (Wilmotte and Golubic, 1991). Molecular techniques based on 16S rRNA gene sequences retrieved from environmental DNA, such as denaturing gradient gel electrophoresis (DGGE) and clone libraries, can be used to visualize the variations in microbial genetic diversity in the studied environment. Nevertheless, these traditional molecular approaches are time consuming, expensive and only provide information on the most abundant members of the microbial communities (Tringe and Hugenholtz, 2008).

Modern technologies, including high-throughput sequencing (HTS), have revealed a much greater diversity of lineages and functions than

previously thought (Huse et al., 2008). Therefore, in my thesis, we applied next-generation sequencing complemented by DGGE and morphological methods to study cyanobacterial community composition in BSCs of the Arctic and Antarctica.

Moreover, it is important to understand the dominant controls on ecosystem development to determine long-term productivity and understand how landscapes become colonized and productive. To achieve this task, we aimed to trace the changes in community structure and associated ecological processes following soil crust development. Even simple descriptions of species distribution and environmental biogeochemistry provide a deeper understanding of the processes which drive the spatial and temporal patterning of microbial communities, and show which factors control their growth, activity and succession.

The main aims of this thesis were:

(i) To summarize the available information about microbial phototrophs in BSCs from the High Arctic;

(ii) To characterize the community of microbial phototrophs (e.g. biovolume of dominant taxonomic groups and molecular diversity) and ecological functions such as photosynthesis rate and nitrogenase activity in Arctic BSCs at different development stages;

(iii) To describe cyanobacterial community structure along 100-year deglaciation gradients in the Arctic;

(iv) To compare cyanobacterial community composition in different nunataks and mountain ridges in the Sør Rondane Mountains, continental Antarctica.

3. Overview of the papers

The High Arctic region includes the territories of Russia, Canada, Greenland and Europe. Such a widespread area results in significant differences in soil microbial communities. In the review paper (Paper I), we assembled information from the available literature about the community structure of microbial phototrophs and the associated ecological processes in Arctic BSCs. Moreover, we summarized the species of cyanobacteria and eukaryotic microalgae that have been reported so far in soil crusts from the High Arctic. Given the lack of knowledge about microbial phototrophs in Arctic soil crusts, in Paper II we described the diversity of cyanobacteria and eukaryotic microalgae within BSCs from Petunia Bay, Svalbard using light microscopy.

Moreover, based on the diversity of phototrophic microorganisms and their chlorophyll fluorescence signals, we classified the studied BSCs into three types: black-brown, brown and grey-brown. In addition, we found that altitude did not affect the diversity of the microbial phototrophs, however their abundance increased with altitude.

Paper III describes an in-depth study of cyanobacterial community composition in the BSCs from Central Svalbard, at different developmental stages. We applied HTS tool such as pyrosequencing 454 to obtain a detailed assessment about cyanobacterial diversity. To the best of our knowledge, this was the first detailed assessment of cyanobacterial community composition in BSCs of the High Arctic based on next-generation sequencing. Moreover, we showed the impact of soil chemistry on cyanobacterial diversity in the studied soil crusts.

Paper IV was a continuation of Paper III where we provided the ecological aspects of the studied BSCs. Using different techniques, we estimated the biovolume of the main taxonomic groups of microbial phototrophs and associated ecophysiological processes (photosynthetic and nitrogenase activities). Moreover, we studied how soil temperature and photosynthetically active radiation (PAR) influence photosynthetic activity. Eventually, the results obtained in Papers III and IV revealed the gradient from poorly-developed to well-developed soil crusts. Moreover, we observed that lichen cover might inhibit the diversity and abundance of microbial phototrophs and, hence, photosynthetic and nitrogenase activities. Nevertheless, eukaryotic microalgae were studied only superficially due to difficulties in detecting them in the coarse soil texture.

Similarly, in Paper V we focused on the cyanobacterial succession of BSCs along a 100-year deglaciation gradient in three glacier forefields in central Svalbard. The sampled transects represented typical chronosequences, composed of BSCs with overall low nutrient contents, but that nevertheless displayed a significant cumulative trend with increasing time since deglaciation. Using both molecular tools and epifluorescence microscopy, we showed how cyanobacterial community composition changes over time and space in such an environment.

In Paper VI, we focused on the BSCs of two nunataks and two ridges in the Sør Rondane Mountains (Dronning Maud Land, East Antarctica). The diversity and abundance of BSC cyanobacteria were studied using epifluorescence microscopy and molecular tools including DGGE and pyrosequencing 454 of the partial 16S rRNA gene. Moreover, the sampling sites serve as control areas for open top chambers (OTCs) which

were placed in 2010 after sample collection. Hence, the present assessment constitutes the baseline data for later comparisons with samples collected from inside the OTCs, which will provide insights on the effects of climate change on Antarctic soil cyanobacteria. In fact, the OTCs were developed as a field manipulation of climate warming as part of the international tundra experiment (ITEX) (Henry and Molau, 1997). Such a manipulative experiment allows a wider range of scientific questions to be answered with appropriate quantitative control of temperature treatments.

4. Results and discussions

The results of this thesis demonstrated the importance of soil crust ecosystems in the polar environment. In agreement with previous studies (Ganzert et al., 2014; Li et al., 2010), we found that pH, total organic carbon and water content were the key parameters shaping BSC communities. Microbial phototrophs are the dominant photosynthetic organisms in the polar soils and play a major role in the carbon and nitrogen cycles (Elster, 2002). Filamentous cyanobacteria (*Leptolyngbya* spp. and *Microcoleus* spp.) were detected by next-generation sequencing as the dominant phototrophs in both Arctic and Antarctic soil crusts. These cyanobacteria are common in soil crusts around the world (Kaštovská et al., 2005; Strunecký et al., 2012; Williams and Eldridge, 2011; Yeager et al., 2004) and, due to mucilage production and motility, are able to live in coarse and unstable soils (Hu et al., 2012). However, their biovolume, as measured by epifluorescence and light microscopy, was lower than the biovolume of unicellular cyanobacteria, a finding which might be due to the different measurement techniques used. The epifluorescence microscopy method breaks up cyanobacterial colonies into separate cells and, therefore, unicellular forms were the most abundant.

Heterocystous cyanobacteria from the order Nostocales were also an abundant group in all studied soil crusts when using both methods. These cyanobacteria are the major source of fixed nitrogen in BSCs and are present around the world (Bastida et al., 2014; Řeháková et al., 2011; Yeager et al., 2004; Zielke et al., 2005). Interestingly, the abundance of Nostocales in the studied soil crusts increased with soil crust development while nitrogenase activity decreased. We assume that other free-living bacteria/cyanobacteria could be responsible for nitrogen fixation.

The community structure of microbial phototrophs at Petunia Bay, Central Svalbard changed according to the development stage of BSCs (Fig.1). Cyanobacterial biovolume and phylotype richness increased with soil crust development until lichens covered the soil surface. Likewise, cyanobacterial biovolume also increased along the deglaciation gradient in BSCs after glacial retreat, being higher in later successional soil crusts. However, the opposite trend was recorded for cyanobacterial richness which decreased with time of deglaciation, presumably, due to competitive exclusion associated with increased cyanobacterial abundance.

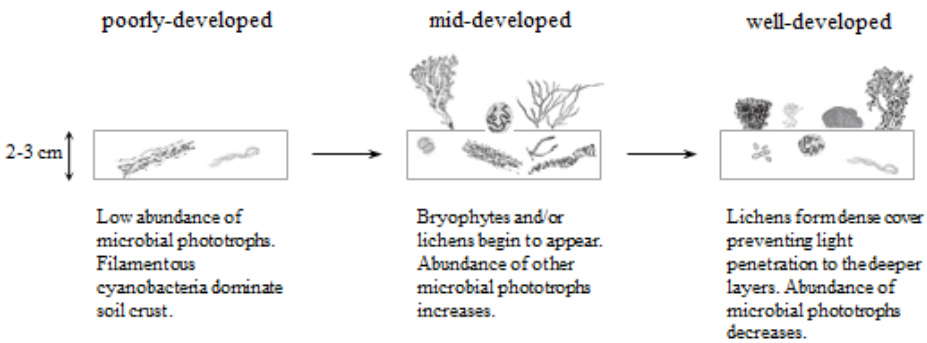

Figure 1. Soil crust development in Petunia Bay, Central Svalbard.

Photosynthetic activity also followed the gradient of soil crust development in Svalbard. In fact, soil temperature was the main factor influencing photosynthetic activity while the effect of PAR was not significant. Indeed, it has been previously shown that temperature is an important parameter for soil microbiota (Rinnan et al., 2009; Yergeau et al., 2012). Higher temperatures led to inhibition of photosynthetic activity and increased energy dissipation, indicating acclimation/adaptation of the BSC photosynthetic microorganisms to the cold Arctic environment.

An interesting finding was the negative influence of lichen cover on the abundance and activity of microbial phototrophs in well-developed soil crusts. The biovolume of microbial phototrophs and richness of cyanobacterial sequences were lower in well-developed lichenized soil crusts than in soil crusts with no or little presence of lichens. Moreover, nitrogenase activity was not detected in soil crusts covered by lichens. We assume that dense lichen cover might inhibit light penetration to the deeper soil layers which is needed for the growth and activity of microbial phototrophs. In addition, lichens can produce chemical substances which

can limit the growth and development of microbial phototrophs in BSCs. However, more studies are needed to prove or refute such an outcome.

5. Conclusions and future perspectives

It is clear that nowadays climate changes rapidly and Polar Regions suffer from it the most. With increasing interest in soil crust ecosystems, it is becoming more feasible to characterize soil crust development in relation to microbial community establishment and nutrient cycling. However, there is still a gap in knowledge about BSC community structure in the Polar Regions. For instance, we found only six publications describing microalgal community structure in Arctic soil crusts. Moreover, the majority of these studies applied only morphological tools to determine species composition.

Using HTS technology in polar environments is particularly challenging because of the relatively low concentrations of microbial biomass, resulting in low recoveries of quality genomic DNA, particularly in young soils. Unfortunately, the HTS technique covers only a small portion of the genes and therefore it gives only an overview about community structure. Hence, it is important that molecular tools should be combined with morphological techniques to obtain a more accurate insight into the soil crust community in the Polar Regions. Moreover, it would also be interesting to know which particular species are responsible for photosynthetic and nitrogenase activities in polar soil crusts at different development stages in respect to the diurnal cycles and seasonality.

BSCs are able to fuel food webs through photosynthesis and nitrogen fixation producing carbon- and nitrogen containing organic compounds, which is particularly important in the Polar Regions and other terrestrial environments with low organic nutrient contents. Nevertheless, while intact BSCs are highly resistant to disturbances such as wind and water erosion, they are particularly vulnerable to mechanical disturbance (e.g. livestock grazing, human intervention, etc.). Once damaged, BSCs are unable to provide key ecosystem services such as resistance to erosion or nitrogen fixation. Moreover, recovery times following disturbance are variable and may require several years. With the known impacts of human disturbance on BSCs, it is difficult to determine the influence of climate change, especially given the uncertainties in the projections. Therefore, it is important to focus on BSC protection to minimize the negative effect of climate change.

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7. Original papers

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REVIEW

A review of the ecology, ecophysiology and biodiversity of microalgae in Arctic soil crusts

Ekaterina Pushkareva[1], Jeffrey R. Johansen[3], Josef Elster[1,2]

[1]Centre for Polar Ecology, University of South Bohemia, České Budějovice, Czech Republic
[2]Institute of Botany, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic
[3]Department of Biology, John Carroll University, USA

Abstract

Biological soil crusts have been extensively studied in arid lands of temperate regions, particularly semi-arid steppes and warm deserts. Arctic soil crusts have received some attention, but they are far less studied than their temperate counterparts. While the tundra zone of Arctic regions has an abundant cover of lichens, mosses and low-growing vascular plants, the High Arctic semi-arid and arid deserts have a much reduced but still very significant cover of biological soil crust dominated by microalgae. This review discusses what is known about Arctic soil crusts with the intention of stimulating study of this sensitive ecosystem. Arctic soil crusts are considered to be one of the most extreme habitat types on earth. Low temperatures and lack of water associated with a wide spectrum of disturbances have a dramatic effect on chemical and physical soil ecological properties (salinity, pH, conductivity and gas content). Microalgae are the keystone microbial species in polar crusts, being significant primary producers, fixing atmospheric nitrogen and secreting polysaccharides that bind soil aggregates together, thereby reducing erosion and water runoff. The biological diversity of soil crust microalgae in the Arctic is high. Soil crusts of the Arctic semi-arid and arid deserts provide a special opportunity to study the environmental factors controlling the diversity, distribution and abundance of the microalgae in the absence of anthropogenic disturbance. However, anthropogenic disturbances and climate change are occurring in the Arctic, and even more transformations are expected in the near future. Therefore, the

ecological study of Arctic ecosystems, including biological soil crusts, is a matter of urgency.

Key words: soil crust, Arctic, cyanobacteria and eukaryotic microalgae

Introduction

Biological soil crusts of arid and semi-arid regions have been studied over the past 35 years. Deserts and semi-arid steppes of temperate regions have received the most attention, where soil crusts have been found to have several key environmental roles, including soil stabilization and the subsequent protection from erosion by wind and water, nitrogen fixation, contribution to soil organic matter, influence on hydrology and infiltration of rain water, increasing soil temperature and affecting the mineral nutrient supply to vascular plants. Extensive work has also been conducted on the disturbance ecology of crusts, including both the impact of various types of disturbance and the recovery or succession following the cessation of the disturbance. Researchers have also been interested in the diversity of all major crust constituents (lichens, mosses, cyanobacteria, eukaryotic microalgae, fungi and heterotrophic prokaryotes). The literature dealing with crusts was reviewed in detail by Belnap and Lange (2001).

Our understanding of the biological soil crusts of polar desert and polar semi-desert regions is far less expansive. Green and Broady (2001) provided a review of the biological soil crusts of Antarctica, but at the time of this review much less was known about the ecosystem function of polar crusts. There has been considerable progress in expanding our understanding of polar biological soil crusts since these seminal reviews, for example, the recent review of soil crusts in Antarctica (Büdel and Colesie 2014). Recent reviews in Arctic terrestrial ecosystems are limited to Stewart et al. (2014) where the authors focused on atmospheric nitrogen exchanges. In addition, the recent article (Pointing et al. 2015) provides information about the biogeography of photoautotrophs in the Arctic and Antarctica.

The purpose of this article is to provide a current review of the biological soil crusts of Arctic semi-arid and arid deserts with a focus on the microalgal community. Here, we use the term ‘‘microalgae’’ to combine both prokaryotic cyanobacteria and eukaryotic microalgae.

Extent and components of Arctic soil crusts

Soil crusts of the Northern Hemisphere are mainly represented in the High Arctic (Fig. 1) and cover the territories of the European, Russian and Canadian Arctic as well as Greenland.

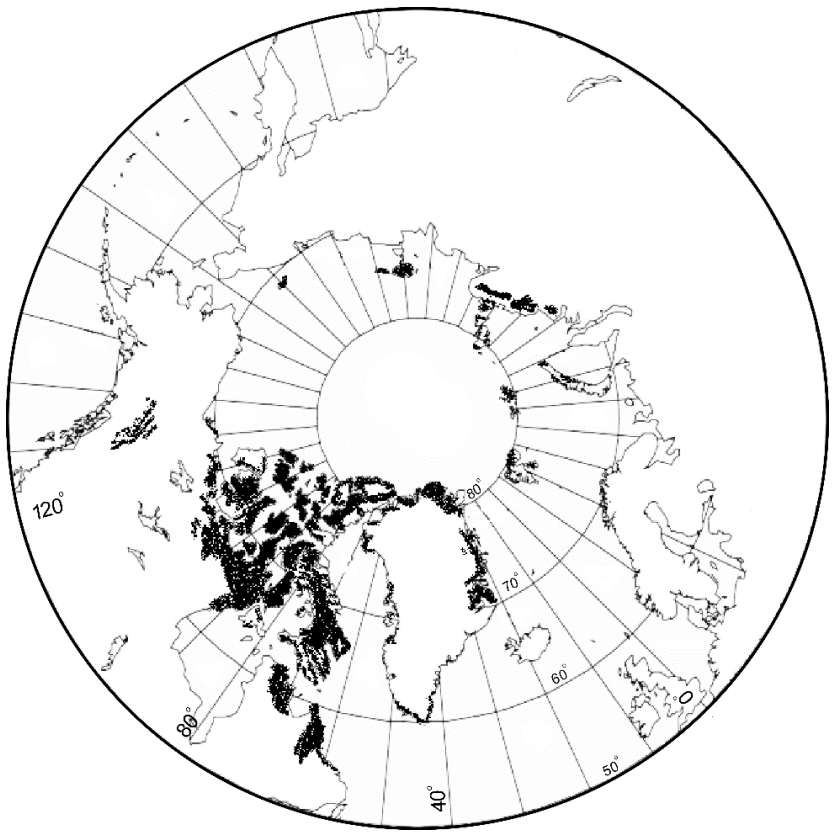

Fig. 1. Map of the High Arctic. Polar semi-desert and desert are colored *black* and represent areas where biological soil crust development is most widespread. In particular geographical areas more than 65°N, high elevation (more than 400–1000 m a.s.l.), lack of precipitation (less than 250 mm), mean air temperature lower than -10 to -15 °C, soil temperature regime (hypergolic) and continentality (eucontinental, hypercontinental), the polar desert and semi-desert with biological soil prevail (see more in the Soil Atlas of the Northern Circumpolar Region, Jones et al. 2009). The highest area with a biological soil crust is located in the Canadian High Arctic (southern part of Nunavut and Canadian Arctic Achipelago). A lesser extent of soil crust is also located in the northeast sea shore of Greenland, northern part of Svalbard, Franz Josef Land, Severnaya Zemlya Archipelago, and higher altitude in Taymyr Peninsula and New Siberian Islands. The southern limit is located in the southeast part of North America (Newfoundland and Labrador, Baffin Island, north part of Hudson Bay).

As shown in Fig. 1, the Arctic desert and semi-desert biome covers large parts of the High Arctic. However, in respect to area, the Canadian Arctic Archipelago and northeast part of the America contain most of this biome. The Canadian Arctic archipelago and neighbouring coastal areas of Greenland constitute an immense geographical region dominated by polar deserts and semi-deserts (Bliss et al. 1984). The area differs in altitude, substratum and other ecological characteristics. In the spring, polar desert soils are supplied by a surge of meltwater, usually followed by longer periods of drought during summer (Elster 2002; Elster and Benson 2004). Thus, the microalgal communities are subject to seasonal extremes of inundation and desiccation, hypo- and hypersalinity, seasonal and diurnal temperature fluctuations, frequent freeze-thaw cycles and, ultimately, deep-freeze temperatures up to -34ºC in winter (Laska et al. 2012).

Even though polar desert occupies 15–20 % of the surface area of the Arctic, only a limited number of studies deal with the Arctic biological soil crust ecosystem (Liengen 1999; Elster et al. 1999; Kaštovská et al. 2005, 2007; Patova and Beljakova 2006; Breen and Levesque 2006, 2008; Yoshitake et al. 2007, 2010, 2014; Andreyeva 2009; Pushkareva and Elster 2013; Steven et al. 2013; Inoue et al. 2014; Pushkareva et al. 2015; Shi et al. 2015). Most of the studies describing microalgal communities in Arctic soil crusts are based on morphology (Elster et al. 1999; Kaštovská et al. 2005, 2007; Patova and Beljakova 2006; Andreyeva 2009; Pushkareva and Elster 2013). A few studies have applied modern molecular methods for microalgal identification, but most of these studies present data for the whole microbial community without a detailed assessment of microalgae (Schutte et al. 2010; Knelman et al. 2012; Steven et al. 2013; Pushkareva et al. 2015). In Table 1, we summarize the microalgal species that have been found in Arctic soil crusts. Greenland is excluded because of the lack of information on crusts of this large and important island.

The dominant components in the soil cyanobacterial community are filamentous forms (Table 1). Representatives from the orders Chroococcales, Pseudanabaenales, Oscillatoriales and Nostocales are the most species-diverse groups found in Arctic soil crusts (Kaštovská et al. 2005, 2007; Patova and Beljakova 2006; Pushkareva and Elster 2013; Pushkareva et al. 2015). *Nostoc* spp. are widely dispersed in all types of soil crusts and primarily inhabit the soil surface (Yeager et al. 2004; Řeháková et al. 2011; Hu et al. 2012; Bastida et al. 2014). Arctic habitats with high humidity, such as areas around streams, pools, lakes, waterfalls

and snow fields, have soils frequently covered by a thick layer of *Nostoc* spp. (Pushkareva and Elster 2013). A high abundance of *Nostoc* spp. might also be explained by decreased nitrogen availability in the soil crusts (Zielke et al. 2005; Stewart et al. 2012). Notably absent from Arctic soils are the genera *Oculatella* and *Hassallia*, which are common in desert soil crusts from temperate and tropical regions (Flechtner et al. 2008; Patzelt et al. 2014; Osorio-Santos et al. 2014).

Table 1. Microalgal genera reported in biological soil crusts, based on seven studies of these communities in the Arctic

	Russian Arctic (Bolshevik Island, Alexandra Land)[1,2]	**European Arctic** (Svalbard)[1,3,4,5]	**Canadian Arctic** (Ellesmere Island, Ellef Ringens Island)[1,6]
CYANOBACTERIA			
Chroococcales			
Aphanocapsa	+		
Aphanothece	+		
Chlorogloea		+	
Chroococcus	+	+	+
Cyanosarcina			+
Gloeocapsa	+	+	+
Gloeocapsopsis	+		
Gloeothece	+		
Rhabdoderma	+		
Synechococcus			+
Synechocystis	+		
Pseudanabaenales			
Geitlerinema		+	
Komvophoron		+	
Leptolyngbya	+	+	+
Phormidesmis		+	
Pseudanabaena		+	
Schizothrix			+
Oscillatoriales			
Microcoleus		+	+
Oscillatoria	+		

Phormidium	+	+	+
Symploca	+		
Symplocastrum	+		
Nostocales			
Anabaena	+	+	
Calothrix	+	+	
Cylindrospermum	+		
Dichothrix	+		
Nodularia		+	
Nostoc	+	+	+
Scytonema	+	+	
Stigonema	+		
Tolypothrix	+	+	
Trichormus		+	
STRAMENOPILA			
Xanthophyceae			
Botrydiopsis			+
Heterococcus			+
Monodopsis			+
Xanthonema			+
CHLOROPHYTA			
Chlorophyceae			
Actinochloris			+
Ascochloris			+
Asterococcus			+
Bracteacoccus	+	+	+
Borodinellopsis			+
Chlamydocapsa	+		+
Chlamydopodium	+		+
Chlamydomonas			+
Chlorolobion			+
Chlorococcum	+	+	+
Chloromonas			+
Chlorosarcina			+
Chlorosarcinopsis		+	+

Chlorosphaeropsis			+
Coccobotrys			+
Coleochlamys		+	
Deasonia			+
Dictyochloris			+
Dictyococcus			+
Diplosphaera			+
Ettlia			+
Gloeococcus		+	+
Gloeocystis		+	+
Halochlorella	+	+	+
Hormotila		+	
Hormotilopsis			+
Macrochloris	+		+
Monoraphidium			+
Mychonastes	+	+	+
Nautococcus			+
Neochloris			+
Neochlorosarcina		+	+
Neospongiococcum			+
Palmellopsis	+		+
Planktosphaeria			+
Pleurastrum			+
Radiosphaera	+		+
Scotiellopsis	+		+
Spongiochloris	+		+
Tetracystis	+	+	+
Trebouxiophyceae			
Chlorella	+	+	+
Choricystis		+	
Coccomyxa	+	+	
Dictyochloropsis			+
Dictyosphaerium			+
Elliptochloris			+
Keratococcus	+		+

Leptosira			+
Muriella	+	+	+
Muriellopsis	+		+
Myrmecia	+	+	+
Parietochloris	+		
Pseudococcomyxa	+	+	+
Schizochlamydella			+
Stichococcus		+	+
Trebouxia		+	+
STREPTOPHYTA			
Klebsormidiophyceae			
Klebsormidium		+	+
Zygnemophyceae			
Actinotaenium			+
Cosmarium			+
Cylindrocystis		+	+
Mesotaenium			+

[1]Andreyeva 2009; [2]Patova and Beljakova 2006; [3]Kaštovská et al. 2005, 2007; [4]Pushkareva and Elster 2013; [5]Pushkareva et al. 2015; [6]Elster et al. 1999.

The extreme conditions of polar regions strongly affect the diversity of eukaryotic microalgae. Coccoid green algae (Chlorophyta) are the most abundant eukaryotic group in Arctic soil crusts (Lange 2001; Andreyeva 2009; Pushkareva and Elster 2013; see Table 1) represented by Chlorophyceae and Trebouxiophyceae (Elster et al. 1999; Andreyeva 2009). Some soils are wet and acidic and support sacoderm desmids as well (see Table 1, Zygnematophyceae). In addition to the coccoid taxa, filamentous taxa can be important as well (e.g., *Klebsormidium*, *Xanthonema* and *Tribonema*; see Elster 2002). Representatives of the stramenopile (heterokont) class Xanthophyceae are also abundant in polarregions (Table 1).

In the Russian Arctic the soil crust ecosystem is well described in tundra zones. However, accessibility to this literature is difficult because most of the papers are in Russian language and consequently the findings of Russian scientists are poorly known. The most important representatives of mosses in the Russian Arctic are *Rhacomitrium lanuginosum*,

Ditrichum flexicaule and *Dicranoweisia crispula* (Matveeva 1979; Afonina and Matveyeva 2003). The lichen flora is dominated by the genus *Cetraria* (*C. delisei*, *C. islandica* var. *polaris*, *C. laevigata*, *C. elenkinii*) (Matveeva 1979; Zhurbenko and Matveyeva 2006). To the best of our knowledge, cyanobacterial species composition of soil crusts in the Russian Arctic was described only on Bolshevik Island, Severnaya Zemlya Archipilago (Patova and Beljakova 2006; see Table 1).

Andreyeva (2009) identified nine species of Chlorophyta from Alexandra Land (Franz Josef Land) and 22 species from Bolshevik Island (Table 1). Common genera for both regions are *Chlamydopodium* sp. and *Chlorococcum* sp.

Ecological factors affecting the polar soil crust ecosystem

Soil crust communities can occur in almost all types of soil, and many biotic and abiotic parameters influence their development (soil geological properties, climate, presence of vascular plants, and animal and human intervention) (Langhans et al. 2009; Fischer and Subbotina 2014; Huang et al. 2014; Pushkareva et al. 2015).

Excellent examples of Arctic semi-desert soil crust can be found in the northern part of Petunia Bay, Billefjorden, Central Svalbard (78°39’22”–78°44’36” N latitude, 16°22’12”–16°49’27” E longitude) (Fig. 2a, b). The highest ground cover is produced by *Dryas octopetala* and the lowest by the *Papaver dahlianum* (Prach et al. 2012). In semi-desert crusts of this area the soil surface can be consolidated by microalgae alone (Fig. 2d, f), a mixture of lichens and cyanobacteria (Fig. 2c, e), or in especially well-established crusts by a rich community of micro- or even macro-lichens (Fig. 2g, h). Such types of crusts are typical in many High Arctic areas (Belnap et al. 2001; Stewart et al. 2011b; Pushkareva et al. 2015).

In more humid habitats, the freeze-thaw cycles, occurring in soils, produce pinnacled and rolling crusts where mosses dominate (Belnap and Lange 2001; Belnap 2008). Water saturation occurs only for a short period of time during snow melt in dry, unstable soils, most frequently in steep slopes at higher elevation. Here, for most of the summer season, water is available only as water vapour from more frequent cloud occurrence. The crusts in these unstable dry soils are dominated by free-living microalgae, which usually become lichenized in less disturbed, more stable habitats (Colesie et al. 2014a). The biological crusts in temperate deserts decrease water evaporation from soil (Xiao et al. 2010; Lichner et al. 2013). This

may be true for polar crusts as well, particularly those that are light-coloured and do not attain elevated temperatures because of higher albedo (Fig. 3). In polar regions, the melting of snow and ice during the spring and summer periods increases the availability of liquid water (Laska et al. 2011); thus, the highest microalgal biomass presence in the Arctic is usually at the time of summer snowmelt (Elster et al. 1999).

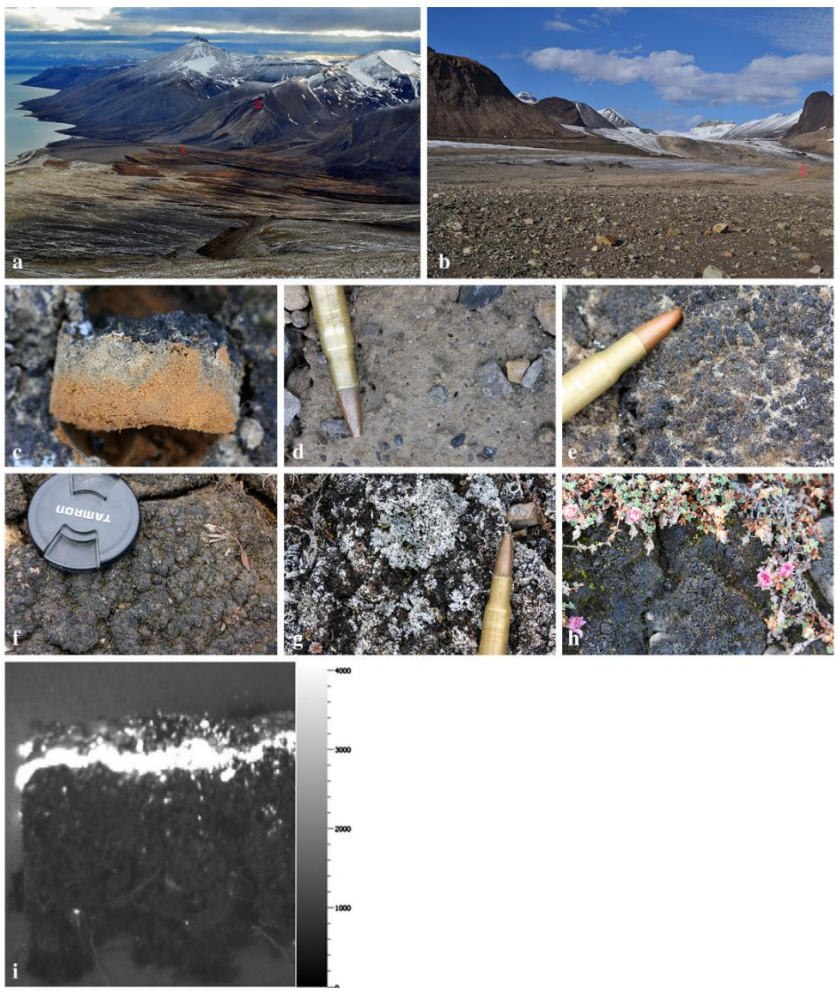

Fig. 2. Northern part of Petunia Bay, Billefjorden, Central Svalbard, Arctic semi-desert soil crust (a). Close-up images (d, e, f, g, h) show development of a soil crust community from quite young bare habitat in front of the Horbye Glacier (b, d) up to more developed (e, f) and partially covered by vascular plants and lichenized soil crust types (g, h). Arrows in images a and b show the top of Mummien Peak (1), araised marine terrace that is characteristic of the early Holocene shoreline of Svalbard (2) and front of the Horbye Glacier (3). Crosssections of a soil crust and its surface (c, i) show algal presence and photosynthetic activity in surface layers. An image (i) taken with the FluorCam 700MF fluorescence imaging camera (Photon Systems Instruments, Czech Republic) has a distinct whitish layer demonstrating the photosynthetically active area of soil crust. The vertical scale demonstrates relative units of imaging fluorometry.

Svalbard is a representative example of the yearly course of soil surface temperature and volumetric water content at a depth of about 2–3 cm below the surface of the soil (Fig. 3). In the Arctic semi-desert (northern part of Petunia Bay, Billefjorden, Central Svalbard) most of the year is both too cold and too dry for the growth of microalgae (October through June, Fig. 3), but both the temperature and moisture at the site shown are quite amenable to the growth of microalgae, lichens and mosses from July through September, with moisture likely being the more limiting factor (Fig. 3).

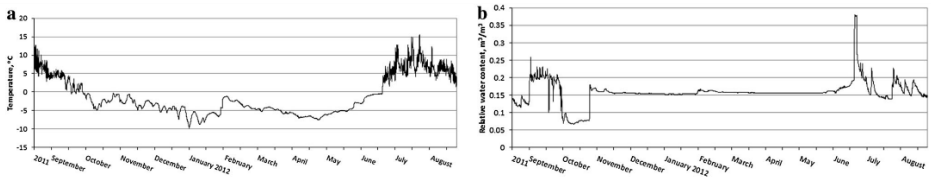

Fig. 3. Soil temperature (a) and volumetric water content (b) at a depth of 2 cm below the surface from the Herbye glacier foreland (northern part of Petunia Bay, Billefjorden, Central Svalbard) in the period from August 2011 to August 2012.

Soil texture greatly influences biological crust communities in polar regions (Colacevich et al. 2009). In contrast to deep soil, the soil crust has a greater proportion of silt and clay on or just below the surface (Breen and Levesque 2008). Thin clay particles adhere to the mucilaginous sheath of microalgae in the soil crust. These particles have a negative charge, which allows them to bind plant micronutrients, which have a positive charge. This process increases soil fertility (Belnap and Lange 2001; Breen and Levesque 2008). A more stable and softer soil texture such as gypsum and silty loams increases the diversity of microalgae, lichens and mosses (Belnap et al. 2001). The presence of unstable and coarse soils negatively influences the abundance and diversity of the structural organisms of soil crusts in the Arctic (Kaštovská et al. 2005). However, filamentous cyanobacteria can be quite abundant in the coarse type of soil crust (Pushkareva et al. 2015). Well-developed soil crusts with a high density of soil crust organisms, mainly microalgae, lichens and mosses, are usually dark in color (Yoshitake et al. 2007), but sometimesthe surface can be paler because of certain lichen species, for example, *Ochrolechia frigida* (Inoue et al. 2014). In dark-colored well-developed biological crusts, there are more available mineral nutrients (such as phosphorus and/or nitrogen) and organic carbon (Chae et al. 2016; Pushkareva et al. 2015). In contrast, a poorly-developed light-colored soil surface is

associated with a low biomass of major soil crust organisms followed by lower photosynthetic activity and lower pigment content (Chae et al. 2016; Pushkareva et al. 2015).

Dark-coloured soil crusts better absorb long-wave radiation, which may increase the temperature of the soil surface (Stradling et al. 2002). Dark soils usually retain warmth for a longer time than light-coloured soils and can positively influence the growth of biological soil crusts and vascular plants. Nevertheless, UV radiation can negatively affect Arctic soil crust organisms (Solheim et al. 2006). Microalgae can enter into a resting stage under such stressful conditions (Chen et al. 2003; Tashyreva and Elster 2012, 2015; Pichrtova et al. 2014). Motile cyanobacteria, such as *Microcoleus vaginatus*, *M. steenstrupii* and other filamentous cyanobacteria, typically occupy a position from 1 to 5 mm below the soil surface and thus can achieve a balance between receiving sufficient light for photosynthesis (Castenholz and Garcia-Pichel 2000; Norris and Castenholz 2006). Non-motile cyanobacteria, such as the heterocytous genera *Scytonema*, produce dark yellowish pigmentation in their sheaths, which acts as a UV screen and darkens the soil surface associated with biological soil crusts (George et al. 2001).

Soil pH is a very important factor for the growth and diversity of microalgae. Soil crusts in temperate and tropical regions with a low pH (<7.0) are usually dominated by green algae (Johansen et al. 1993; Hastings et al. 2014), while the pH optimum for cyanobacteria is between 7.4 and 8.0 (Burja et al. 2002). Lower pH was found to promote higher microalgal abundance in Arctic soil crusts (Kaštovská et al. 2007). However, an increase of pH results in dominance of filamentous cyanobacteria (Pushkareva et al. 2015).

Organisms living on the soil surface are more easily exposed to and affected by wind compared to organisms inhabiting other parts of the ecosystem (Jia et al. 2012). Because of the sparse vegetation in polar regions, the soil crust organisms lack protection from the wind. Soil movements generated by freeze-thaw processes limit development of vascular plants, but likely are less limiting to microbial components of biological soil crusts (Jia et al. 2012). However, the responses of polar desert and/or semidesert biological soil crust to wind are still largely unexplored.

Adaptation of soil crust microalgae to polar conditions

In polar soil crust ecosystems, microalgae have developed a wide range of adaptive strategies that allow them to avoid, or at least minimize, the injurious effects of extreme and fluctuating environmental conditions (Elster 2002). Soil microalgae are poikilohydric microorganisms (having no mechanism to prevent desiccation), but are extremely tolerant to drought and are able to quickly return to an active state upon rehydration. They can survive long periods of desiccation and strong fluctuations in temperature (Moquin et al. 2012; Pichrtova et al. 2014; Tashyreva and Elster 2015) and have very different life strategies with respect to their susceptibility to low temperatures and freezing. This could be because cyanobacteria and several microalgae (mainly terrestrial Chlorophyceae) do not contain vacuoles, which in many eukaryotic microalgae and plants are the cellular component responsible for water control. For example, in the marine polar ecosystem vacuoles have been recorded in microalgae quite frequently (Kirst 1990; Kirst and Wiencke 1995), while there is scarce information about the presence of vacuoles in polar soil microalgae. The external environment directly manages the metabolic activity of poikilohydric organisms by affecting the presence or absence of water in either liquid or vapour form. Cyanobacteria are particularly well adapted to the severe and changeable conditions involving cycles of desiccation, rehydration, low to high salinity and freeze-thaw episodes. High light intensity during freezing and desiccation has been shown to negatively influence the survival of *Leptolyngbya* spp. (George et al. 2001). In contrast, *Scytonema* spp. usually live on the surface of biological soil crusts, requiring the production of the UV-screen sheath pigment scytonemin (Quesada et al. 1999). However, the biomass of *Scytonema* sp. can decrease where mosses occur and compete for light (Lan et al. 2012).

Microalgae are the most important photosynthetic organisms of soil crusts in the polar regions (Elster 2002). However, they often have a low concentration of photosynthetic pigments per unit area (Elster et al. 1999) as a consequence of the harsh climatic and environmental conditions (Colacevich et al. 2009). Figure 2c, i shows photosynthetic organisms (mainly microalgae) living on the soil surface or slightly below it. Because of structural and functional differences in photosynthetic organelles, eukaryotic microalgae have higher rates of photosynthesis and lower resistance to freeze-thaw cycles than cyanobacteria (Šabacká and Elster 2006; Pichrtova et al. 2014). A good example of structural changes in cell

morphology that provide resistance to freeze-thaw injuries was documented in a study of the annual development of mat-forming conjugating green algae, *Zygnema* sp. (Pichrtova et al., in press), showing that in summer *Zygnema* sp. cells contain stellate chloroplasts and large hyaline vacuoles. As summer progressed, *Zygnema* sp. produced pre-akinetes, overwintering cells, with high lipid content and reduced chloroplast lobes. Such cells have been found to be able to survive winter conditions and other extremes. However, features responsible for resistance against low temperature, cryoinjuries, desiccation and salinity stress are taxonomically specific at the species and ecoform levels (Elster and Benson 2004). Cyanobacteria, in contrast to eukaryotic microalgae, have lower rates of photosynthesis, but their biomass production is higher than their subsequent decomposition. Accumulation of cyanobacterial biomass in Arctic soil crust habitats is, in addition to the low rate of decomposition, influenced by low grazing pressure by invertebrates. However, these processes are still poorly understood, and accumulation of cyanobacterial biomass in particular polar habitats needs further research. For example, soil crusts dominated by *Nostoc* spp. rapidly rebuild (during several hours or days) after freezing and desiccation (Hawes et al. 1992). Extracellular polysaccharides in the sheath and colonial investments of cyanobacteria often persist in the environment and can serve to aggregate soil particles and stabilize the soil surface even after the cellular components of the soil have died (Elster and Benson 2004; Tashyreva and Elster 2015).

Soil crust microalgae, due to a diverse range of ecological and physiological life strategies, manifest an ability to tolerate stressful conditions. There are three main strategies for survival in polar soil crust habitats: avoidance of stress, protection from stress and forming partnerships with other organisms. Water availability and state (liquid-ice-vapour) connected with all types of mechanical disturbances or instability are decisive factors that determine whether avoidance, protection or formation of a partnership prevails. In conditions where there is a more regular occurrence of water in the liquid form, avoidance or protection is more common. In contrast, protection or forming of partnerships is quite common in habitats where water in liquid form occurs only for a limited time or where water is primarily available only in vapour form (Elster 2002).

Some soil crust microalgae are motile in vegetative and/or reproductive cell stages. Mobility can facilitate avoidance of the most stressful

conditions and propel the organism to a more favourable environment. Motile microalgae can also react to a wide spectrum of environmental conditions (soil properties, temperature, moisture). The mucilage protects them against fluctuations in water status by inhibiting water loss from cells. When soil is wet, the mucilage of cyanobacteria swells and trichomes migrate out of their sheaths (Hu et al. 2012). After each migration, new sheath material is formed, thus extending the filament length. Repeated swelling leaves a complex network of empty sheath material, which maintains the soil structure after the organisms have become dehydrated and decreased in size.

Other strategies for survival in severe conditions are the development of resting (dormant) vegetative stages as well as reproductive stages that affect an organism's ability to adapt to seasonal environmental fluctuations (Tashyreva and Elster 2012, 2015). We have already mentioned production of pre-akinetes in Arctic populations of *Zygnema* sp. (Pichrtova et al. 2014). In contrast, *Phormidium* sp. (Oscillatoriales, Cyanobacteria) forms perennial populations, with a high proportion of cells able to survive winter without specialized cells being produced (Tashyreva and Elster 2016). This is a very important adaptive feature for living in polar conditions. Physiological changes preceding dormancy include accumulating high concentrations of soluble carbohydrates that substitute for water molecules during dehydration (Elster and Benson 2004). These intracellular carbohydrates stabilize the structure and function of macromolecules, membranes and cellular organization. The presence of protective sugars in cells enables vitrification of cytoplasm on drying and supports the formation of a high-viscosity, metastable glassy state. This preserves cell viability during dry frozen storage by immobilizing cellular constituents and suppressing deleterious chemical or biochemical reactions that threaten survival (Sun and Leopold 1994).

An important feature of soil crust communities in polar regions is the production of life-form associations such as mutualistic symbiosis, which offer protection against unstable extreme conditions on the soil surface layer. They are traditionally defined as the living together of two or more unlike organisms to the benefit of all partner organisms. The life associations that commonly occur in the polar terrestrial environment, with the exception of physical protection, also have physiological and metabolic advantages.

The most frequent and most ecologically important association in the polar terrestrial environment is the association of microalgae with fungi in

lichens (Bjerke 2011; Inoue et al. 2011, 2014). Fungal hyphae are frequent components of the cryptogamic crust. Through their filamentous hyphae, fungi in crusts contribute to soil stability by aggregating soil particles (Abed et al. 2012). This symbiosis helps to protect them from low temperatures and water fluctuations (Stewart et al. 2011b). Lichens that use green algae as photobionts are usually dominant in polar soil crusts. Lichens that have the green alga *Trebouxia* sp. as the photobiont are known to be extremely cold-resistant in the dry as well as hydrated states (Leal 2000), and several lichen species are known to be able to attain significant net photosynthetic rates at subzero temperatures.

Another important and common example of association in Arctic soil crusts is epiphytic occurrence of cyanobacteria on moss surfaces (Stewart et al. 2011b). Probably, the benefit for cyanobacteria in this kind of association may be the supply of carbohydrates and protection against desiccation and UV radiation while mosses can get nitrogen fixed from the cyanobacteria (Zielke et al. 2005).

Ecological role of microalgae in soil crusts

Soil crust organisms are involved in important processes of polar soil ecosystems such as nitrogen fixation, moisture retention, stabilizing of soil and increasing of soil organic carbon (Breen and Levesque 2008; Stewart et al. 2012, 2014; Büdel and Colesie 2014; Chae et al. 2016). Stabilization of the soil surface by soil crust organisms helps to protect the soil from erosion through both aggregation of the soil and speeding the rate of water infiltration (Colesie et al. 2014b). By increasing surface roughness, they reduce runoff and, as a consequence, increase infiltration and the amount of water stored for plant use. Cyanobacteria secrete polysaccharides that bind soil, thus influencing the soil stability, erosion, runoff and growth of soil crust components such as lichens (Colesie et al. 2014a).

A major feature of cyanobacteria is the ability of heterocystous species to fix atmospheric nitrogen (Solheim et al. 2006; Maqubela et al. 2009; Stewart et al. 2011a). They start to fix nitrogen as soon as the temperature in the thallus rises above 0^{o}C. In addition to accumulation of organic carbon and other elements in cells, nitrogen fixation is one of the most important ecological contributions to cyanobacteria-rich Arctic soil crusts. Nitrogen-fixing bacteria (including cyanobacteria) are a significant source of fixed nitrogen for plants and edaphic heterotrophic microorganisms. Both free-living forms and those associated with species of vascular plants

have been reported as being important nitrogen sources in High Arctic locations (Zielke et al. 2005; Breen and Levesque 2006). In nature, nitrogen-fixing cyanobacteria are abundant in areas deficient in nitrogen.

Nitrogen fixation depends on the water availability, temperature, soil chemistry and other parameters (Zielke et al. 2005; Kvıderova et al. 2011). For example, soil crusts with a sandy cover can contain higher concentrations of nitrogen compared with non-sand-covered soil crusts (Williams and Eldridge 2011). However, the relation of the rate of nitrogen fixation and texture and the type of soil substrate has not yet been studied. Phosphate limitation can negatively affect nitrogen fixation by decreasing the rate of photosynthesis and consequently inhibiting nitrogenase by reducing the photosynthates required for the energy-intensive process of nitrogen fixation (Hartley and Schlesinger 2002; Stewart et al. 2011a).

Cyanobacteria are often responsible for the majority of carbon fixed in Arctic soils, providing fixed carbon to soil crusts, which likely subsidizes food webs there (Yoshitake et al. 2010; Darby et al. 2010). In addition, the abundance of cyanobacteria increases with crust development, resulting in a higher carbon concentration in well-developed soil crusts rather than in poorly developed ones (Kaštovská et al. 2007; Pushkareva et al. 2015).

Endemism or cosmopolitanism?

The question about endemism of polar terrestrial microorganisms is still unsettled (Lawley et al. 2004; Casamatta et al. 2005; Rybalka et al. 2009; Strunecky et al. 2012) because many isolates found in the Arctic and the Antarctic share similar morphology with microalgae from other geographic regions. Microorganisms can be transported by dispersed vectors such as atmospheric circulation, ocean currents, animals (particularly migratory birds) and humans (Lawley et al. 2004; Strunecky et al. 2010). However, recent molecular analyses have not detected totally identical genotypes between particular species of polar regions and other locations (Lawley et al. 2004; Rybalka et al. 2009; Schmidt et al. 2011; Strunecky et al. 2010, 2012). It is very possible that the unique challenges of polar ecosystems prevent establishment by most microbial introductions and that the microorganisms inhabiting these regions have become specially adapted.

The increase in scientific and tourist activities in polar regions has a potentially huge impact on the geographical distribution of microalgae

genotypes. Anthropogenic activities promote species movement and their subsequent reproduction and spread at the new locations (Walther et al. 2002). Newly transported genotypes may adversely interfere with indigenous species and endanger their subsistence. Human presence in all polar regions has increased, thereby extending threats to biodiversity. However, up to now we have had very limited information about anthropogenic impacts on the geographical distribution of microalgae. There is a complete lack of data on alien microalgae dispersal and their subsequent occupation of particular localities and habitats. With the concomitant rapid climate change occurring in polar areas (particularly the Arctic and northwest Antarctic Peninsula region), the locally adapted species may lose their competitive advantage and be displaced by invasive species (Frenot et al. 2005). However, recent research on the dispersal of morphologically simple airborne eukaryotic microalgae, *Klebsormidium* (Streptophyta), *Chlorella* and *Stichococcus* (Chlorophyta), to polar regions showed ubiquitous distribution on a global scale (Hodac et al., in press; Rysanek et al., in press). Almost 80 % of all Arctic *Klebsormidium* strains were included within the cosmopolitan superclade B sensu Rindi et al. (2011). Similarly, Hodac et al. (in press) found that psychrotolerant strains of *Chlorella* and *Stichococcus* are without exception conspecific (or closely related) with strains originating from the temperate zone. A warming climate can promote immigration of alien species into the polar regions and could cause shifts in species abundance and distribution. The combined effects of invasive species and climate change on the biodiversity of soil organisms could modify the polar ecosystem.

Conclusion

This article gives a brief overview of the microalgal ecology, physiological ecology and biodiversity of soil crust ecosystems in polar regions with a focus on Arctic soil crusts. Low temperature, low availability of liquid water and instability are characteristic for these terrestrial ecosystems. A combination of ecological properties, including geochemical and physical factors, light availability, grazing pressure by invertebrates, anthropogenic impacts and invasive species, influences various microalgal processes in soil crust ecosystems. The severe conditions influence the diversity, abundance and ecological manifestation of organisms in soil crusts. Soil microalgae have developed diverse ecological and physiological life strategies and behaviors that help

them to avoid stressful conditions in the upper soil layer. Survival strategies in these microorganisms (similar to organisms living in temperate and tropical regions) have occurred because of the considerable evolutionary pressures experienced and an exceptionally long period of predictably unpredictable climatic conditions. Study of the community composition and main processes in Arctic soil crusts is necessary for better prediction of the future climate change.

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Biodiversity and ecological classification of cryptogamic soil crusts in the vicinity of Petunia Bay, Svalbard

Ekaterina Pushkareva[1*], Josef Elster[1,2]

[1]*University of South Bohemia, České Budějovice, Czech Republic*
[2]*Institute of Botany, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic*

***Corresponding author:** E. Pushkareva <puekse@gmail.com>

Abstract

The objective of this study was to describe various types of Arctic soil crust that were collected in the vicinity of Petunia Bay, Svalbard in the 2012 summer season. The photosynthetically active area of different soil crust samples was estimated by a chlorophyll fluorescence imaging camera. Biodiversity of cyanobacteria and microalgae from the collected soil crusts was analyzed using a stereomicroscopy and light microscopy. In most cases, cryptogamic crusts were dominated by cyanobacteria such as *Gloeocapsa* sp., *Nostoc* sp., *Microcoleus* sp., *Scytonema* sp., and *Chroococcus* sp. The dominant green microalgae were *Coccomyxa* sp., *Hormotila* sp., and *Trebouxia* sp. which commonly occurred in a lichenised soil crust. Soil crusts that were located in conditions with high water content were dominated by *Nostoc* sp. Cryptogamic soil crusts from the studied area can be divided into three different types and classified: (1) black-brown soil crusts (with low diversity of cyanobacteria and microalgae), (2) brown soil crusts (with high diversity of cyanobacteria and microalgae) and (3) grey-brown soil crusts (with low diversity of cyanobacteria and algae). The occurrence of similar soil crust types was compared at different altitudes. Altitude does not affect the biodiversity of cyanobacteria and microalgae. However, cyanobacteria and microalgae abundance increases with altitude.

Key words: soil crust, microalgae, cyanobacteria, photosynthetic area, variable chlorophyll fluorescence, diversity.

Introduction

In polar deserts, soil cyanobacteria and microalgae can form distinct visible biotic crust layers on the ground surface which are called cryptogamic crusts (Broady 1996, Elster et al. 1999). They consist of water-stable, surface soil aggregates held together by cyanobacteria, microalgae, fungi, lichens and mosses. These layers protect the soil from wind and water erosion (Leys et Eldrige 1998), and contribute to plant growth (Belnap et Lange 2001). In cold regions, the higher species diversity of biological crusts positively effects structural diversity of vascular vegetation. Soil crusts accumulate organic carbon and nutrients which are used by plants for their growth.

These communities influence key ecosystem processes and their characteristics such as water infiltration, moisture holding capacity, organic matter content, CO_2 fluxes, and nitrogen fixation and transformations (Bond et Harris 1964). Therefore, biological soil crusts are important in maintaining ecosystem structure and functioning in dry lands. However, biological soil crusts have only recently been recognized as having a major influence on terrestrial ecosystems (Belnap et Lange 2001).

Composition of biological soil crusts is very diverse. In many arid and semi-arid communities, there are often many more species associated with the biological soil crust at a given site than there are vascular plants (Ponzetti et al. 1998). As harsh environmental conditions limit vascular plant cover, a greater cover of crusts probably occurs at lower elevations in spite of, but not because of, these conditions (Belnap et al. 2001).

Soil cyanobacteria and microalgae play a major role in the initiation of crust development and the early stages of its growth. Algal components of cryptogamic crusts are found in the upper few centimetres of soil. The low biomass of cyanobacteria and microalgae is associated with a colourless soil surface. On the contrary, with higher cyanobacteria and microalgal biomass, the soil surface is usually covered by variously coloured patches, including lichenised communities and mosses (Lange et al. 1992, Zaady et al. 2000). Such types of crusts have a higher photosynthetic activity, because of high pigment content (Housman et al. 2006).

Crusts are formed by living organisms and their by-products, creating a surface crust of soil particles bound together by organic material. Surface crust thickness can reach up to 10 cm (Belnap et Lange 2003). The general

appearance of the crusts in terms of color, surface topography, and surficial cover varies.

Biological soil crusts have considerable photosynthetic potential (Evans et Johansen 1999). Water content and temperature can influence their photosynthetic activity (Yoshitake et al. 2010). Cyanobacteria, together with some green algae, are the most conspicuous elements of cryptogamic crusts (Elster et al. 1999). *Nostoc* sp. is widely found in all types of soil crusts and usually located on the surface (Belnap et Lange 2001). In most cases, cryptogamic crusts are also dominated by filamentous cyanobacteria. *Microcoleus* sp., *Phormidium* sp., *Plectonema* sp., *Schizothrix* sp., *Tolypothrix* sp. and *Scytonema* sp. are the most common genera found in both hot and cold deserts worldwide (Johansen 1993). They particularly have been shown to be important in binding surface soil particles (Anantani et Marathe 1974). Cyanobacteria and microalgae appear to play important roles in the northern and southern polar ecosystems, including the nitrogen economy of certain environments (Dickson 2000).

The objective of this study was to describe different types of Arctic soil crusts that were collected in the Petunia Bay, central Svalbard using various methods. We hypothesized that there would be crust type- and altitude-dependent differences in their biodiversity and photosynthetic capacity.

Material and Methods

Crust samples were collected during August 2012 in various sites across the vicinity of the Petunia Bay (78°40'60" N, 16°33'0" E), the north-western branch of Billefjorden, Dickson Land, Svalbard. Each site contained different types of soil crust that were selected by ocular observation using visible features of the crusts. Three soil subsamples were taken in each site by a corer (diameter of 5 cm) with a depth of 2-3 cm.

The same types of soil crust were taken from various altitudes and compared. Soil crusts from four different areas were studied: 350, 500, 700, and 800 m a.s.l.

The photosynthetic area of different samples was estimated using 2D epifluorescence images of the visible crust using a FluorCam 700MF fluorescence imaging camera (Photon Systems Instruments, Czech Republic). Circles of soil crusts were put into the dark adaptation

compartment of the device to allow photosynthetic organisms, their reaction centers of photosystem II (RCs PS II), to open. Then, using the measurements of single Kautsky kinetics, images of the photosynthetic area were obtained.

The diversity of cyanobacteria and microalgae from collected soil crusts was studied using an Olympus SZX-ZB7 stereomicroscope and Olympus BX-51 light microscope (Olympus C&S, Japan). By using the stereomicroscope, various parts of each soil crust were chosen for measuring of several parameters, including morphological characteristics of the soil, photosynthetic area, the presence of lichens, and *Nostoc* colonies on the crust surface.

Using light microscopy, the diversity of cyanobacteria and microalgae was observed in the chosen parts of the soil crusts. Dominant species were identified by morphological characteristics such as colony or cell habitats, size, colour, shape of colonies and cells, presence of akinetes, heterocysts and sheath (Komárek et Anagnostidis 1999, Komárek et Anagnostidis 2005, Ettl et Gartner 1995). Microphotographs of samples were taken using an Olympus DP71 digital camera (Olympus C&S, Japan) and processed using the Quick Photo Camera 2.3 software (Promicra, Czech Republic).

Results and Discussion

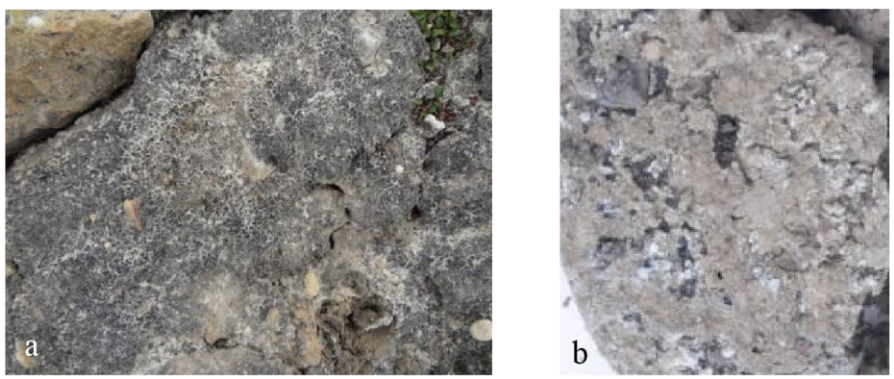


Fig. 1. Dark (a) and light (b) types of soil crust.

Based on data from the literature (Dunne 1989), our study has confirmed that the dark colour of soil crusts is due to the density of the organisms and their dark colour: cyanobacteria, lichens, and mosses (Fig. 1a). Soil crusts that contain a low amount of these organisms usually have a light

colour (Fig. 1b). Crusts generally cover all soil spaces which are not occupied by vascular plants. They may account for 70% or more of the ground cover (Belnap et Lange 2003).

Chlorophyll fluorescence analysis

The study of the fluorescent area showed the parts of soil crust that contained photosynthetic organisms (Fig 2). The higher intensity of colour (*i.e.* Chl. fluorescence signal) shows higher concentration of photosynthetic pigments. This method allows for determining the location of organisms such as microalgae, cyanobacteria, mosses and lichens that are capable to photosynthesize. Lichens and mosses have a higher intensity of colour such as the light blue (marked on a Fig. 2 by green circles). It can be considered that higher absolute values of chlorophyll fluorescence come from lichens and mosses rather than from free-living microalgae and cyanobacteria. On this base, i.e. when substracting lichens and mosses, we can estimate the approximate area of free microalgae and cyanobacteria over the sample area measured by chlorophyll fluorescence.

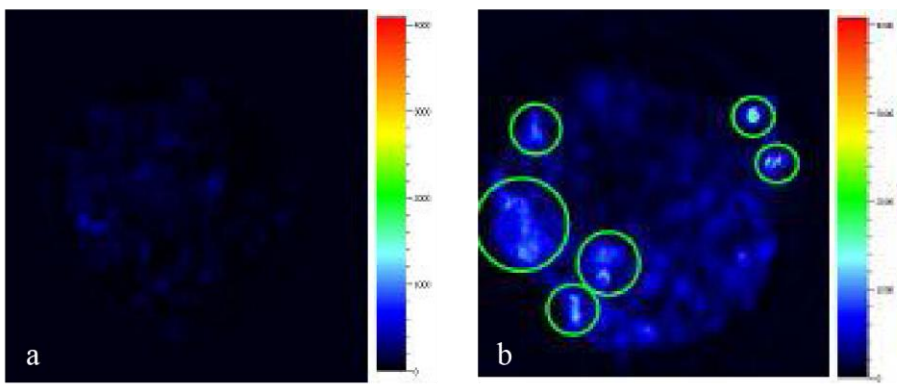


Fig. 2. Soil crusts with low (a) and high (b) variable chlorophyll fluorescence (F_V).

On the base of visual observation, the soil crusts from the studied area can be divided into three types: (1) black-brown soil crusts with low diversity of cyanobacteria and microalgae, (2) brown soil crusts with a high diversity of cyanobacteria and microalgae, and (3) grey-brown soil crusts with a low diversity of cyanobacteria and microalgae (Fig. 3).

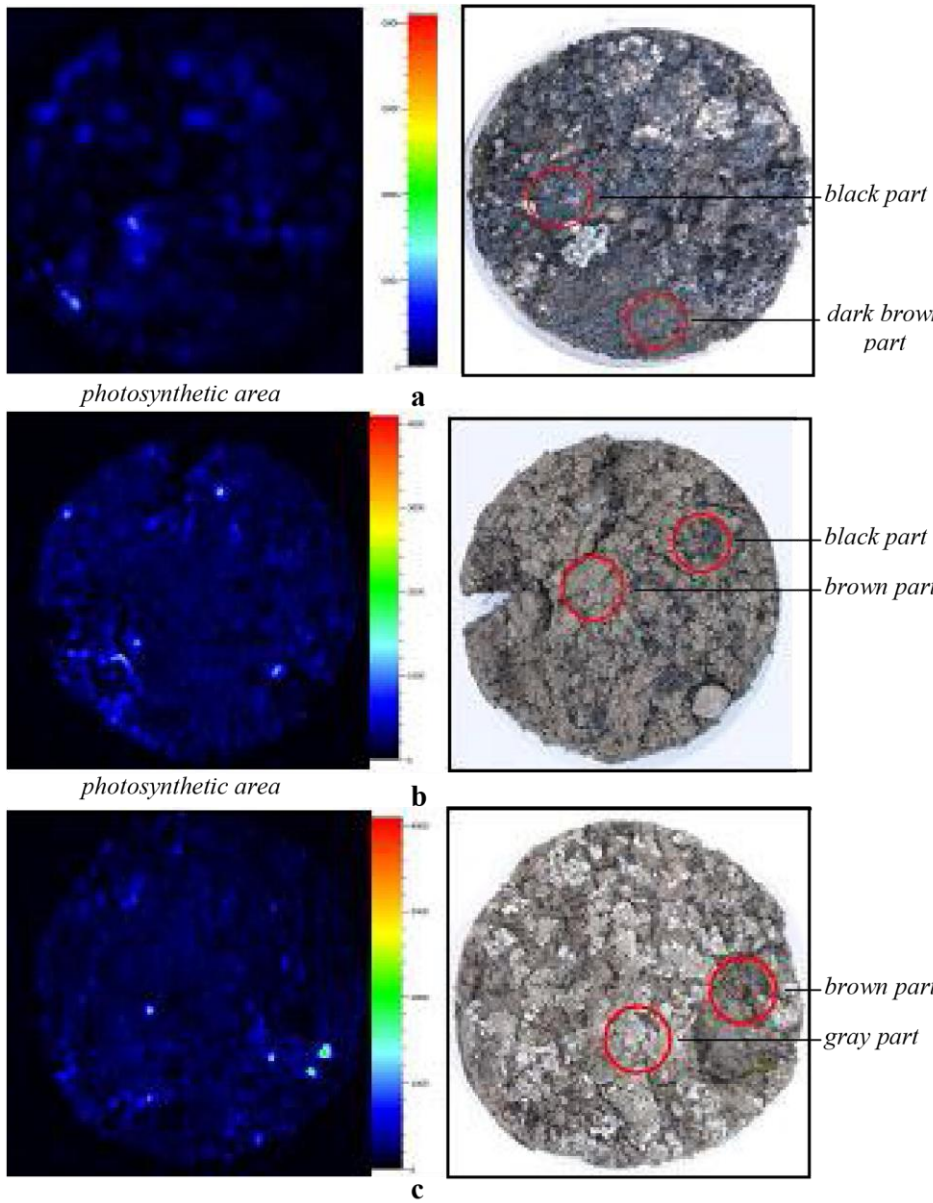


Fig. 3. Classification of soil crust types from the Petuniabukta, Svalbard: black-brown soil crust (a), brown soil crust (b) and gray-brown soil crust (c). Bright light blue spots on pictures with photosynthetically active areas indicate the presence of lichens or mosses.

Microscopic analyses

The most common species of cyanobacteria and microalgae are presented in Fig. 4, 5, 6.

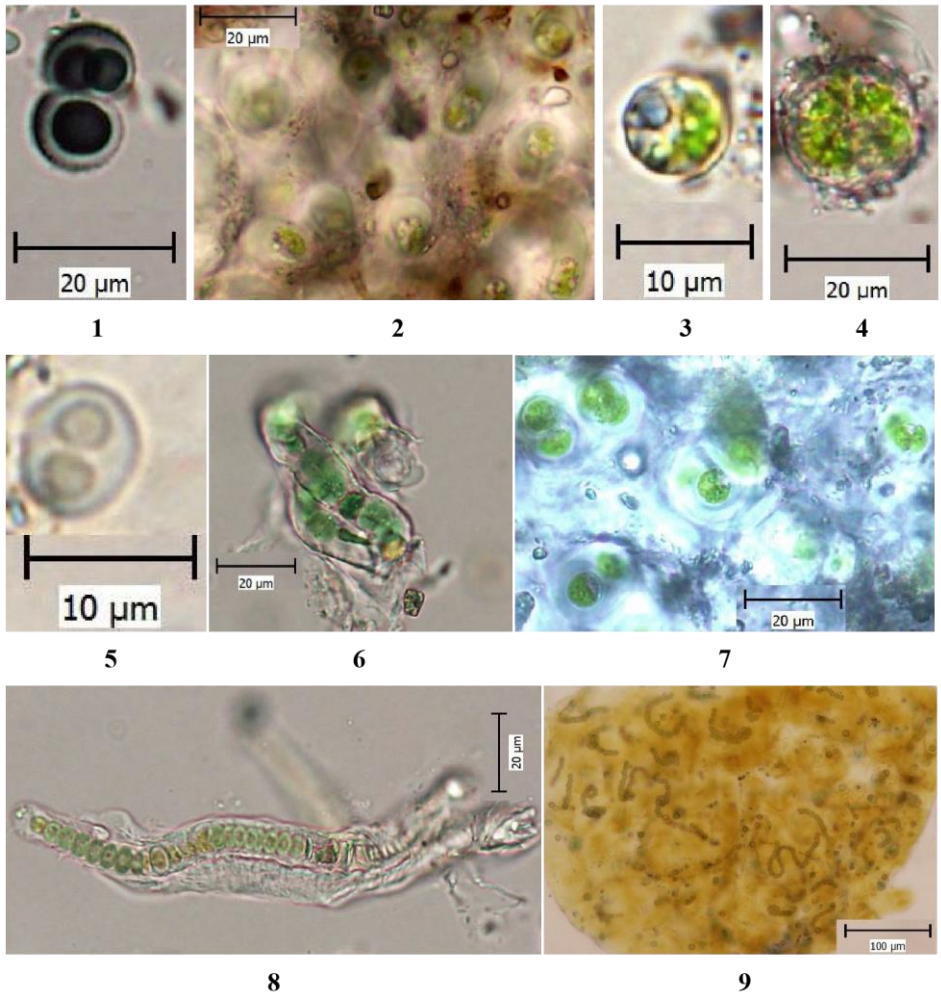
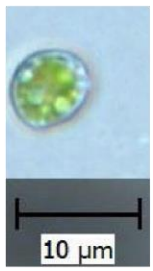


Fig. 4. ↑ Microalgae and cyanobacteria diversity of black-brown soil crusts. Dominant species:1 - *Chroococcus* sp., 2 - *Hormotila* sp., 3, 4 - Unidentified green balls, 5 - *Gloeocapsa* sp., 6 - *Tolypothrix* sp., 7 - *Myrmecia* sp., 8 - *Nodularia* sp., 9 - *Nostoc* sp.

Fig. 5. ↓ Microalgae and cyanobacteria diversity of brown soil crusts. Dominant species: 1, 2 - Unidentified green balls, 3 - *Gloeocapsa* sp., 4 - *Hormotila* sp., 5 - *Myrmecia* sp., 6 – *Scytonema* sp. (initial stage), 7 - *Nostoc* sp., 8 - *Nodularia* sp., 9 - *Scytonema* sp., 10 - *Asterocapsa* sp., 11 - *Geitlerinema* sp., 12 - *Phormidesmis* sp., 13 - *Calothrix* sp.



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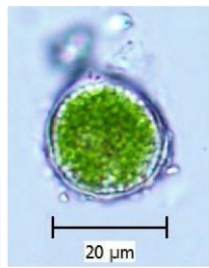

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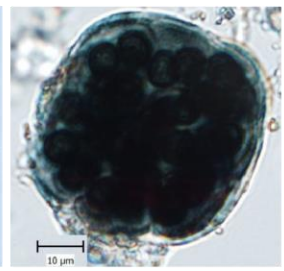

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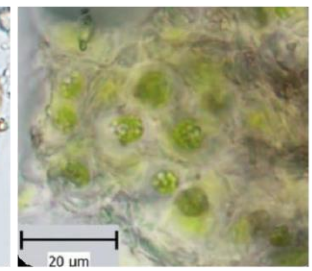

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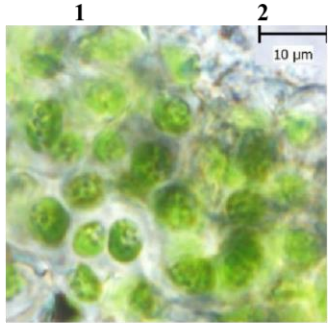

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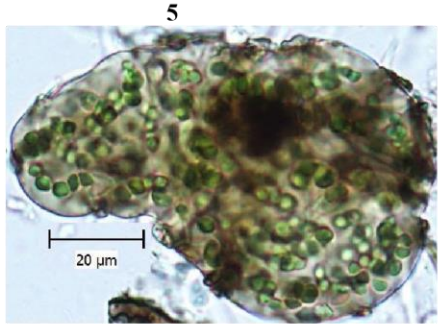

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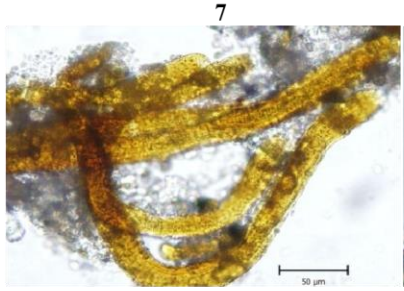

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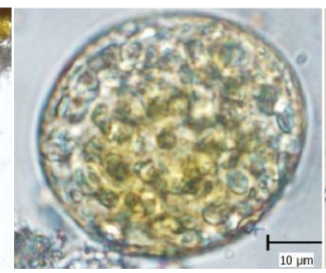

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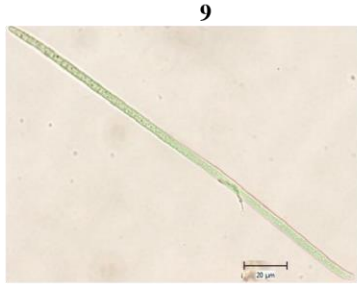

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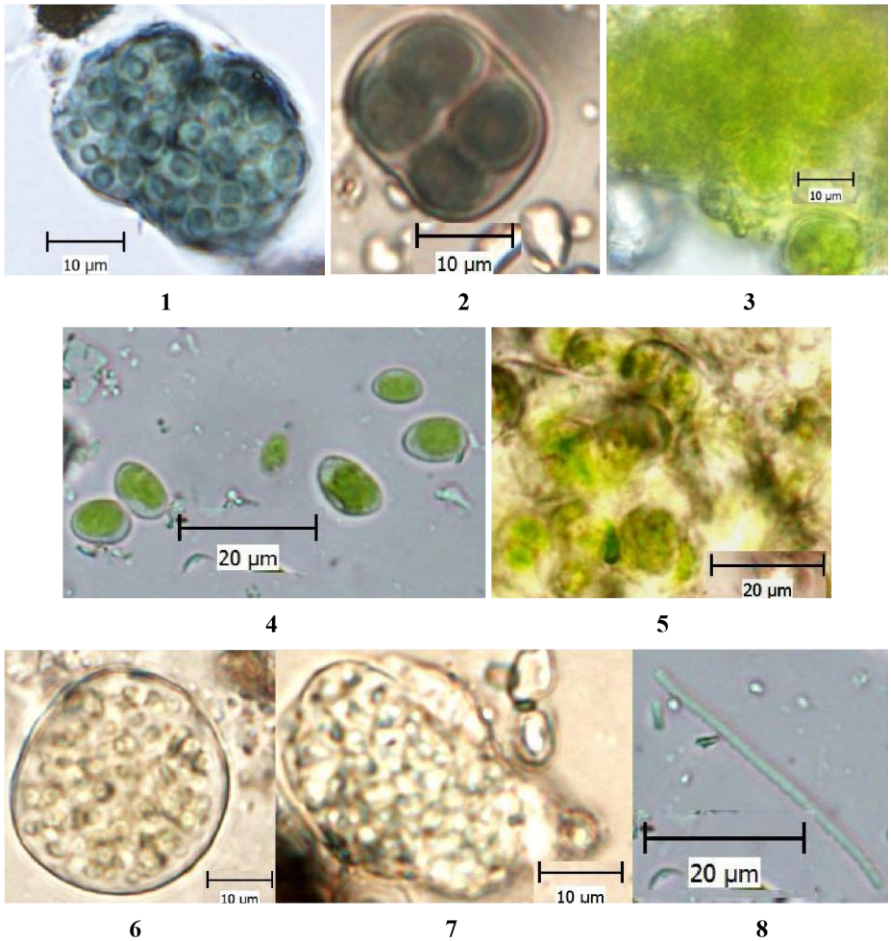

Fig. 6. Microalgae and cyanobacteria diversity of grey-brown soil crusts. Dominant species: 1, 2 - *Gloeocapsa* sp., 3 - *Trebouxia* sp., 4 - *Coccomyxa* sp. 5 - Unidentified green balls, 6 – *Asterocapsa* sp., 7 - *Chlorogloea* sp., 8 - *Pseudanabaena* sp.

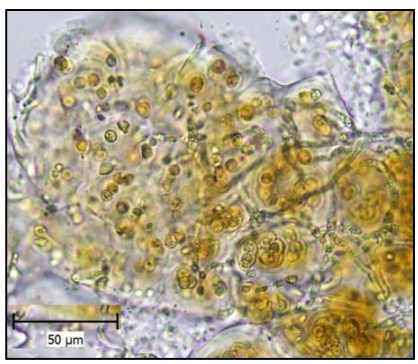

Fig. 7. The initial phase of lichenization.

In most cases, cryptogamic crusts were dominated by cyanobacteria such as *Gloeocapsa* sp., *Nostoc* sp., green algae such as *Hormotila* sp. and *Trebouxia* sp. Green microalgae are usually present as the photobionts of lichens (Fig. 7) (Nash 1987). In three types of soil crusts, the green microalgae, which are very difficult to identify by morphological analysis, were found as dominant species.

Also, we observed soil crusts that were located in the places with high water content, including areas near shallow wetlands or in the places with melting snowfields. The stereomicroscope analysis showed that these areas were usually covered by *Nostoc* sp. (Fig. 8a). These areas had also a high potential of photosynthetic activity (Fig. 8b). The presence of a high amount of *Nostoc* sp. might also be explained by an increased nitrogen demand of the soil crust (Lan et al. 2012).

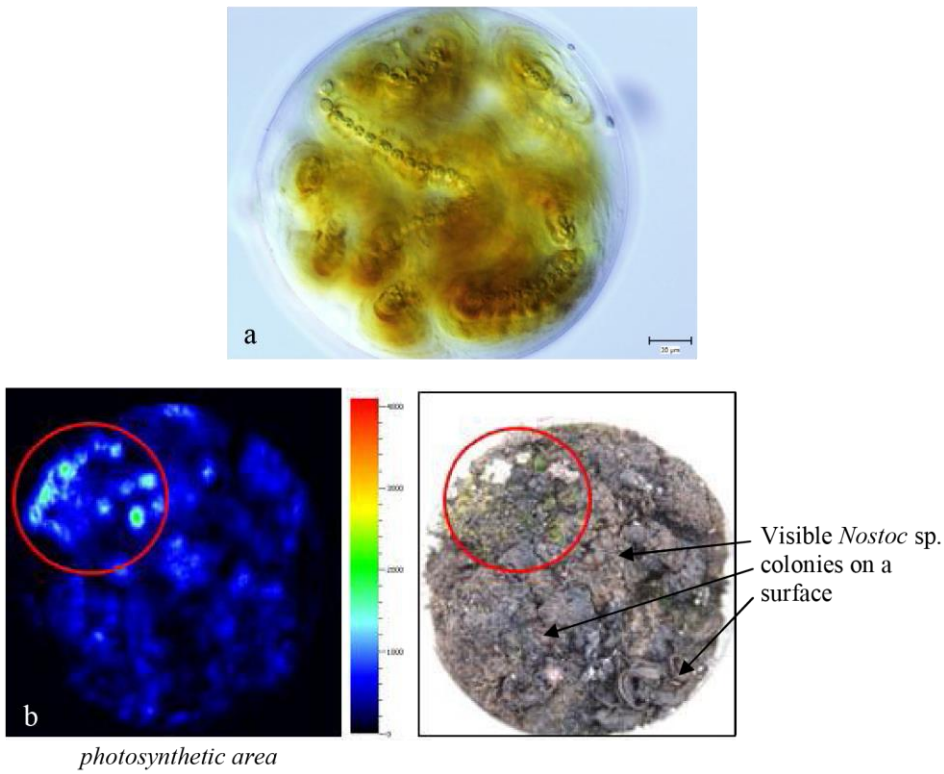

Fig. 8. *Nostoc* sp. (a) from a soil crust, (b) in an area with high humidity. Red circles show the area covered by mosses.

Altitude effect

The same types of soil crust were compared from different altitudes. *Microcoleus* sp. was the most dominant species among the microalgae and cyanobacteria at all studied altitudes (Fig. 9-1). When it is wet, the filaments glide out of their sheaths and, in a phototactic response, move towards the soil surface (Belnap et Lange 2001). Other common species are presented in Fig. 9. Differences in altitude do not affect the biodiversity of cyanobacteria and microalgae. However, their amount increases with increasing altitude. This is probably connected with the soil water content on mountain upper parts and presence of melting snowfields.

Concluding remarks

Different types of soil crusts are presented in the studied area. The presence of a high diversity of cyanobacteria and microalgae allows photosynthetic activity the potential of which was indicated by chlorophyll fluorescence measurements. While the diversity of cyanobacteria and microalgae differs among the various types, there are some common species for all types of soil crust. Water content is important ecological parameter influencing abundance, diversity and photosynthetic performance of cryptogamic soil crusts ecosystem.

Chlorophyll fluorescence measurements of soil crust ecosystem are effective and promising tool for ecological and ecophysiological studies of soil crust ecosystem. Further study of the soil crust should be focused on detailed chlorophyll fluorescence parameters measurements, potential and effective quantum yields in particular, followed by proper taxonomical and ecological studies of soil crust ecosystem.

Acknowledgements

The work was supported by the Grant Agency of the Ministry of Education of the Czech Republic (LM – 2010009 CzechPolar, CZ.1.07/2.3.00/20.0064). We are very grateful to Mrs. Jana Šnokhousová for her technical assistance and Dr. Jana Kvíderová who introduced us to fluorescence imaging camera measurements. Prof. Jiří Komárek helped us with cyanobacteria and algae identification. We also appreciate the help given by Keith Edwards for language revision.

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Cyanobacterial community composition in Arctic soil crusts at different stages of development

Ekaterina Pushkareva[1*], Igor S. Pessi[2], Annick Wilmotte[2] and Josef Elster[1,3]

[1]Centre for Polar Ecology, University of South Bohemia, České Budějovice, Czech Republic
[2]Centre for Protein Engineering, University of Liege, Belgium
[3]Institute of Botany, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic

***Corresponding author:** E. Pushkareva <puekse@gmail.com>

Abstract

Cyanobacterial diversity in soil crusts has been extensively studied in arid lands of temperate regions, particularly semi-arid steppes and warm deserts. Nevertheless, Arctic soil crusts have received far less attention than their temperate counterparts. Here, we describe the cyanobacterial communities from various types of soil crusts from Svalbard, High Arctic. Four soil crusts at different development stages (ranging from poorly-developed to well-developed soil crusts) were analysed using 454 pyrosequencing of the V3-V4 variable region of the cyanobacterial 16S rRNA gene. Analyses of 95660 cyanobacterial sequences revealed a dominance of OTUs belonging to the orders Synechococcales, Oscillatoriales and Nostocales. The most dominant OTUs in the four studied sites were related to the filamentous cyanobacteria *Leptolyngbya* sp. Phylotype richness estimates increased from poorly- to mid-developed soil crusts and decreased in the well-developed lichenized soil crust. Moreover, pH, ammonium and organic carbon concentrations appeared significantly correlated with the cyanobacterial community structure.

Key words: soil crust; Arctic; cyanobacteria; 454 pyrosequencing; 16S rRNA gene; soil chemistry.

Introduction

Soil crusts are considered to be characteristic of arid environment and are known to harbour diverse microbial communities (Elster et al. 1999). Cyanobacteria are the main primary producers in these formations (Belnap and Lange 2001), and many biotic and abiotic parameters are known to influence their development, such as water availability, soil geological properties, climate, presence of vascular plants and animal and human intervention (Elster 2002; Hu, Gao and Whitton 2012).

Soil cyanobacteria are well adapted to severe conditions, having developed a wide range of adaptive strategies that allow them to avoid the effect of extreme polar conditions such as long periods of desiccation and large temperature fluctuations (Moquin et al. 2012). For example, *Microcoleus* spp. are known to produce thick polysaccharidic sheaths which protect them against fluctuations in water availability and prevent desiccation (Belnap 2008). Mobility is another example of a survival strategy. Some soil cyanobacteria are motile and able to migrate to a more favourable environment, avoiding stressful conditions. However, the abundance and diversity of some cyanobacteria, mainly unicellular cyanobacteria, can be reduced under extreme conditions such as unstable and coarse soils (Rossi et al. 2012).

The community composition and nutrient availability of soil crusts can influence crust development (Li et al. 2010). Colourless soil crusts, which are usually poorly developed, are associated with a low biomass of major soil crust organisms, as well as low nutrient concentration (Housman et al. 2006). On the other hand, dark-coloured well-developed soil crusts have higher biomass and diversity of soil crust organisms as well as higher amounts of mineral nutrients (such as phosphorus and nitrogen) and organic carbon (Belnap 2008).

In high-latitude environments, soil crusts are common in the High Arctic, and have been reported in territories of the Russian, Canadian, and European Arctic as well as Greenland. Arctic soil crusts are known to be highly diverse, hosting complex microbial communities (Elster 2002). Next-generation sequencing technologies have greatly improved our knowledge about the microbial diversity of Arctic soil crusts, enabling a more comprehensive understanding (Chu et al. 2010; Schutte et al. 2010; Knelman et al. 2012). However, the identification of soil crust cyanobacteria has rarely been performed (Patova and Beljakova 2006; Strunecky, Elster and Komarek 2010; Pushkareva and Elster 2013). In

general, these studies have shown that Arctic soil crusts are composed by cyanobacteria from the orders Chroococcales, Synechococcales, Oscillatoriales and Nostocales. However, much more information is needed to accomplish a full description of the cyanobacterial diversity in Arctic soils, and to better understand the impact of environmental factors on cyanobacterial diversity. Thus, here we aimed to describe the cyanobacterial communities in soil crusts from Petunia Bay (Svalbard) at different stages of development, using a comprehensive sampling scheme, and assess how nutrient availability influence their abundance and distribution.

Material and Methods

Study site and sampling

Petunia Bay is located in the northwestern branch of Billefjorden (Dickson Land, Svalbard). Average annual air temperature is approximately –6.5^{o}C. Air temperatures above 0^{o}C are generally recorded from June until the end of August ormiddle of September, with usual temperatures of 5 – 7 ^{o}C (Rachlewicz, Szczucinsky and Ewertowski 2007). Annual precipitation in Petunia Bay is about 200 mm (Laska, Witoszova and Prosek 2012).

Soil crust samples were collected in August 2013 from four sites in the vicinity of Petunia Bay (78^{o}40.961' —78^{o}41.895' N, 16^{o}26.661' — 16^{o}26.361' E; Fig. 1a). Bedrocks are characterized as fluvial and glaciofluvial deposits from the Pleistocene and Holocene. Soil crusts covered around 80% of the area, but sparse vegetation was also present, including vascular plants such as *Polygonum viviparum*, *Salix polaris*, *Carex rupestris* and *Dryas octopetala*.

Soil crusts differed macroscopically between the four sites and presented different levels of developmental growth. The sampling sites were located along 2 km walk in a homogenous area and appeared to be subjected to similar environmental conditions. (Fig. 1b). Soil crust in site SC1 was poorly developed and colonized by cyanobacteria alone. Sites SC2 and SC3 had soil crusts in mid development stage with a mixture of lichens and cyanobacteria. Finally, soil crust in site SC4 was well developed and had a rich lichen community dominated by the genera *Cladonia* and *Collema*. For pyrosequencing, nine soil crust samples of 2–3 cm depth were taken in each site within a 25m^{2} area. These multiple samples were

intended to take into account any possible patchiness of the communities. For chemical analysis, three soil crust samples at the same depth were collected in each site. Samples were kept at –20°C and transported in dry ice to the laboratory in Trebon, Czech Republic.

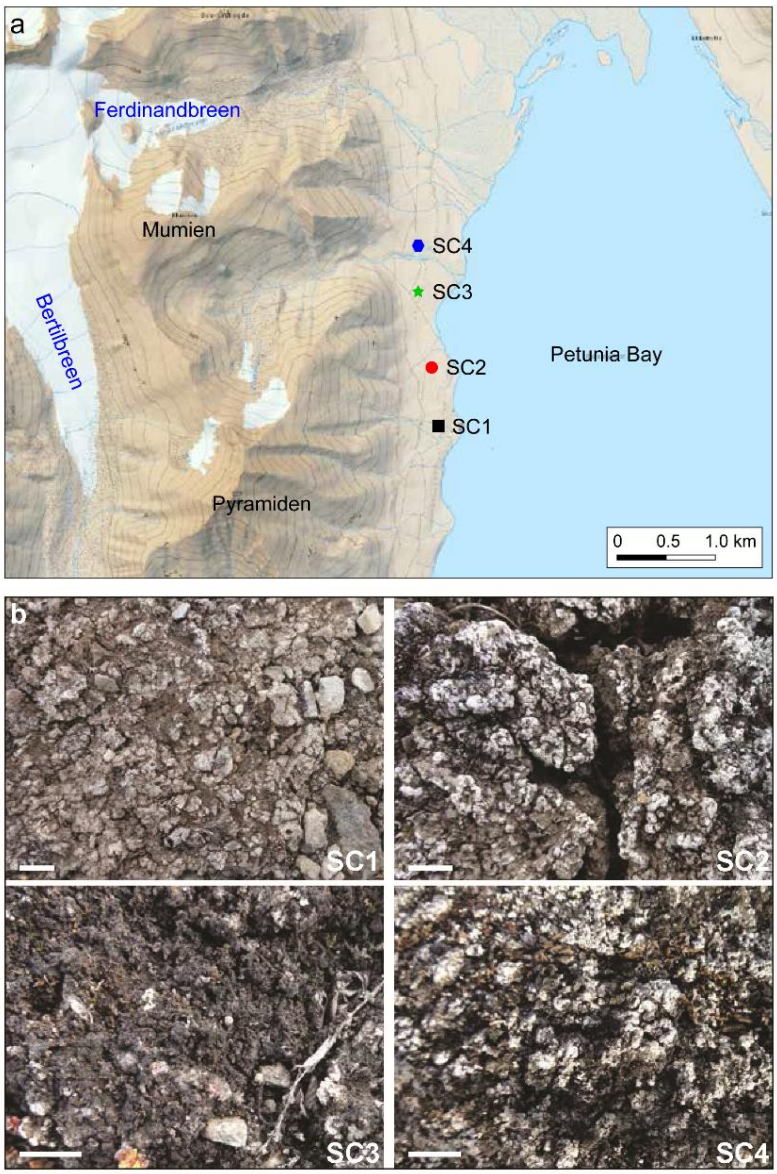

Fig. 1. (a) Map of Petunia Bay, Billefjorden, central Svalbard showing the location of the sampling sites and (b) pictures of the studied soil crusts. Scale bar on picture (b) represents one centimeter.

Chemical analysis

The three samples collected from each site were mixed and passed through a sieve (2 mm mesh). Analysed parameters included pH (in KCl buffer), conductivity, water content, total organic carbon, $N\text{-}NH_4$, $N\text{-}NO_3$, $P\text{-}PO_4$, Ca^{2+}, Mg^{2+}, Na^+ and K^+. All analyses were performed according to the methodology described in Czech and European Union standards (ISO 10390, ISO 10523, CSN EN 27888, ISO 11465, CSN EN ISO 11732, CSN ENISO 13395 and CSN EN ISO 15681-1). Significant differences in chemical properties between sites were determined using one-way ANOVA followed by Tukey's pairwise posthoc tests using the PAST 3 software (Hammer, Harper and Ryan 2001).

DNA extraction, PCR and pyrosequencing

Pyrosequencing analyses were carried out following the protocol of Pessi et al. (in preparation). DNA was extracted from the 36 soil crust samples using the PowerSoil DNA Isolation Kit (MOBIO, Carlsbad, CA, USA) according to manufacturer instructions. The V3-V4 region of the 16S rRNA gene was amplified using the cyanobacteria-specific primers 359F and 781Ra/781Rb (Nübel, Garcia-Pichel and Muyzer 1997) in separate reactions for each reverse primer (Boutte et al. 2006). A barcode sequences were added to both forward and reverse primers and were specific to each sample. PCR reactions consisted of 1× PCR buffer with 1.5 mM $MgCl_2$, 1 mg ml^{-1} BSA, 200 μM of each dNTP, 0.2 μM of each primer, 1 U SUPER TAQ 'plus' DNA polymerase (HT Biotechnology, Cambridge, UK) and 4 ng μl^{-1} DNA in a final volume of 50 μl. The amplification comprised an initial denaturation step of 94ºC for 2 min, followed by 35 cycles of 94ºC for 45 s, 57ºC for 45 s and 68ºC for 45 s (for primer 781Rb) or 45 cycles of 94ºC for 45 s, 60ºC for 45 s and at 68ºC for 45 s (for primer 781Ra), and a final extension of 68ºC for 5 min. PCR reactions were performed in triplicates, which were pooled prior to purification. The 36 amplified PCR products were purified using the GeneJet PCR Purification Kit (Thermo Scientific, Waltham, MA, USA) and quantified using the Quant-iTPicoGreen dsDNA Assay Kit (Life Technologies, Carlsbad, CA, USA). Libraries were pooled in equimolar concentrations and sent to Beckman Coulter Genomics (Takeley, UK), where sequences were obtained using the 454 GS FLX+ Titanium platform (454 Life Sciences, Branford, CT, USA).

Pyrosequencing data analysis

Quality control of reads, removal of chimeric sequences and operational taxonomic unit (OTU) clustering were performed using UPARSE (Edgar 2013) according to Pessi et al. (in preparation). Two and zero mismatches were allowed to the primer and barcode sequences, respectively, and reads were required to have a maximum expected error of 0.5 and a length of 370 bp. Quality-filtered sequences were clustered into OTUs at 97.5% similarity, according to Taton et al. (2003). OTUs were classified using CREST (Lanzen et al. 2012) based on the Greengenes database (McDonald et al. 2012), and non-cyanobacterial OTUs were removed from the datasets. Furthermore, a manual inspection of the taxonomic classification was carried out in order to comply with the cyanobacterial taxonomy recently published by Komarek et al. (2014). In this classification, filamentous cyanobacteria from families Pseudanabaenaceae and Leptolyngbyaceae were placed into the order of Synechococcales. The order Chroococcales was considerably reduced, and some species were placed into the order of Synechococcales. The latter has thus become a large order with both unicellular and filamentous types and thus, in this work, we divide it into 'Synechococcales (coccoid forms)' and 'Synechococcales (filamentous forms)' for a better description of diversity.

The most closely related isolates and uncultured sequences for each OTU (based on themost abundant sequence type inside each OTU cluster) were retrieved using the SeqMatch tool from RDP (Cole et al. 2014). To avoid the inclusion of artefact OTUs, low abundance OTUs that had less than 97.5% similarity with their best SeqMatch isolate hit and were present in only one sample were discarded. A distance tree was constructed with the software package TREECON for Windows 1.3b (Van de Peer and De Wachter 1997), with an alignment of 412 positions obtained with MUSCLE (https://www.ebi.ac.uk/Tools/msa/muscle/) (Edgar 2004) containing our OTUs, and the two most related sequences of isolates indicated by SeqMatch. The dissimilarity values were corrected for multiple substitutions by the model of Jukes and Cantor (1969) and were used to calculate a distance matrix. A distance tree was constructed by the Neighbour-joining method (Saitou and Nei 1987). Indels were not taken into account. A bootstrap analysis was performed that involved construction of 500 resampled trees. A manual curation based on such a phylogenetic analysis and a BLAST analysis had previously allowed to

detect 13 OTUs that were either clusters of sequences of bad quality but related to an existing OTU ('bad variants'), or seemed to be artefactual based on their isolation and low similarities. OTUs' representative sequences have been deposited in Gen-Bank under the accession numbers listed in Table S1 (Supporting Information).

To account for uneven sequencing depths across samples, diversity calculations were carried out after rarefying datasets to 1000 sequences. Samples SC3-4, SC3-5, SC3-6, SC4-1, SC4-2, SC4-7 and SC4-8, which consisted of less than 1000 sequences, were thus excluded from downstream analysis. Alpha diversity indices [Good's coverage, richness, Chao1 and Shannon's diversity index (H')] were calculated using QIIME (Caporaso et al. 2010). Changes in phylotype richness and relative abundances of cyanobacterial orders across sites were assessed by Kruskal–Wallis non-parametric analysis of variance followed by Mann–Whitney posthoc tests using the PAST 3 software. Cyanobacterial community dissimilarities at the phylotype level were examined by non-metrical multidimensional scaling (NMDS) and significant differences in community structure across sites were assessed by a PERMANOVA routine using the Vegan package in R (Oksanen et al. 2013). For this, OTU abundance data were square root transformed and submitted to Wisconsin double standardization, and dissimilarities were calculated based on Bray–Curtis distances. The contribution of environmental parameters to changes in beta diversity was assessed by indirect gradient analyses (Spearman's rank correlation coefficients) using the Vegan package in R. For this, soil physico-chemical parameters were $\ln^{+1}$ transformed (except pH). Differences in OTU relative abundances across the sites were assessed using log-likelihood ratio tests in QIIME.

Results

Soil parameters

Chemical analysis of the studied soil crusts is shown in Table 1. For some parameters, samples were found to represent a gradient corresponding to the level of soil crust development observed macroscopically (Fig. 1b). Total carbon content was lowest in the poorly developed, cyanobacteria-dominated soil crust (site SC1). It increased gradually and reached the highest value in the more developed, lichen-dominated soil crust (site SC4). The same gradient was detected for water

content whereas an inverse relationship (from more- to less-developed) was observed for pH. In site SC2, the highest conductivity and Ca^{2+} concentration were observed. No significant difference was found in N-NH_4, N-NO_3 and P-PO_4 concentrations between sites.

Table 1. Chemical parameters of the studied soil crusts.

Parameter	SC1	SC2	SC3	SC4
pH	8.1±0.01 [a]	8.0±0.08 [a]	7.8±0.06 [a,b]	7.5±0.05 [b]
Water content (%)	19.6±2.4 [a]	30.8±3.9 [b]	35.3±3.0 [b]	34.5±0.1 [b]
Conductivity (µS cm^{-1})	101±5 [a]	365±136 [b]	242±31 [a,b]	269±34 [a,b]
Total organic carbon (%)	4.8±0.2 [a]	11.5±2.0 [b]	15.6±2.7 [b,c]	16.9±0.8 [c]
N-NH_4 (µg kg^{-1})	3.5±0.6 [a]	4.0±1.5 [a]	5.7±1.7[a]	3.4±0.6[a]
N-NO_3 (µg kg^{-1})	0.7±0.5 [a]	0.4±0.1 [a]	0.7±0.1[a]	4.4±0.8[b]
P-PO_4 (µg kg^{-1})	19.1±0.0 [a]	15.6±1.1 [a]	18.3±4.3 [a]	19.4±0.3 [a]
Ca^{2+} (µg g^{-1})	5.8±0.3 [a]	16.5±1.2 [b]	7.6±0.2 [c]	8.2±0.2 [c]
Mg^{2+} (µg kg^{-1})	808±39 [a]	534±54 [b]	799±40[a]	417±14[c]
K^{+} (µg kg^{-1})	144±5 [a]	208±16 [b]	322±40[c]	221±13 [b]
Na^{+} (µg kg^{-1})	36±12 [a,b]	39±10 [a,b]	45±4[a]	18±0.3[b]

Values are shown as the average (±SD) of the measurements of the three independent samples per site.

Same letters indicate no statistical difference between groups according to one-way ANOVA followed by Tukey's pairwise post-hoc tests ($p < 0.05$)

Cyanobacterial community structure

Pyrosequencing analysis of partial 16S rRNA gene amplicons generated 292210 sequences from the 36 soil crust samples. After bioinformatic analysis and manual removal of spurious sequences, 94592 cyanobacterial sequences remained, representing 136 OTUs at 97.5% similarity level. A distance analysis shows the large spread of the OTUs along the cyanobacterial radiation, their mutual relationships and to the most related isolates (Fig. S1, Supporting Information).

Taxonomic assignment of sequences revealed the presence of six cyanobacterial orders across the four studied sites (Fig. 2; Table S2, Supporting Information). Synechococcales (32 OTUs, 43.1%–58.7% of the sequences) was the dominant order, followed by Oscillatoriales (36 OTUs, 13.8%–35.5% of the sequences), Nostocales (25 OTUs, 9.9%–

25.8% of the sequences), Chroococcidiopsidales (8 OTUs, 1.1%–5.1% of the sequences), Gloeobacterales (8 OTUs, 1.5%–3.6% of the sequences) and Chroococcales (14 OTUs, 0.03%–1.2% of the sequences). However, between 1.1% and 9.0% of the sequences (13 OTUs) could not be assigned to any cyanobacterial order by the used bioinformatic tool.

Table 2. List of OTUs with statistically different relative abundances across the four sites.

OTU	Mean relative abundance (%)				Best SeqMatch isolate hit; accession number (% ID)
	SC1	SC2	SC 3	SC 4	
OTU1	13.8	**21.7**	**21.5**	12.1	*Leptolyngbya* sp. LLi18; DQ786166 (97.5%)
OTU2	**16.4**	**13.2**	2.8	3.0	*Leptolyngbya antarctica* ANT.L67.1; AY493572 (98.9%)
OTU3	0.5	**7.5**	**8.6**	**9.9**	*Leptolyngbya nostocorum* UAM 387; JQ070063 (98.4%)
OTU4	**7.6**	**6.8**	0.6	2.4	*Calothrix* sp. KVSF5; EU022730 (95.1%)
OTU5	**11.9**	3.9	2.7	0.3	*Coleofasciculus chthonoplastes* EcFYyyyy00; KC463190 (95.1%)
OTU8	**9.2**	3.5	1.1	3.0	*Leptolyngbya subtilissima* EcFYyyy700; KC463197 (99.5%)
OTU9	0.3	**2.6**	1.2	**2.7**	*Leptolyngbya* sp. CENA112; EF088337 (96.2%)
OTU11	**6.8**	1.9	0.8	0.1	*Oscillatoria* sp. PCC 7112; AB074509 (100.0%)
OTU12	1.3	2.5	**6.2**	0.3	*Stigonema ocellatum* SAMA 35; GQ354275 (98.9%)
OTU14	0.2	**3.9**	0.1	0.5	*Microcoleus* sp. HTT-U-KK5; EF654070 (95.1%)
OTU17	0.2	0.4	5.3	**26.5**	*Phormidium* sp. CYN64; JQ687330 (98.6%)
OTU18	0.3	0.5	**6.1**	3.1	Unidentified cyanobacterium Ni2-C1; AB275351 (93.4%)
OTU21	4.8	2.9	**6.5**	1.5	*Nostoc* sp. CCAP 1453/28; HF678493 (99.5%)
OTU24	0.3	0.7	**3.1**	0.7	*Chroococcidiopsis* sp. CC1; DQ914863 (98.1%)
OTU25	0.1	**1.7**	**1.4**	0.3	*Oscillatoria duplisecta* ETS-06; AM398647 (95.6%)
OTU28	0.2	0.7	**2.5**	1.5	*Loriellopsis cavernicola* LF-B5; HM748318 (98.6%)
OTU31	**2.1**	0.7	0.1	0.1	*Gloeobacter violaceus* PCC 8105; AF132791 (92.9%)
OTU32	0.0	1.2	1.2	**2.3**	Oscillatoriales cyanobacterium EcFYyy200; KC463201 (99.5%)
OTU34	**1.7**	0.1	0.5	1.1	*Phormidium* sp. LEGE 07317; HM217043 (96.4%)
OTU41	0.0	0.6	**2.2**	1.1	*Stigonema ocellatum* SAMA 35; GQ354275 (94.2%)
OTU52	0.1	0.2	**1.5**	0.8	*Chroococcidiopsis* sp. CC1; DQ914863 (94.2%)
OTU127	**7.7**	1.2	2.7	2.4	*Leptolyngbya tenuis* PMC304.07; GQ859652 (99.5%)

Numbers in bold indicate OTUs with higher relative abundances in specific sites according to log-likelihood test ($p < 0.05$, Bonferroni-corrected)

Differences in the relative abundance of cyanobacterial orders were observed across the sites. Site SC3 presented a considerably lower abundance of Synechococcales and Oscillatoriales and a higher abundance of Nostocales in comparison to the other sites. The highest abundance of

filamentous cyanobacteria from the order Synechococcales was found in site SC2. Filamentous cyanobacteria from the order Oscillatoriales were more abundant in site SC4 than in the others (Fig. 2; Table S2, Supporting Information).

Good's coverage estimates ranged from 98.1% to 99.6%, indicating that the large majority of the cyanobacterial diversity was captured in the analysis. Phylotype richness ranged from 32 to 71 OTUs per sample (Fig. 3). The highest phylotype richness was observed in samples from sites SC2 and SC3. The same trend was observed with the Chao1 and Shannon's diversity index estimates.

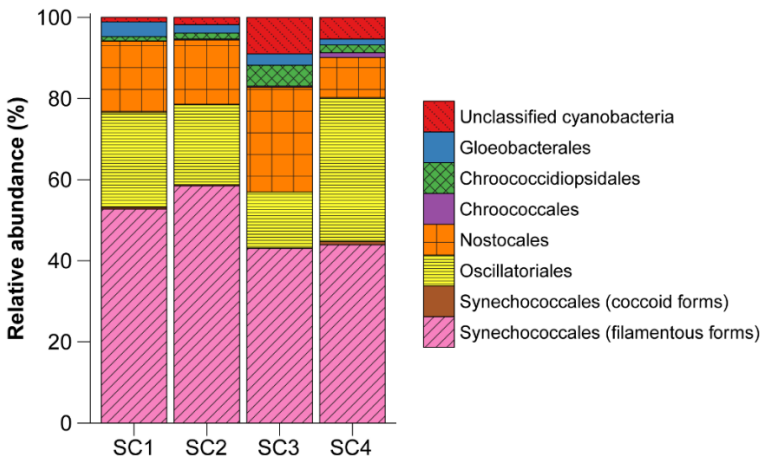

Fig. 2. Taxonomic assignment at the order level of OTUs found in the four soil crust samples.

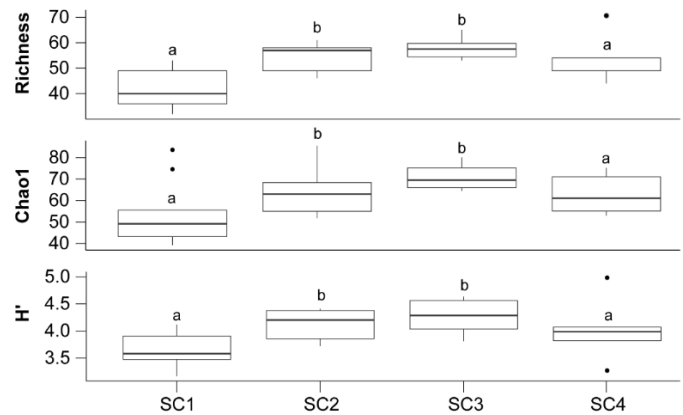

Fig. 3. Cyanobacterial richness and diversity estimates calculated for the four soil crust samples. Identical letters indicate that there is no statistical difference between the groups according to a Kruskal–Wallis analysis of variance test followed by Mann–Whitney posthoc tests ($P < 0.05$, Bonferroni-corrected).

Beta-diversity analysis at the phylotype level showed that samples from the same site harbour cyanobacterial communities more similar to each other than to samples from the other sites (Fig. 4). Moreover, community dissimilarities were explained to some extent by the soil chemical properties, particularly total organic carbon, pH and water content. No influence was observed for Na^+, $N\text{-}NO_3$ and $P\text{-}PO_4$.

Log-likelihood ratio tests identified OTUs with relative abundances that differed statistically across the four sites (Table 2). OTU1 (97.5% identity to *Leptolyngbya* sp. LLi18) was present in all sites but was the dominant OTU in sites SC2 and SC3 with statistically higher relative abundances. OTU2 (98.9% identity to *Leptolyngbya antarctica* ANT.L67.1) was the dominant phylotype in site SC1, but was also quite abundant in site SC2. Finally, OTU17 (98.6% similar to *Phormidium* sp. CYN64) was the dominant OTU in site SC4, but was also found at lower abundances in the other sites. In general, cyanobacterial communities were dominated by phylotypes related to the form-genera *Leptolyngbya* (94.8%–99.5% similarity), *Calothrix* (95.1% similarity), *Coleofasciculus* (95.1% similarity), *Oscillatoria* (100% similarity), *Stigonema* (98.9% similarity), *Microcoleus* (95.1% similarity) and *Phormidium* (96.4%–98.6% similarity).

Discussion

Cyanobacterial diversity in polar soil crusts may be restricted due to low water availability, high solar radiation, temperature fluctuations and frequent freeze-thawing cycles (Elster 2002). There are not a lot of studies dealing with Arctic soil crusts. However, it has been shown that cyanobacterial morphotype diversity and abundance there are limited compared to tropical and temperate regions (Kaštovská et al. 2005, 2007; Patova and Beljakova 2006). Here, we report the cyanobacterial diversity found in soil crust samples from Petunia Bay, Svalbard (Fig. 1) based on pyrosequencing of partial 16S rRNA gene sequences. Moreover, multiple samples were taken from four sites at different development stages, to take into consideration any patchiness of the cyanobacterial communities and the influence of the environmental conditions. To the best of our knowledge, this is the first in-depth assessment of cyanobacterial diversity in Arctic soil crusts based on next-generation sequencing.

The level of soil crust development observed macroscopically in the studied sites was related to chemical parameters of the soil (Fig. 1b, Table

1). Collection of nine soil crust samples in each site allowed us to obtain a more global image of the cyanobacterial diversity present there and assess the influence of pH and nutrient availability on cyanobacterial community composition, as these parameters play a major role in soil crust development (Belnap and Lange 2001). Soil crusts at different stages of development and disturbance have been extensively studied in Australia (Eldridge, Semple and Koen 2000; Eldridge, Freudenberger and Koen 2006; Thompson, Eldridge and Bonser 2006). These studies showed a relation between soil crust components (lichens and mosses) and crust development, where bare soils hosted the most extensive and diverse cryptogamic communities. However, information about soil crusts in polar regions is limited. The most related study using pyrosequencing investigated the global bacterial diversity of High Arctic soil crusts in relation to water pulses (Steven et al. 2013). Their main finding was that Oscillatoriales were more abundant inside the water tracks than outside. Interestingly, the three cyanobacterial sequences that belonged to their 25 most abundant OTUs were either closely related to OTU703 (*Nostoc*) or to OTU11 (*Oscillatoria*) and OTU35 (*Arthronema*) of this study (98%–99% 16S rRNA similarity on the 370 common nucleotides). Colesie et al. (2014) illustrated the importance of water availability for soil crusts in extreme Antarctic terrestrial sites, but the emphasis was on the lichens and mosses and no cyanobacteria were observed in the very dry biotopes of the Diamond Hill. The few studies describing and comparing cyanobacterial communities in Arctic soil crusts at different stages of development, generally, focus on the succession of microbial communities that grow after glacier retreat (Kaštovská et al. 2005, 2007; Schutte et al. 2010; Knelman et al. 2012). However, these studies present whole microbial community without a detailed assessment of cyanobacteria, or the cyanobacterial community is only described using morphological methods.

Taxonomic assignment of the obtained sequences revealed a dominance of the orders Synechococcales, Oscillatoriales and Nostocales across all samples (Fig. 2; Table S2, Supporting Information), based on the most recent cyanobacterial classification (Komarek et al. 2014). Obtained results agreed with previous studies which have shown that Arctic soil crusts are dominated by filamentous cyanobacteria that can survive in extreme conditions due to their motility and mucilage production (Kaštovská et al. 2005; Strunecky, Elster and Komarek 2010). Filamentous cyanobacteria belonging to the Leptolyngbyaceae family

were the most abundant in the four studied sites (relative abundances between 42.17%–57.44%). Heterocyst-forming cyanobacteria were mostly represented by *Nostoc* sp., *Calothrix* sp., *Stigonema* sp. Moreover, Nostocales are very important for nitrogen fixation in the Arctic (Zielke et al. 2005). In addition, they are widely distributed around theworld in different types of soil crusts (Belnap and Lange 2001; Yeager et al. 2004; Řeháková, Chlumska and Dolezal 2011; Hu, Gao and Whitton 2012; Bastida et al. 2014). Unicellular cyanobacteria (mainly Chroococcales) were rare in all soil crust samples, whereas *Gloeocapsa* sp. together with *Microcoleus* sp. were observed in cyanobacterial soil crusts of the Dry Valleys in Antarctica (Colesie et al. 2014). However, Chroococcales were slightly more conspicuous in the well-developed soil crust (site SC4). The dominance of Chroococcales in lichenized soil crusts have been already shown in temperate regions (Bastida et al. 2014). On the other hand, the relative abundance of Oscillatoriales and Synechococcales (coccoid forms) gradually decreased from poorly developed soil crust (site SC1) to mid-developed soil crusts (sites SC2 and SC3, respectively) but greatly increased in the well-developed soil crust (site SC4). The opposite trend was observed for the relative abundance of Chroococcidiopsidales which increased from site SC1 to site SC3, and slightly decreased in site SC4. Based on 16S rRNA clone libraries, *Chroococcidiopsis* sequences appeared to be abundant in more developed soil crusts (Redfield et al. 2002; Yeager et al. 2004).

At the phylotype level, all soil crust samples were dominated by OTUs related to filamentous cyanobacteria from the genus *Leptolyngbya* (Table 2; Table S2, Supporting Information), common cyanobacteria in soil crusts around the world (Yeager et al. 2004; Kaštovská et al. 2005; Newsham, Pearce and Bridge 2010; Williams and Eldridge 2011; Strunecky, Elster and Komarek 2012; Osorio-Santos et al. 2014). The widespread filamentous cyanobacteria from the genus *Phormidium* were most abundant in the well-developed soil crust (site SC4). The sequence of OTU2, dominant in site SC1, was 98.9% similar to *L. antarctica* ANT.L67.1 isolated from an Antarctic microbial mat (Taton et al. 2006). This morphospecies is ubiquitous in Antarctic lakes (Anagnostidis and Komarek 1988) and has been suggested as endemic to the Antarctic continent (Komarek 2007). However, the sequence of OTU2 has already been found in lake from an Himalayan cold desert (Singh et al. 2014b) and in a freshwater stromatolite sample from Spain (Santos et al. 2010), but had not yet been reported in soil crusts. The OTU28 was related to a strain

of *Loriellopsis cavernicula* isolated from a cave in Spain (98.6% similarity) (Lamprinou et al. 2011), which has likewise never been reported in polar soil crusts. On the other hand, several dominant OTUs (4, 12, 21 and 41), identified as heterocyst-forming cyanobacteria from the order Nostocales, have been previously observed in Arctic soils on the basis of their morphology (Elster et al. 1999; Zielke et al. 2005; Patova and Beljakova 2006; Řeháková, Chlumska and Dolezal 2011).

Relative abundances of OTU2 (98.9% similarity to *L. antarctica*), and OTU11 (100% similarity to *Oscillatoria* sp.) decreased with soil crust development. Apparently, these species are able to live in coarse and unstable soil crusts and they even dominated site SC1. This might be due to the reduced competition with other soil cyanobacteria. Interestingly, a sequence very similar to OTU11 has been found amongst the 25 most dominant bacterial OTUs in the biocrusts over permafrost soils of the High Arctic polar desert (Steven et al. 2013). On the contrary, the relative abundance of OTU3 (98.4% similar to *L. nostocorum*) and OTU17 (98.6% similar to *Phormidium* sp.) increased with soil crust development. The morphospecies *L. nostocorum* has already been found and isolated from lichenized well-developed soil crusts in USA (Flechtner, Johansen and Belnap 2008). Similarly, morphotypes related to the Oscillatoriales were more abundant in vegetated soil crusts than in poorly developed ones (Kaštovská et al. 2005).

An important caveat of trying to connect molecular data with morphotypes is that the databases of 16S rRNA gene sequences are still lacunous. If the sequence corresponding to a morphotype is unknown because no culture has yet been sequenced, it might be retrieved from environmental samples but will remain unidentified or loosely affiliated with a known morphotype, though it, in fact, corresponds to a quite different morphology. Another problem is that the strain identification linked to sequences in databases is not always documented with a morphological description.

A surprising result obtained in this study was the presence of OTUs assigned to the order Gloeobacterales in studied soil crusts. Only two species belonging to this order have been described so far: *Gloeobacter violaceus*, isolated fromcalcareous rock in Switzerland (Rippka, Waterbury and Cohen-Bazire 1974) and *G. kilaueensis*, found in epilithic biofilm in a lava cave in Kilauea Caldera, Hawaii (Saw et al. 2013). Gloeobacter-like sequences have been already reported in Ellesmere Island (High Arctic) from mats of Ward Hunt Lake (Jungblut, Lovejoy

and Vincent 2010; Lionard et al. 2012), but have never been recorded in Arctic soil crusts. However, given that OTUs obtained in this study had 95.4% or lower identity to the database sequences of Gloeobacterales, their classification within this order cannot be claimed with confidence. Nevertheless, the possibility of Gloeobacterales being present in Arctic soil crusts should not be completely disregarded.

Phylotype richness estimates differed greatly between the four sites (Fig. 3). Site SC1 with poorly developed soil crust had the lowest phylotype richness, probably as a consequence of low concentration of nutrients, which greatly influence abundance of soil microbial community (Housman et al. 2006; Newsham, Pearce and Bridge 2010; Pietrasiak et al. 2013). Phylotype richness estimates were highest in the mid-developed soil crusts (sites SC2 and SC3) consolidated by a mixture of lichens and cyanobacteria, and decreased in site SC4 with stable lichenized soil crust. In lichenized soil crusts, cyanobacteria might compete with lichens for water, nutrients and light. Competition could be an important factor shaping the structure of microbial communities, and might explain the decrease in phylotype richness observed in well-developed soil crust, despite the higher availability of nutrients. However, there is no knowledge how competition of soil crust components influences the cyanobacterial community composition.

Multivariate analysis at the phylotype level showed that the four sites harbour distinct cyanobacterial communities (Fig. 4), mirroring the differences in chemical characteristics (Table 1) and development stage of soil crusts (Fig. 1b). Community dissimilaritieswere mostly explained by pH, total organic carbon and water content, as it was found by other authors (Li et al. 2010; Ganzert, Bajerski and Wagner 2014). Although a neutral to slightly alkaline pH interval is considered to be the optimum for cyanobacterial growth (Burja et al. 2002; Singh et al. 2014a), apparently, even small fluctuations in pH can influence cyanobacterial community composition. It has been suggested that the abundance of filamentous cyanobacteria increases with increasing pH (Nayak and Prasanna 2007). Our results agree with this observation, given the higher relative abundance of several OTUs related to filamentous cyanobacteria from the orders Synechococcales (OTUs 2, 8, 34 and 127) and Oscillatoriales (OTUs 5 and 11) in samples with higher pH (Table 2). Water availability is an important factor for Arctic soil crusts and positively influences soil crust community growth (Fischer and Subbotina 2014). Lack of water might cause a deceleration of the carbon production processes as already

shown at 20% water content in High Arctic soil crusts, whereas a water content of 50% appeared optimal for photosynthesis and respiration rates (Yoshitake et al. 2010). Thus, it seems that soil crusts in this study were at the lower end of the values needed for growth (19.6%–35.3%). We can also infer that the nitrogen-fixation could be potentially more active in the later developmental stages due to a higher water content there (Stewart et al. 2014). In addition, the total organic carbon also increased with crust development (from site SC1 to SC4). Cyanobacteria are physiologically active only when wet (Belnap and Lange 2001) and, thus, it is logical to observe this parallel increase.

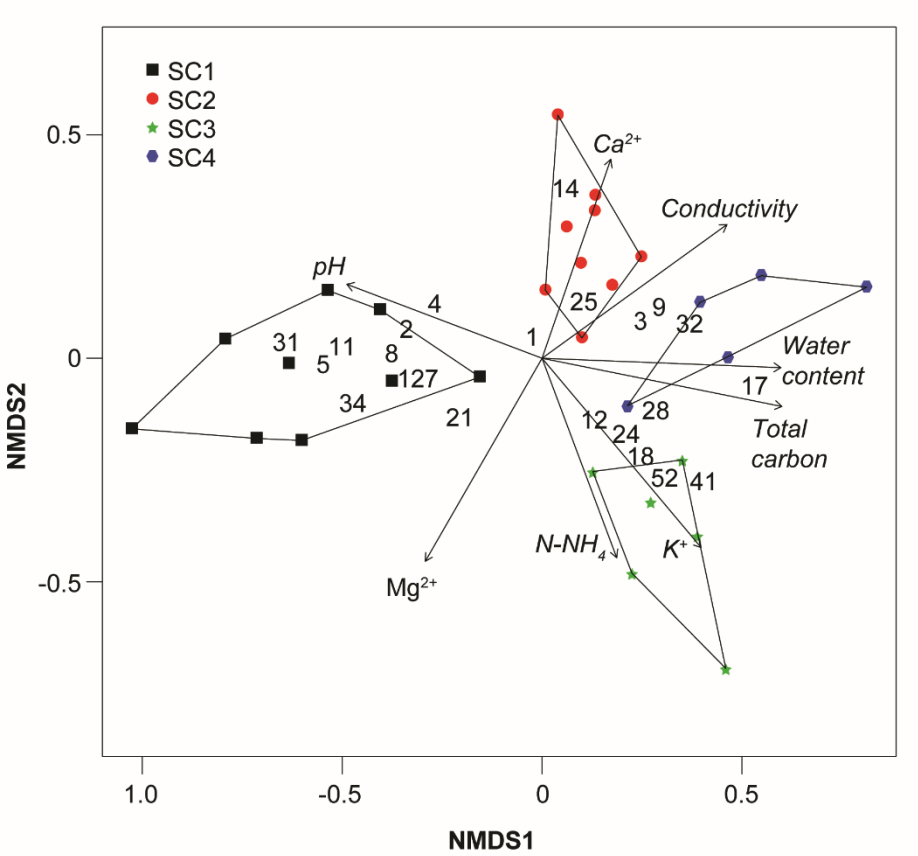

Fig. 4. Non-metric multidimensional analysis. Numbers indicate OTUs with statistically different relative abundances across the four sites (Table 2).

Conclusions

Our results indicate that the cyanobacterial community composition of Arctic soil crusts from the same type of substrate is diverse and changes considerably with the stage of development. Moreover, we have found that pH, total organic carbon and water content were the key parameters shaping the cyanobacterial communities. Lower concentration of total organic carbon, lower water content and higher pH resulted in lower cyanobacterial richness in poorly developed soil crusts where filamentous cyanobacteria were dominant. The increase of water content and concentration of total organic carbon and decrease in pH resulted in higher cyanobacterial richness in mid developed soil crusts, where, despite the dominance of *Leptolyngbya* sp, other filamentous and heterocyst-forming cyanobacteria were highly abundant. However, in stable well-developed soil crusts, in spite of the higher nutrient availability, the cyanobacterial richness appears to decrease, probably due to the competition with dense populations of lichens.

Supplementary data

Supplementary data are available at FEMSEC online.

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Nitrogen fixation and diurnal changes of photosynthetic activity in Arctic soil crusts at different development stage

Ekaterina Pushkareva [a, *], Jana Kvíderova [a, b], Miloslav Simek [c], Josef Elster [a, b]

[a] *Centre for Polar Ecology, University of South Bohemia, České Budějovice, Czech Republic*
[b] *Institute of Botany, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic*
[c] *Institute of Soil Biology, Biology Centre CAS, České Budějovice, Czech Republic*

***Corresponding author:** E. Pushkareva <puekse@gmail.com>

Abstract

Nitrogen fixation and photosynthesis provided by microbial phototrophs (cyanobacteria and eukaryotic microalgae) are important processes occurring in Arctic soil crusts. Here, we describe and compare these processes in biological soil crusts from Central Svalbard at different stages of development. The gradient from poorly-developed to well-developed soil crusts was accompanied by the changes in biovolume of microbial phototrophs, nitrogenase and photosynthetic activity. The lowest biovolume of microbial phototrophs was detected in poorly-developed soil crusts as a consequence of the initial stage of soil colonization. The biovolume initially increased during the soil crust development but decreased in well-developed lichenized soil crusts. However, nitrogenase activity decreased from poorly to more developed soil crusts. Diurnal courses of photosynthetic activity differed among the soil crust types showing shifts in diurnal minima and maxima; the poorly-developed soil crust reacted faster to changes in temperature and PAR. In spite of different microclimatic conditions during the measurements, temperature was the main factor influencing photosynthetic activity while the effect of PAR was not significant. Higher temperatures led to inhibition of photosynthetic activity and increased energy dissipation, indicating acclimation/adaptation of the soil crust photosynthetic microorganisms to a cold environment.

Key words: Soil crust; Arctic; Photosynthetic activity; Nitrogen fixation; Microbial phototrophs; Soil crust development.

1. Introduction

Biological soil crusts are one of the most important components in Arctic desert and semi desert ecosystems [1]. These crusts consist of soil particles held together by the main soil crust components such as microbial phototrophs (the term includes both cyanobacteria and eukaryotic microalgae), fungi, mosses, liverworts and lichens [2]. Soil crust organisms provide important processes for the Arctic desert and semi desert ecosystems, for instance, atmospheric nitrogen fixation as a source of nitrogen and photosynthesis as a source of organic carbon, and hence energy, for consumers and decomposers [3].

Low temperature, lack of water and high photosynthetically active radiation (PAR, 400-700 nm)/UV-radiation are typical stressors in the extreme polar environments where biological soil crusts are frequently present [1,4]. These factors inhibit biological processes and cause changes in diversity, abundance and ecophysiological performance of soil crust communities in the upper layers of soil crusts [5-7]. However, soil crust microbial phototrophs, due to a diverse range of ecological and physiological life strategies, manifest an ability to tolerate stressful conditions [1]. For example, the heterocystous cyanobacteria *Scytonema* sp. and *Nostoc* sp, produce the dark yellowish pigment scytonemin in their sheaths which acts as an UV-screen [8]. These taxa consequently can survive on the soil surface, and are the organisms primarily responsible for the darkening of the soil surface associated with biological soil crusts [9]. Another strategy to avoid or minimize stressful conditions is vertical migration of filaments beneath the soil surface. This behavior is typical for cyanobacteria from the order Oscillatoriales, because they are capable of a gliding movement and can achieve a balance between receiving sufficient light for photosynthesis and avoiding harmful UV radiation and photooxidation [10,11]. Similarly to cyanobacteria, eukaryotic microalgae, e.g. *Klebsormidium* sp. and *Zygnema* sp., form multi-layered structures or are interwoven within the upper millimeters of soil for protection from UV radiation [12,13]. Increased PAR/UV radiation causes serious damage to photosystem II (PSII) in photosynthetic microorganisms and, hence, decreased photosynthetic efficiency [14]. The depression of photosynthetic efficiency usually occurs at midday [15].

Thus, the prolonged day during the summer season in the Arctic results in decreased photosynthetic activity [16]. These changes occur rapidly, either due to short-term oscillations caused by clouds, or due to diurnal irradiance cycles. Therefore, variable chlorophyll fluorescence-based approaches may provide valuable data on changes in photosynthetic activity [16]. For detailed and rapid insight into the light energy utilization, i.e. either its utilization in photosynthetic reactions, or its dissipation as heat, the chlorophyll fluorescence fast-transient analysis (OJIP transient) seems to be suitable [17]. Moreover, knowledge of the diurnal changes in photosynthetic activity is crucial to establish reliable primary productivity models [18].

Soil crust community composition and abundance varies depending on the disturbance rate (water-wind erosion, soil cryodisturbance, anthropogenic activities, animal grazing, etc.) from poorly-developed to well-developed soil crusts [19]. Although the process of soil crust development in time and space has been described [2], little is known about the factors driving small scale patterns of soil crust community structure and associated ecological processes. The poorly-developed soil crusts usually have low nutrient content and low abundance of microbial phototrophs dominated by cyanobacteria. These soil crusts are light in colour and have low capability to mitigate damage caused by UV radiation. In the next step of development mosses and lichens begin to appear and the soil crusts acquire darker colour due to higher abundance of microbial phototrophs which increase net photosynthetic exchange rates. In the latest development stage moss and soil lichen crusts form mosaic patterns with higher small-scale variation [20].

Here, we hypothesized that composition and biovolume of microbial phototrophs and associated ecological processes (photosynthesis and nitrogenase activity) might follow the gradient of soil crust development in the High Arctic. Moreover, we studied how individual environmental parameters (soil temperature and PAR) influence photosynthetic (expressed as variable chlorophyll fluorescence parameters) and nitrogenase activities as proxies of primary production and nitrogen fixation, with respect to diurnal cycles. Since the application of the OJIP transient measurement is proposed as possible approach for study of photosynthetic processes, for the first time we applied it in the Artic soil crusts correlating the shared parameter (maximum quantum yield) with the standard protocol.

2. Material and Methods

2.1. Field site description and sampling

The sampling sites were located in the vicinity of Petunia Bay, the northwestern branch of Billefjorden, Dickson Land, Svalbard. Soil crusts cover a substantial part of the area. The mean annual air temperature is approximately -6.5 ºC. An active period when liquid water is available normally starts in June and lasts until the end of August or middle of September (90-100 days) with mean soil temperatures of 4-6 ºC and air temperatures of 5-7 ºC [23].

The field experiments and sample collection were conducted in the end of July (for sites SC1 and SC2) and the beginning of August (for sites SC3 and SC4) 2013. Soil crusts were selected in the field (area of 5 x 5 m per each site) based on their distinct macroscopic features and represented gradient (from poorly-developed to well developed) based on chemical parameters and cyanobacterial community composition listed in Table 1 [19]. Pictures of studied soil crusts and their detailed description including soil chemistry have been already presented in a previous publication [19].

In order to determine the effect of environmental factors on the photosynthetic activity of biological soil crusts, temperature and photosynthetically active radiation (PAR) were measured at the soil surface in each sampling site. The soil temperature was measured using Minikin T dataloggers (Environmental Monitoring Systems, Czech Republic) positioned on the soil crust surface. PAR was measured using a PU-550 light meter (Metra Blansko, Czech Republic) equipped with a custom-made quantum probe.

Table 1. Sampling sites description is taken from the previous publication [19].

Site	GPS coordinates	Soil crust description	Chemical parameters				The most relatively abundant cyanobacterial species
			pH	Corg, %	N-NH_4, µg kg^{-1}	Water content, %	
SC1	78º 40′ 57″ N 16º 26′ 39″ E	Poor-developed, light-colored, no lichen presence	8.1	4.8	3.5	19.6	*Leptolyngbya* sp., *L. antarctica*, *L. subtilissima*, *Coleofasciculus chthonoplastes*
SC2	78º 41′ 31″ N 16º 26′ 47″ E	Mid-developed, brown, with presence of lichens	8.0	11.5	4.0	30.8	*Leptolyngbya* sp., *L. antarctica*, *L. nostocorum*, *Calothrix* sp.
SC3	78º 41′ 36″ N 16º 26′ 12″ E	Mid-developed, brown, with presence of lichens	7.8	15.6	5.7	35.3	*Leptolyngbya* sp., *L. nostocorum*, *Stigonema ocellatum*, *Nostoc* sp.
SC4	78º 41′ 54″ N 16º 26′ 22″ E	Well-developed, dark-colored, dense lichen cover	7.5	16.9	3.4	34.5	*Phormidium* sp., *Leptolyngbya* sp., *Leptolyngbya nostocorum*

After measurements of photosynthetic activity in the field, the same soil crust samples (diameter of 1 cm, depth of 2 cm, weight of around 4 g) were collected and brought to the Czech Station in Svalbard to measure nitrogenase activity. After that, samples were placed into new zip bags, kept at 20°C and transported in dry ice to the Czech Republic.

2.2. Biovolume of microbial phototrophs in soil crusts

Biovolume of microbial phototrophs in soil crusts was estimated by light and epifluorescence microscopy (Olympus BX 51). A non-staining method was employed using chlorophyll autofluorescence according to Kaštovská et al. [6]. 1 g of soil was diluted in 4 ml of distilled water and mixed thoroughly. A total of 20 ml of the soil solution was used for the microscopy. Filter cube (Olympus, MWB) with blue excitation at 450-480 nm (emission 515+ nm) was tested for eukaryotic microalgae and filter cube (Olympus, MWG) with green excitation at 510-550 nm (emission 590+ nm) was tested for cyanobacteria in soil crust samples. However, due to coarse soil texture it was not possible to distinguish eukaryotic microalgal cells by the filter with blue excitation. Thus, only the filter with green excitation was used, which allowed the identification of cyanobacteria into three groups according to their cell morphology: unicellular, filamentous and heterocystous cyanobacteria. Under this filter it was also possible to distinguish diatoms and coccoid microalgae (green algae Chlorophyta and yellow-green algae Xanthophyceae) within the eukaryotic microalgae. Basic geometric equations for cylinders with hemispherical ends and spheres were applied to calculate the biovolume per soil samples.

2.3. Nitrogenase activity

Nitrogenase activity was measured by the acetylene-ethylene reduction assay [24] in nine soil crust samples per each site (36 soil samples in total). First, 5 ml of commercial-grade acetylene was added by syringe to 100 ml glass flasks containing soil crust samples (3.85 g each) and closed with butyl rubber stoppers. Headspace samples of 5 ml were collected by syringe and transferred into 5 ml evacuated LABCO vials after 30, 90 and 180 min of incubation. Ethylene in the headspace samples was quantified with a Hewlett Packard 5890 gas chromatograph (Hewlett Packard, USA)

equipped with a flame ionization detector. Nitrogenase activity was expressed in nmol C_2H_4 g^{-1} soil crusts h^{-1}.

2.4. Photosynthetic measurement

Photosynthetic activity of the soil crusts was measured in nine replicates in each site every 6 h (36-48 h in total) under field conditions using a hand-held FluorPen FP 100 fluorometer (Photon Systems Instruments, Czech Republic). To minimize the error due to different days of measurements, we tried to choose similar weather conditions to conduct experiments. Two parameters, maximum quantum yield (F_V/F_M) and actual quantum yield (Φ_2), were determined using the FluorPen QY protocol. After each measurement of Φ_2, soil crust samples were covered to avoid exposure to light, dark-adapted for 15 min and then the F_V/F_M was measured.

The parameters were calculated according to Rohacek [25]. The F_V/F_M, which reflects the actual physiological state, was calculated using the equation:

$$F_V/F_M = (F_M - F_0)/F_M \quad (1)$$

In which F_0 is the minimum fluorescence and F_M is the maximum fluorescence after dark adaptation.

Φ_2 reflects the actual photosynthetic performance of photosystem II in light and was calculated according to the equation:

$$\Phi_2 = (F'_M - F_S)/(F'_M) \quad (2)$$

In which F_S is the steady-state fluorescence and F'_M is the maximum fluorescence in ambient light.

The Φ_2 values were used to calculate the relative electron transport rate (rETR), which is a very raw proxy of photosynthesis, based on the equation present in Maxwell and Johnson [26]:

$$rETR = 0.5 \times \Phi_2 \times PFD \quad (3)$$

In which *PFD* is the actual irradiance (PAR) and 0.5 is a factor reflecting the partitioning of energy between photosystems.

In order to screen the photosynthetic processes in detail, the OJIP transient was measured using the FluorPen OJIP protocol lasting two seconds. The studied parameters were calculated according to Strasser et al. [17]. The parameters and equations are summarized in Suppl. Table 1.

In order to distinguish between the QY and OJIP maximum quantum yields, F_V/F_M was used for values obtained using the QY protocol while φ_{Po} was used for values obtained using the OJIP protocol due to differences between the measurement procedures [27]. To find any diurnal

courses of individual fluorescence parameters, the measured fluorescence parameters were normalized to their initial state at the beginning of the measurement period. The mean values of the fluorescence parameters and PAR for each measurement time was used for fitting by a sine function using SigmaPlot 10 (Systat Software, USA). The general equation was

$$y = a + b \times \sin(t + c) \quad (4)$$

In which *t* is time, *y* is the calculated value of a fluorescence parameter or PAR, and *a*, *b* and *c* are parameters describing the shape of the curve.

2.5. Statistical analysis

Differences in microalgal abundance, nitrogenase activity and the photosynthetic parameters between the four sites were determined using one-way ANOVA followed by Tukey's pairwise posthoc tests using R software [28].

3. Results

3.1. Microclimate during in situ measurements

Soil temperature during the measurement period varied from 4.2 to 11.6 ^{o}C and was positively correlated to PAR (Suppl. Fig. 1). The lowest soil temperature as well as the lowest PAR were detected in site SC1. However, these parameters increased in sites SC2 and SC3 and slightly decreased in site SC4. In site SC4 relationship between irradiance (PAR) and soil surface temperature differed from sites SC1, SC2 and SC3. These differences could be caused by light reflectance contrasts since soil surface in site SC4 was covered by pale lichens.

3.2. Microalgal abundance

Microalgal biovolume differed across the sites (Fig. 1) with total biovolume ranging from 10^8 to 10^9 µm^3 g^{-1} soil (Fig. 1a). Cyanobacteria were the most abundant group of microbial phototrophs contributing from 67 to 95% of the quantified cells per site and were considerably more abundant in sites SC2 and SC3 (Fig. 1b). Moreover, unicellular cyanobacteria were the dominant in studied soil crusts except site SC1 where filamentous cyanobacteria were the most abundant. The biovolume of coccoid eukaryotic microalgae increased with soil crust development.

However, their percentage to other microbial phototrophs within the sites was low except for site SC4 where they accounted for 14% of the total biovolume.

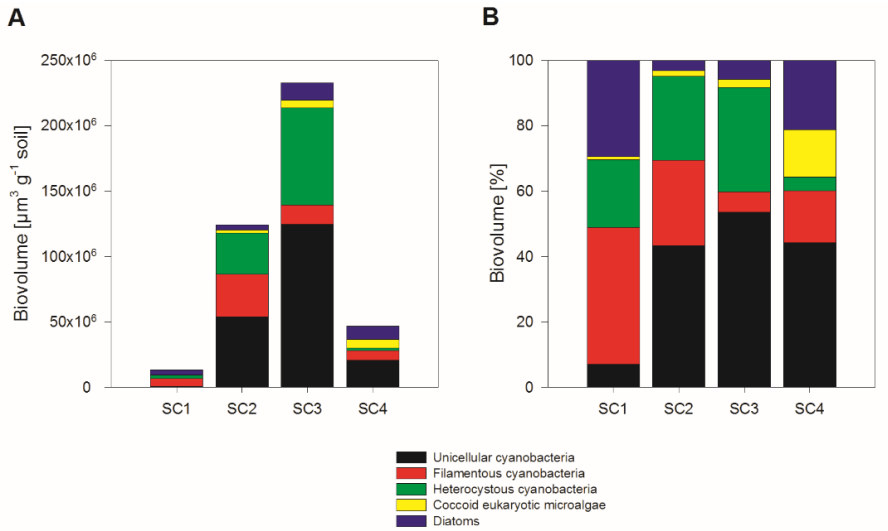

Fig. 1. Biovolume of microbial phototrophs in soil crust samples expressed in $mm^3\ g^{-1}$ soil (A) and % (B).

3.3. Nitrogenase activity in soil crusts

Nitrogenase activity gradually decreased from poorly developed (site SC1) to mid-developed soil crust (site SC3) (Fig. 2). Site SC3 had significantly lower nitrogenase activity (0.39 nmol g^{-1} h^{-1}) than in sites SC1 and SC2. However, measurements for nine soil crust samples from the site SC4 presented values equal to 0.

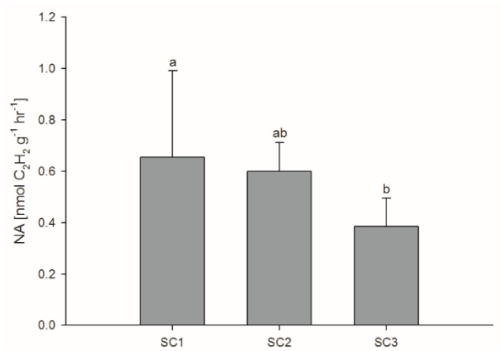

Fig. 2. Nitrogenase activity (mean ± SD, n ¼ 9) in different soil crusts. Site SC4 is not present in the figure since the values were equal to 0. The same letters indicate no statistical difference between groups according to one-way ANOVA followed by Tukey's pairwise post-hoc tests ($p < 0.05$).

3.4. Photosynthetic activity and its diurnal changes in the soil crusts

3.4.1. F_V/F_M, Φ_2 and rETR

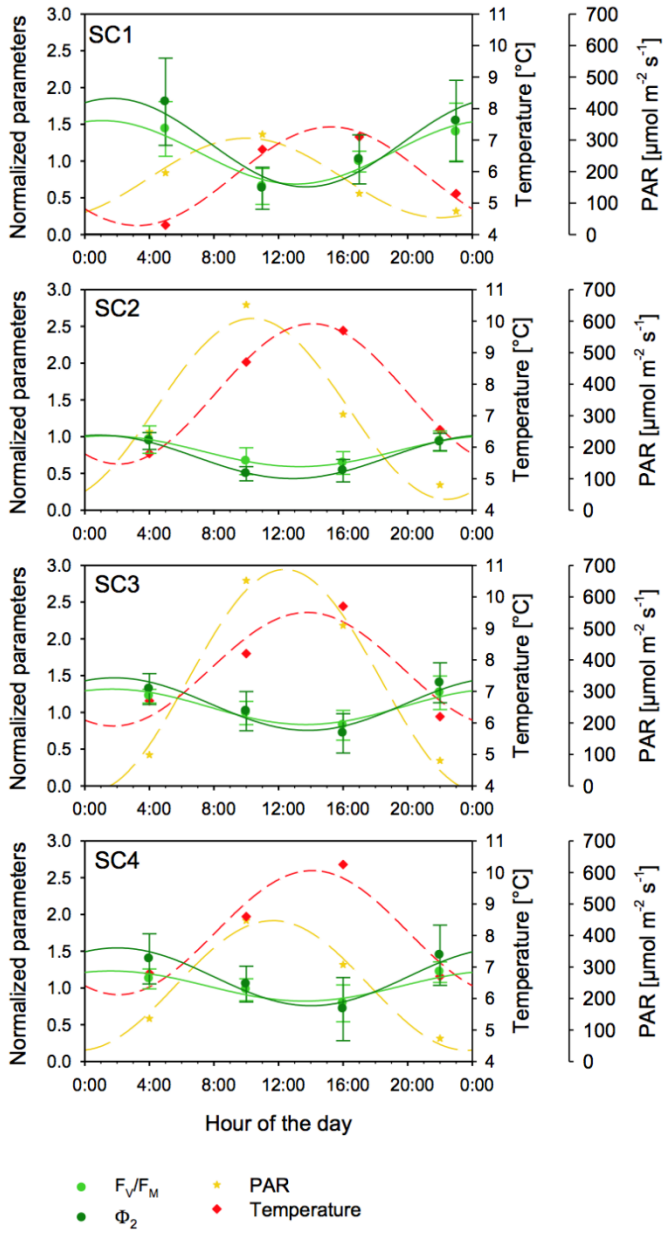

Fig. 3. The diurnal changes of FV/FM and F2 (both mean ± SD, n ¼ 9) in each soil crust type and their relation to temperature and PAR changes. The data were normalized to initial stage (initial stage = 1). Lines indicate the predicted diel cycle.

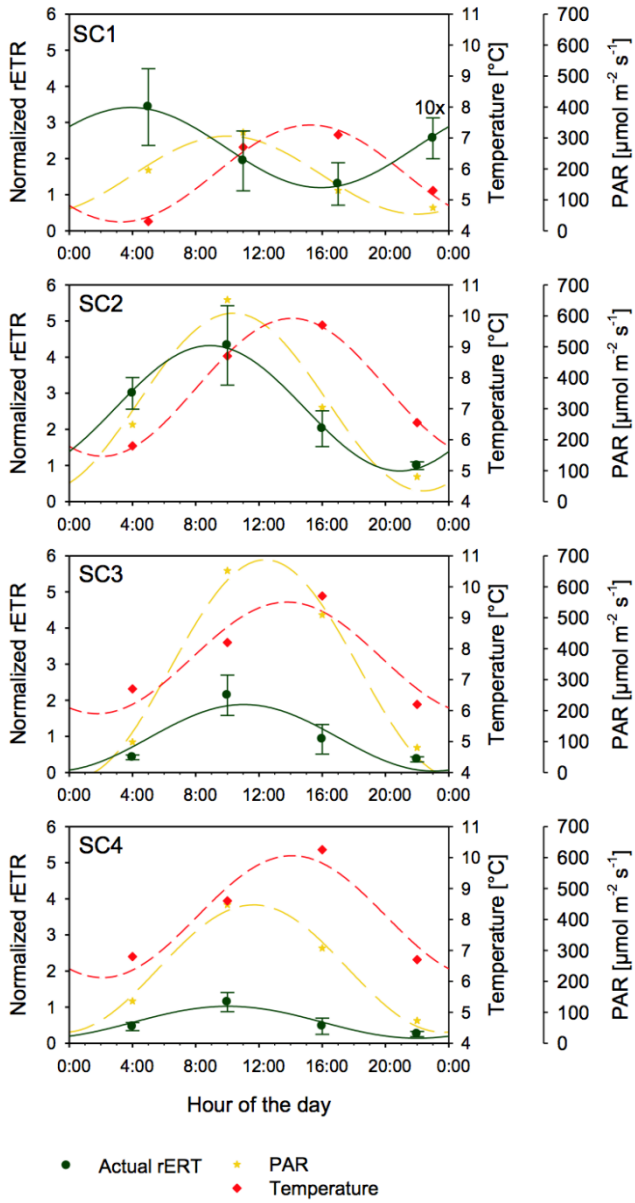

Fig. 4. Diurnal changes of actual rETR (both mean ± SD, n = 9) in each soil crust type and their relation to temperature and PAR changes. The data were normalized to initial stage (initial stage = 1). Lines indicate the predicted diel cycle. Numbers at the error bars indicate the multiplication factor for a given error bar. If the multiplication factor is not mentioned, it is equal to 1.

The F_V/F_M and Φ_2 values were relatively low, ranging from 0.25 to 0.47 and from 0.22 to 0.34, respectively, indicating serious stress encountered

in the field. Both F_V/F_M and Φ_2 followed the diurnal cycles of temperature and irradiance (Fig. 3) and these changes were significant in all studied soil crusts. Both parameters were negatively correlated to temperature and PAR (Fig. 3). Several hours delay was observed between the maximum PAR and the minima of the parameters, while the temperature maximum corresponded well to their minima. No statistically significant correlation was observed between the fluorescence parameters and PAR, with the exception of a strong negative correlation between PAR and F2 ($r = -0.856$, $P = 0.030$) in site SC2.

The maximum actual rETR in site SC1 was observed around midnight and early morning, indicating possible photoinhibition, in spite of the lower irradiances encountered during the measurement. These diurnal changes were significant in all soil crust types ($F = 35.61$, $P < 0.001$ for SC1; $F = 3137$, $P < 0.001$ for SC2; $F = 67.44$, $P < 0.001$ for SC3; $F = 22.79$, $P < 0.001$ for SC4). On the other hand, the maximum actual rETRs were observed in the highest PARs in sites SC2, SC3 and SC4, despite decreased F_V/F_M and Φ_2. Therefore, these types of crust were probably not photoinhibited even at relatively high irradiances (Fig. 4). The rETR was strongly positively correlated to PAR in sites SC2 ($r = 0.935$, $P = 0.002$) and SC3 ($r = 0.957$, $P = 0.003$). Other correlations were not significant.

3.4.2. OJIP parameters

The OJIP parameters in the studied soil crusts also followed the temperature and PAR diurnal cycles, however, not all responses to these diurnal changes were significant in all soil crust types. All soil crusts showed significant changes in parameters such as φ_{Po}, F_M/F_0 and F_V/F_0, and no reaction was detected for V_J, M_0, ψ_0 and TR_0/RC (Tables 2 and 3). The OJIP parameters were predominantly significantly correlated to temperature, while the correlations with PAR were not usually significant (Suppl. Table 2).

As in the case of F_V/F_M, Φ_2 and rETR, the range of the photosynthetic activity response was different between the studied sites (Table 2). Despite the relatively low temperature and PAR variability (2.9 ^oC and 281 µmol m^{-2} s^{-1}, respectively) during the measurements in site SC1 with poorly-developed soil crust, changes in the photosynthetic activity were usually more notable there in comparison with the more developed soil crust types. The variability sometimes reached tens of percents of the initial value at the beginning of the measurement (Table 2; Suppl. Fig. 2-7).

Table 2. The statistical significance (P-value and F-value) of diurnal changes of fluorescence parameters in different types of soil crusts (one-way ANOVA; n=9 in each soil crust type). The statistically significant changes (P<0.05) are marked bold. For parameter definition and physiological meaning, refer to Supplementary material 1.

	Development stage			
	poor →			well
Parameter	SC1	SC2	SC3	SC4
F_V/F_M	**P<0.001** F=20.60	**P<0.001** F=12.70	**P<0.001** F=10.70	**P<0.001** F=22.79
Φ_2	**P<0.001** F=18.00	**P<0.001** F=47.50	**P<0.001** F=19.72	**P<0.001** F=30.68
φ_{Po}	**P<0.001** F=13.48	**P<0.001** F=8.387	**P<0.001** F=4.971	**P=0.001** F=3.965
F_M/F_0	**P<0.001** F=16.46	**P<0.001** F=6.568	**P<0.001** F=5.314	**P=0.026** F=2.477
F_V/F_0	**P<0.001** F=13.11	**P<0.001** F=8.024	**P<0.001** F=4.669	**P=0.006** F=3.163
V_J	P=0.072 F=2.178	P=0.059 F=2.175	P=0.303 F=1.223	P=0.149 F=1.610
V_I	**P=0.004** F=3.984	**P=0.018** F=2.830	P=0.586 F=0.805	P=0.087 F=1.885
M_0	P=0.699 F=0.601	P=0.470 F=0.945	P=0.762 F=0.589	P=0.877 F=0.434
ψ_0	P=0.050 F=2.407	P=0.063 F=2.138	P=0.316 F=1.200	P=0.553 F=0.848
φ_{Eo}	**P=0.038** F=2.576	**P=0.044** F=2.331	P=0.177 F=1.519	P=0.720 F=0.641
φ_{Do}	**P<0.001** F=16.23	**P<0.001** F=5.778	**P<0.001** F=5.782	P=0.060 F=2.068
ABS/RC	**P=0.026** F=2.819	P=0.226 F=1.413	P=0.426 F=1.020	**P=0.033** F=2.372
TR_0/RC	P=0.765 F=0.513	P=0.824 F=0.475	P=0.862 F=0.456	P=0.998 F=0.097
ET_0/RC	P=0.390 F=1.067	P=0.971 F=0.213	P=0.746 F=0.610	**P=0.018** F=2.656
DI_0/RC	**P=0.007** F=3.680	**P=0.044** F=2.332	P=0.137 F=1.651	**P=0.011** F=2.914

In all soil crust types, the decrease in photosynthetic activity, i.e. lower values of photochemical quantum yields, was accompanied by increased energy dissipation (Suppl. Fig. 2-7). A slightly reduced V_J and elevated ψ_0 might indicate faster electron transport due to increased temperatures (Suppl. Fig. 4-5). However, the response to environmental changes was more delayed in more developed soil crust types (Suppl. Fig. 2-7).

Table 3. The highest positive and negative differences in fluorescence parameters in each soil crust type (in %). The values were normalized to initial state at the beginning of the measurements. For parameter definition and physiological meaning, refer to Supplementary material 1.

	Soil crust development stage poor → well							
	Site SC1		Site SC2		Site SC3		Site SC4	
	Difference		Difference		Difference		Difference	
Parameter	positive	negative	positive	negative	positive	negative	positive	negative
φ_{Po}	39.6	-27.3	5.4	-39.8	18.8	-30.3	14.9	-24.9
F_M/F_0	12.1	-7.3	2.2	-17.0	9.8	-12.5	12.8	-18.2
F_V/F_0	57.9	-31.8	9.3	-48.1	31.7	-35.5	32.3	-35.5
V_J	21.9	0.0	10.3	-17.1	30.0	0.0	18.8	-8.7
V_I	14.7	-4.3	6.8	-10.4	10.2	-1.6	10.3	-0.9
M_0	46.6	0.0	59.1	-11.6	39.9	-8.3	32.1	-2.7
ψ_0	1.5	-17.4	17.2	-6.8	0.0	-20.5	12.4	-14.0
φ_{Eo}	18.0	-23.9	0.0	-29.8	5.7	-29.5	13.5	-13.8
φ_{Do}	8.2	-10.6	21.9	-1.4	16.2	-8.4	27.7	-8.3
ABS/RC	74.6	-24.2	106.0	0.0	51.8	-11.2	53.7	-11.8
TR_0/RC	18.6	-9.9	38.5	0.0	10.8	-16.2	6.0	-2.5
ET_0/RC	16.9	-19.9	25.6	0.0	0.0	-26.8	18.5	-15.7
DI_0/RC	91.1	-31.4	157.9	0.0	85.8	-16.6	109.6	-15.1

3.4.3. QY and OJIP protocols comparison

Due to the different measurement methods of the maximum quantum yield, the values of F_V/F_M and φ_{Po} for each measurement were compared. The values showed strong positive correlation between the F_V/F_M and φ_{Po} for each soil crust type, $r = 0.892$ and $P < 0.001$ for SC1, $r = 0.894$ and $P < 0.001$ for SC2, $r = 0.793$ and $P < 0.001$ for SC3, and finally, $r = 0.850$ and $P < 0.001$ for SC4 (Fig. 5). In general, the values of F_V/F_M were slightly higher than those of φ_{Po}, however the difference between the values did not exceeded 15% on average in all soil crust types.

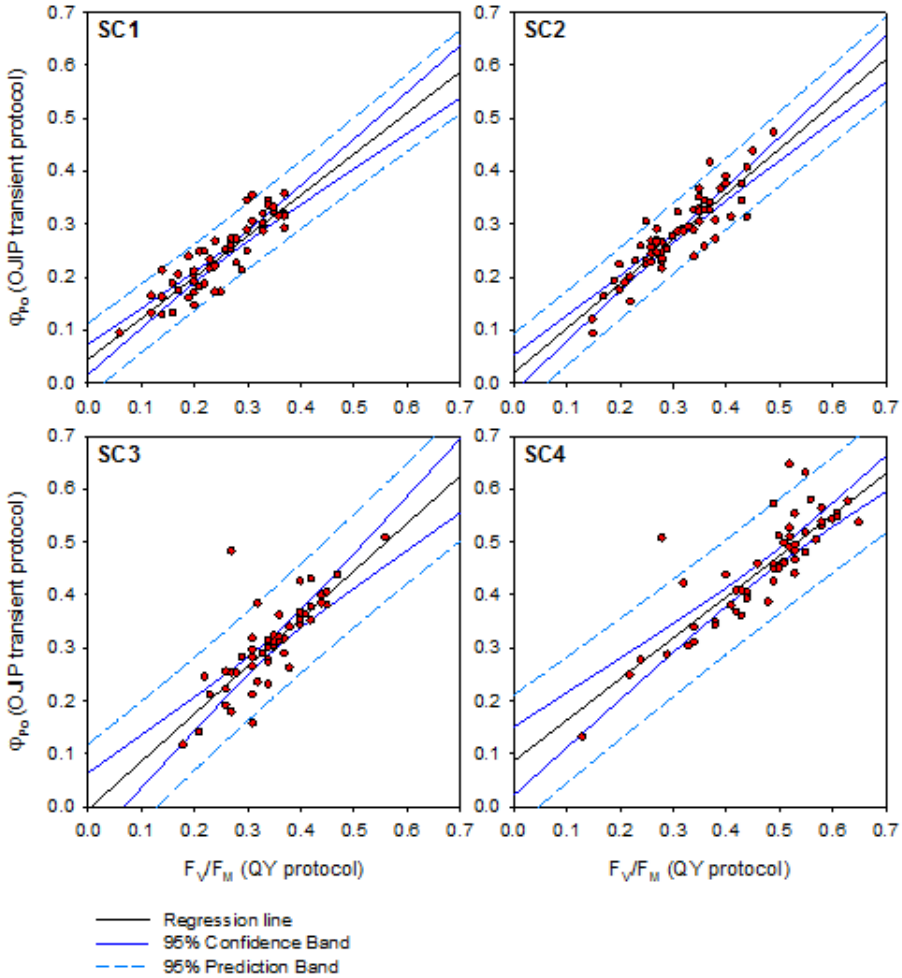

Fig. 5. Correlations between the maximum quantum yield values obtained using either the QY (F_V/F_M) or OJIP transient protocols (φ_{Po}). Each data point represents one measurement.

4. Discussion

The development of biological soil crusts in the High Arctic starts from the more/less abiotic stage of soil crusts with low diversity of microbiota and continues up to a richer and more diverse climax state [1]. Here, we expanded our previous work [19] to provide the knowledge about community structure of microbial phototrophs and associated photosynthetic activity including diurnal cycles and nitrogenase activity in a gradient of soil crust development in the High Arctic.

4.1. Abundance of microbial phototrophs

Biovolume of microbial phototrophs was significantly lower in the poorly-developed soil crust (site SC1). Probably, low nutrient concentrations inhibited growth of the biological soil crust community as it has been similarly shown for the soil crusts formed after glacier retreats [6,33]. The highest biovolume of microbial phototrophs was found in mid-developed soil crusts (site SC3 and SC2, respectively). Although soil crusts from site SC4 were well-developed, the total biovolume of microbial phototrophs decreased, likely due to the dense lichen cover. However, lichens may use microalgae as photobionts, thus, the abundance of unicellular cyanobacteria and coccoid eukaryotic microalgae was relatively high within the site.

Cyanobacteria were the dominant group in all four studied sites (Fig.1). They are the major component of photosynthetic organisms in soil crusts in Svalbard [1,6,19,29,30] and in other polar regions [31,32]. The total biovolume of cyanobacterial cells showed the same trend as cyanobacterial richness of the previously recorded cyanobacterial sequences [19] in the soil samples increasing from poorly-developed soil crusts (site SC1) to more developed soil crusts (sites SC2 and SC3, respectively) before slightly decreasing in well-developed lichenized soil crusts (site SC4). Moreover, unicellular cyanobacteria had the highest biovolume in the soil crusts at mid- or well-developed stages (sites SC2, SC3, SC4) while the relative abundance of the recorded sequences corresponding to unicellular cyanobacteria was low [19]. This apparent discrepancy could be due to the different measurement techniques used. The epifluorescence microscopy method breaks up cyanobacterial colonies into separate cells and, therefore, unicellular forms were the most abundant. The opposite trend was observed for filamentous cyanobacteria. Next-generation sequencing showed their dominance in all four soil crust samples [19], while in this study filamentous cyanobacteria had a lower biovolume in sites SC1, SC2 and SC3 than unicellular and heterocystous forms. Again, this was influenced by the different measurement techniques as mentioned above. However, expectedly, poorly-developed soil crust (site SC1) was dominated by filamentous cyanobacteria, which due to their motility and mucilage production are able to live in frequently disturbed soils [2].

It has been shown in a desert of Mongolia that the biomass of *Nostoc* sp. and eukaryotic microalgae increases following a soil crust succession [34].

Alike, *Microcoleus* sp., *Nostoc* sp. and *Scytonema* sp. were more abundant in dark-coloured than in light-coloured soil crusts from the Colorado Plateau (USA) [22]. Our results showed a similar trend of increasing cyanobacterial biovolume with soil crust development until lichens cover the surface.

4.2. Nitrogenase activity

Nitrogenase activity in the studied soil crusts decreased from the poorly to more developed soil crusts (from site SC1 to site SC3) (Fig. 2). This is in agreement with previous observations of decreasing nitrogenase activity from 3 to 13 years old soil crusts [39]. However, these results differ from another study where greater nitrogenase activity was found in the later successional stages of biological soil crust development [40]. Interestingly, in our study nitrogenase activity in well-developed soil crust (site SC4) was not recorded. We assume that it was caused by dense lichen cover of *Cladonia* sp. which use green algae (*Trebouxia* sp.) as photobionts and thus are not capable to fix nitrogen. Moreover, nitrogenase activity decreases in the dark conditions [41] and therefore lichen cover might prevent penetration of light needed for nitrogen fixing cyanobacteria located under.

Nostocales are the main microorganisms involved in the nitrogen fixation process in the Arctic [36]. In addition, they are widely distributed around the world in different types of soil crusts [9,42,43], but are more abundant in well-developed stages [44]. However, there was the negative correlation of nitrogenase activity and the abundance of heterocystous cyanobacteria in the studied soil crust samples (Figs. 1 and 2). Perhaps, in the poorly-developed soil crust (site SC1), other free-living bacteria/cyanobacteria were responsible for nitrogen fixation and, due to lower competition with cyanobacteria/microalgae and lichens, they dominated the less-developed crusts. Soil nitrogen availability may also affect nitrogenase activity. Nitrogen fixation may decrease in higher nitrogen concentrations. Unfortunately, no data on nitrogen content in the soils under study are available. Another possible explanation of negative correlation is an inhibitory effect of atmospheric oxygen on nitrogen fixation [45]. Thus, in the sites with high biovolume of microbial phototrophs which produce oxygen via photosynthesis the nitrogenase activity was low.

4.3. Photosynthetic activity

In the High Arctic (central Svalbard), metabolic activity of microbial phototrophs in soil crusts usually starts from early snow melt (late May and early June) and lasts as long as water is available in a liquid state (usually middle or the end of September) [1,23]. Despite of the continuous day light in Svalbard during the summer period, there is a large variation in the incoming irradiance and, hence, PAR [23]. This greatly affects the photosynthetic activity of the phototrophic community in the soil ecosystem. The microalgae often have a low concentration of photosynthetic pigments as a consequence of the harsh environmental conditions [46] and the basic indicator of their physiological status, F_V/F_M, is usually low as well. Normally, F_V/F_M under unstressed conditions is about 0.6 for cyanobacteria [47] and about 0.7 for eukaryotic microalgae [27,48]. In our study, F_V/F_M varied significantly during the measured period (from 0.25 to 0.47) being lower due to the stressful conditions of the Arctic environment. The obtained F_V/F_M values were comparable to those measured in *Nostoc* colonies during in situ manipulation experiments [49,50]. However, the Φ_2 values were lower in our study than in the other one from Svalbard [16]. This may be caused by differences in soil crust composition and/or different microclimate conditions during the measurements.

Comparative studies between soil crusts types are rare. F_V/F_M was found not to differ between light- and dark-coloured soil crusts in hot desert [22]. On the other hand, other study showed the increase of photosynthetic parameters with soil crust succession [51]. In our study, the diurnal course of photosynthesis, expressed as rETR, which is considered as a photosynthesis proxy, was depressed in late afternoon in poorly-developed light-coloured soil crusts (site SC1), while the maximum rETR occurred at the highest irradiances in better developed soil crusts (sites SC2, SC3 and SC4). The values of the fluorescence parameters in site SC1 might indicate more stressful conditions and, indeed, the measurements in less developed soil crusts were conducted under lower temperatures and irradiance than in the other sites (Suppl. Fig. 1).

The low temperature may lead to photoinhibition at lower irradiances [52], since photosynthesis is strongly affected by temperature [53]. A negative correlation was observed between temperature and the indicators of photosynthetic efficiency (F_V/F_M and Φ_2) in all studied soil crusts (Fig. 3). That is in agreement with study where authors found the same

correlation in soil crusts from Petunia Bay, Svalbard [16]. The lack of a temperature effect on the rETR in well-developed soil crusts might indicate photosynthetic acclimatization to low temperatures and good photoacclimation [54], as seen from the time difference between the maximum PAR and rETR decrease. The dark colour of the crust and high abundance of Nostocales might indicate the presence of scytonemin, which provides protection against excessive radiation [1,55,56].

The OJIP transients were proposed for field measurements or routine screenings [57]. Our study marks the first time that these transients were used to study photosynthetic activity in soil crusts of the Arctic. In all soil crusts, we observed a decline of light energy utilization in the photochemical reactions and increased non-photochemical energy dissipation in the afternoon. This may be related to increased temperature and excessive PAR, as has been already recorded before in higher plants [58]. Since the rate and efficiency of electron transport increased at higher temperatures, there should not be increased reduction or even over-reduction of the acceptor side of the PSII even in high PAR [57,59]. The soil crust types differed in the amplitude delay of the response. Generally, the amplitude was lower and the response delay lasted longer in the more developed crusts, probably due to better adaptation/acclimation and more efficient active and passive protection mechanisms.

The comparison of the maximum quantum yield values obtained from the QY and OJIP transient protocols proved that the OJIP transients could be a valuable tool for in situ measurements. However, detailed laboratory experiments should be performed, either with a natural community or isolated strains, to decipher the fluorescence signal correctly.

Data on the diurnal periodicity of photosynthesis in the polar regions are rare [16,18,53]. Our observations confirmed periodical changes in photosynthetic activity. The mid-day/afternoon decrease in photosynthesis, caused by a lower photochemical energy utilization decline and non-photochemical energy dissipation, is a common phenomenon [16,18]. The slight differences observed in the diurnal courses of some OJIP parameters, especially in fluxes per active RC, reflected, probably, variability in the OJIP transients due to a different structure of the photosynthetic apparatus in different photosynthetic microorganisms [60] and, hence, different photoacclimation strategies [61,62]. However, the observed differences might be the result of local microclimatic conditions, especially thermal regime.

Despite of the continuous irradiance in the polar summer, diurnal cycles of photosynthesis do occur in marine [18] and terrestrial ecosystems ([16]; this study). Therefore, the following factors must be considered in field measurements: (1) maximum and minimum photosynthesis may shift in daytime during the vegetation season [18]; (2) the microclimate may significantly influence the results ([16,49]; this study); and (3) community structure and the development stage of soil crusts may affect the response to the environment (this study).

5. Conclusions

The results of this study demonstrate the difference between soil crusts at different stages of development in the same type of substrate. Biovolume of microbial phototrophs, dominated by cyanobacteria, increased with soil crust development. However, lichen cover negatively influenced their growth which resulted in a decrease of microbial phototrophs abundance. The opposite trend has been reported for nitrogenase activity, which was the highest in poorly-developed soil crusts. However, no nitrogenase activity was detected in well-developed soil crust, probably due to dense lichen cover with green algae as photobiont. The results indicate that soil crust type influences the response of photosynthesis to diurnal PAR changes. The more profound changes were observed in less developed soil crust types due to a thinner soil crust layer. The shift of photosynthetic maximum/minimum activity in more developed soil crusts may be caused either by the PAR microclimate of the site or biovolume and species composition.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejsobi.2017.02.002.

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Marked primary succession of cyanobacterial communities following glacier retreat in the High Arctic

Igor S Pessi[1], Ekaterina Pushkareva[2], Yannick Lara[1], Fabien Borderie[1,3], Annick Wilmotte[1], and Josef Elster[2,4]

[1]InBioS – Centre for Protein Engineering, University of Liège, Liège, Belgium;

[2]Centre for Polar Ecology, University of South Bohemia, České Budějovice, Czech Republic;

[3]Laboratoire Chrono-environnement, UMR 6249 CNRS Université Bourgogne Franche-Comté UsC INRA, Besançon, France;

[4]Institute of Botany, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic

Abstract

Here we report on the successional trajectories of cyanobacterial communities in biological soil crusts (BSCs) along a 100-year deglaciation gradient in three glacier forefields in central Svalbard, High Arctic. Distance from the glacier terminus was used as a proxy for time since deglaciation and cyanobacterial abundance and community composition were evaluated by epifluorescence microscopy and pyrosequencing of partial 16S rRNA gene sequences. A general increase in BSC nutrient content and cyanobacterial abundance was observed with increasing time since deglaciation. Succession was further characterized by a decrease in phylotype richness and a marked shift in community structure, resulting in a separation between early (10–20 years since deglaciation), mid (30–50 years), and late (80–100 years) communities. Community turnover was explained by a combination of spatio-temporal (time since deglaciation) and environmental (moisture, SOC, SMN, K, and Na) factors, which accounted together for 46.9% of the variation. Phylotypes associated with early communities were related either to potentially novel lineages (< 97.5% similar to sequences currently available on GenBank) or lineages predominantly restricted to polar and alpine biotopes, suggesting that the initial colonization of proglacial soil

is accomplished by cyanobacteria transported from nearby glacial environments. Late communities, on the other hand, included more widely distributed genotypes, which are likely able to establish only after the microenvironment has been modified by the pioneering taxa.

Keywords: cyanobacteria; glacier forefield; High Arctic; high-throughput sequencing; primary succession; proglacial soil

Introduction

Most glaciers in the High Arctic have been retreating and thinning almost uninterruptedly since the end of the "Little Ice Age" (LIA) in the late 19th century [1]. Glacier retreat exposes new terrestrial and aquatic habitats which are readily colonized by pioneering organisms. This results in a spatio-temporal gradient of soil development, where distance from the glacier terminus can be used as a proxy for time since deglaciation. This space-for-time substitution – also known as chronosequence – approach has been widely used to investigate the successional patterns of plant communities in glacier forefields [2]. Nevertheless, we know comparatively little about the earlier successional stages, prior to the establishment of plant communities, where microbial processes prevail.

Microorganisms are dominant in proglacial soil close to the glacier front, where low nutrient content and high levels of physical disturbance (e.g. glaciofluvial activity and frost weathering) preclude the establishment of larger organisms [2–3]. Cyanobacteria are typically recognized as common pioneers in these environments [4–6], and are often found in association with other bacteria, eukaryotic microalgae, fungi, and lichens, forming complex communities known as biological soil crusts (BSCs) [7–8]. Filamentous cyanobacteria such as *Leptolyngbya*, *Phormidium*, and *Microcoleus* have a pivotal role in BSC structuring due to the production of extracellular polymeric substances (EPS), which promote the stabilization of the soil surface, moisture retention, and protection against erosion [7, 9]. EPS protects the cells from the physical damages of desiccation and freezing, and is thus a crucial adaptive feature enabling cyanobacteria to thrive in extreme conditions [10]. In many cyanobacteria, EPS sheaths are also rich in UV-screening pigments such as scytonemin, which protect the cells from the high UV radiation in the soil surface [11]. BSC cyanobacterial productivity is an important factor promoting the accumulation of organic matter in High Arctic ecosystems, constituting

the trophic foundation for other organisms [12]. BSCs also facilitate the establishment of plant communities in later successional stages, at which point BSC cover declines due to increasing competition for nutrients, ground cover, and light [5, 13].

Insights into the successional dynamics of cyanobacterial communities in glacier forefields have been mostly limited to large-spectrum investigations targeting whole microbial communities, of which cyanobacteria only make up a small fraction. These studies have shown that cyanobacterial communities are important components of proglacial soil communities in the High Arctic [14], Antarctica [15], the Swiss Alps [16–18], and the Peruvian Andes [19–20]. Few works dedicated specifically to the study of cyanobacterial succession have so far been carried out, mainly applying microscopic observations, culture-dependent, and/or traditional molecular fingerprinting techniques [21–22]. High-throughput sequencing (HTS) allows the investigation of rare, low-abundance taxa and thus provides better estimations of species richness and turnover along environmental gradients in comparison to traditional techniques [23]. Moreover, phylum-specific community profiling has advantages over community-wide surveys, as the use of universal primers can lead to under- or overrepresentation of specific groups [24]. HTS coupled with cyanobacteria-specific primers has recently been shown as a useful tool for the targeted assessment of cyanobacterial diversity in polar environments [25–26], but has never been applied to an in-depth investigation of cyanobacterial successional in glacier forefields.

A more thorough knowledge on how cyanobacteria respond to changing environmental conditions is key to better understand the effects of climate change on the functioning of polar ecosystems. The study of microbial succession in high latitude ecosystems is of special interest given that deglaciated areas are likely to expand in the future, according to general circulation models which predict enhanced warming in the Arctic [27]. This study provides an in-depth assessment of the successional trajectories of cyanobacterial communities in BSCs formed after the retreat of three glaciers in central Svalbard, High Arctic. By focusing on a short deglaciation gradient corresponding to 100 years of glacier retreat, we describe the early successional stages where vegetation cover is nearly absent and cyanobacteria are the most important primary producers. First, we were interested in determining if cyanobacterial communities undergo any discernible compositional shift along this short spatio-temporal gradient. We then set forth to investigate how shifts in community

structure correlate with spatio-temporal and environmental parameters. Finally, we identify individual phylotypes associated with early and late communities, in order to gain further insights into the dynamics of cyanobacterial communities following the colonization of recently deglaciated habitats.

Materials and methods

Study area and field sampling

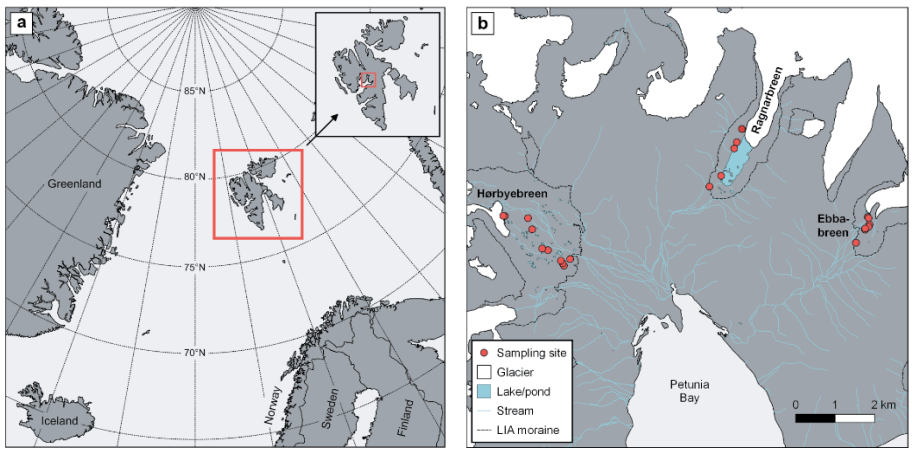

Fig. 1. (a) Location of the Svalbard archipelago and the Billefjorden region (inset), and (b) map of Petunia Bay showing the location of the sampling sites. A more detailed representation can be found in Suppl. Fig. S1. Maps were created using open data from the Alaska Geobotany Centre (University of Alaska, Fairbanks, USA) and Norwegian Polar Institute (Tromsø, Norway).

The investigated glacier forefields are located in Petunia Bay (78.68°N, 16.52°E), which is part of the northwestern branch of Billefjorden, central Spitsbergen, Svalbard archipelago (Fig. 1). The climate is typical of high latitude environments, with an annual mean air temperature of –4.5°C. The active growing season when liquid water is available typically lasts from mid-June to early October and is characterized by a mean air temperature of 5°C. Wind circulation in Petunia Bay is influenced by the local topography, with strong katabatic winds from northeast and east along the Ragnar- and Ebbabreen valleys, respectively, and southerly winds from Billefjorden [28]. Ebbabreen (78.73°N, 16.95°E) and Hørbyebreen (78.76°N, 16.28°E) are valley glaciers with marginal zones consisting of an ice front, and Ragnarbreen (78.75°N, 16.70°E) is an outlet glacier terminating on land with a marginal lake. The three glaciers present

negative mass balances and have lost, respectively, 5.2, 27.7, and 14.9% of their surface area since the end of the LIA until the beginning of the 21st century. Glacier fronts have retreated a total of 1030, 1525, and 1468 m in this period, at an average linear retreat rate of 10, 15, and 14 m y^{-1}, respectively [29]. Bedrocks are composed of Carboniferous/Permian clastic (conglomerates, sandstones, and mudstones) and carbonate (limestones and dolomites) rocks [30].

Sampling was performed according to the chronosequence concept, in which distance from the glacier terminus is used as a proxy for time since deglaciation. Despite being an indirect approach, chronosequences are useful for studying mid- and long-term successional changes (over decades, centuries, or millennia) due to the unfeasibility of direct, repeated observations over these time scales [2]. Transects and sampling sites were determined according to the glacier retreat rates established by Rachlewicz et al [29] based on field measurements, topographical maps, aerial photography, and older scientific reports. BSC samples were collected in August 2014 in the Ebba-, Hørbye-, and Ragnarbreen forefields along transects of ca. 0.7, 2.1, and 1.7 km, respectively (Fig. 1, Suppl. Fig. S1). Ground cover throughout the transects was characterized by unconsolidated glacial debris, with poorly developed BSCs and isolated plant patches, mainly *Saxifraga oppositifolia*, *Dryas octopetala*, *Salix polaris*, and *Bistorta vivipara*. Samples were taken at sites corresponding to 20, 30, 40, and 100 years since deglaciation in Ebbabreen, 10, 20, 50, 80, and 100 years in Hørbyebreen, and 10, 20, 30, 50, and 100 years in Ragnarbreen. Sampling sites were established in elevated areas (top of hillocks) to minimize the impact of meltwater, and away from ephemeral streams and ponds. In Ebba- and Hørbyebreen, two replicate samples were collected at each time period in order to account for spatial variability within the forefields. To minimize biases due to soil spatial heterogeneity, each sample consisted of three pooled subsamples (5 x 5 cm each, ca. 2–3 cm depth) taken randomly at each site. Samples were put into plastic bags using a spatula and transported in dry ice to the laboratory, where they were kept at –20°C until processing.

Determination of soil chemical composition

Soil chemical parameters were measured according to Czech Republic and European Union standards (ISO 10390, ISO 10523, ČSN EN 27888, ISO 11465, ČSN EN ISO 11732, ČSN EN ISO 13395, and ČSN EN ISO

15681-1). Moisture content was estimated after drying at 105°C for 6 hours. pH was measured in 1M KCl solution (1:5) and conductivity in demineralized water (1:5). Soil organic carbon (SOC) content was measured by wet oxidation with acidified dichromate. Ammonium and nitrate concentrations were measured using a QuikChem 8500 Series flow injection analysis system (Lachat Instruments, Loveland, CO, USA). K, Na, Ca, and Mg contents were analysed using a ContrAA atomic absorption spectrometer (Analytik Jena, Jena, Germany). The sum of ammonium and nitrate contents is expressed here as soil mineral nitrogen (SMN).

Estimation of cyanobacterial biovolume

Cyanobacterial biovolume was determined using light and epifluorescence microscopy according to Kaštovská et al [6]. Briefly, 1 g of sample was dissolved in 4 mL of distilled water and observed on a BX51 epifluorescence microscope (Olympus, Tokyo, Japan) equipped with a U-MWG filter cube (green excitation at 510–550 nm and emission at 590+ nm). Cyanobacteria were discriminated according to their basic morphology into unicellular, filamentous, and heterocystous morphotypes, and geometric equations were applied in order to calculate the biovolume of each morphological group [31].

Assessment of cyanobacterial diversity

Cyanobacterial community composition was investigated by pyrosequencing of partial 16S rRNA gene sequences according to Pessi et al [26]. In summary, DNA was extracted from ca. 0.5 g of sample using the PowerSoil DNA Isolation Kit (MOBIO, Carlsbad, CA, USA) and partial 16S rRNA gene sequences were obtained by PCR using the primer set CYA359F and CYA781R(a)/CYA781R(b), which amplify the V3-V4 region of the cyanobacterial 16S rRNA gene [32]. Sequencing was performed using the 454 GS FLX+ Titanium platform (454 Life Sciences, Branford, CT, USA) at the Beckman Coulter Genomics facilities (Danvers, MA, USA).

Quality control of reads, removal of chimeric sequences, and operational taxonomic unit (OTU) clustering were performed using UPARSE [33] according to Pessi et al [26]. Briefly, two and zero mismatches were allowed to the primer and barcode sequences, respectively, and reads were

required to have a maximum expected error of 0.5 and a length of 370 bp after the removal of primer and barcode sequences. Quality-filtered sequences were clustered into OTUs at 97.5% similarity according to Taton et al [34]. OTUs were classified using CREST [35] based on the Greengenes database [36], which follows the suprageneric classification of Hoffmann et al [37]. In this system, filamentous non-heterocystous cyanobacteria are included in the orders Pseudanabaenales (comprising generally thin filamentous taxa with parallel thylakoids such as Leptolyngbya and Pseudanabaena) and Oscillatoriales (larger filamentous taxa with radial thylakoids such as Microcoleus and Phormidium). Unicellular taxa are assigned to the orders Gloeobacterales (no thylakoids), Synechococcales (parallel thylakoids), and Chroococcales (radial thylakoids). Finally, all heterocystous taxa are grouped in a single order, the Nostocales.

Non-cyanobacterial OTUs – consisted of sequences assigned to plastids of eukaryotic phototrophs (3.6% of quality-filtered sequences) and other bacterial phyla such as Acidobacteria, Chloroflexi, and TM7 (0.5%) – were removed from the dataset. Downstream analyses were carried out after subsampling the pyrosequencing dataset to 1393 sequences per sample.

Statistical and phylogenetic analyses

Significant shifts in environmental (soil chemical composition) and biotic (cyanobacterial biovolume and phylotype richness) parameters along the deglaciation gradient were assessed by simple linear regression using STATISTICA 12 (StatSoft, Tulsa, OK, USA).

β-diversity analyses were performed in PRIMER 7 (Primer-E, Plymouth, UK). This did not include samples from Ragnarbreen, which were removed due to a lack of replicated time periods. Community dissimilarities were computed based on unweighted UniFrac distances using QIIME [38–39]. The overall variability in community structure within each forefield was first examined using unweighted pair group method with arithmetic mean (UPGMA) and principal coordinates (PCO) analyses. Based on the observed clustering patterns, samples were then categorized into three community groups according to time since deglaciation (early, 10–20 years; mid, 30–50 years; and late, 80–100 years). Significant differences between community groups were evaluated

using permutational ANOVA (PERMANOVA) [40] and canonical analysis of principal coordinates (CAP) [41].

A combination of distance-based linear models (distLM) and variation partitioning [42] was used to determine the amount of variation in community structure which could be attributed to spatio-temporal (time since deglaciation) and environmental (soil chemistry) variation. Prior to the analyses, predictor variables were log(x+1) transformed (except pH) and standardized by subtracting the mean and dividing by the standard deviation of the variable. First, a model building with forward selection based on the adjusted R2 criterion and 1000 permutations was used to select a subset of physicochemical variables which best explain the variation in community structure. These were included along with the spatio-temporal variable in a new distLM routine combined with variation partitioning to distinguish between the amount of variation explained by (i) the selected physicochemical variables, (ii) the spatio-temporal variable, and (iii) both set of factors combined.

Pearson correlation between OTU distribution data and the CAP axes was used to identify phylotypes associated with each community group (referred to as 'indicator phylotypes'), which were submitted to further phylogenetic and biogeographic analyses. A representative sequence was selected for each indicator phylotype as being the most abundant unique sequence in the cluster. In order to verify the current biogeographic distribution of indicator phylotypes, all closely related (≥ 97.5% similarity) cultured and uncultured sequences were retrieved from GenBank using BLAST, and information relative to the source and country of isolation of each hit was obtained using in-house UNIX scripts. For phylogenetic analyses, indicator phylotypes were aligned using MUSCLE [43] along with their closest BLAST isolate hits. A maximum likelihood tree based on the Kimura 2-parameter model was then computed using MEGA 7 [44].

Sequence data

Raw sequencing data are available in the NCBI Sequence Read Archive (SRA) database under the accession numbers SRR5382144–SRR5382167.

Results

Communities are dominated by filamentous cyanobacteria

A total of 294 469 pyrosequencing reads with an average length of 410 bp were obtained (average of 12 269 sequences per sample). After removal of low-quality, chimeric, and non-cyanobacterial sequences, 152 541 sequences (51.8% of the initial pyrosequencing reads) were grouped into 148 OTUs at 97.5% similarity. Cyanobacteria were the most dominant phototrophs, with plastid sequences from eukaryotic organisms accounting for only 3.6% of the quality-filtered reads. Pseudanabaenales was the most phylotype-rich order (96 OTUs), followed by Chroococcales (13 OTUs), Oscillatoriales (7 OTUs), Synechococcales (6 OTUs), Nostocales (5 OTUs), and Gloeobacterales (3 OTUs). Eighteen OTUs were not classified at the order level, as well as most phylotypes at the genus level (118 OTUs). Phylotypes which could be successfully classified were assigned to the genera *Leptolyngbya* (21 OTUs), *Phormidium* (3 OTUs), *Nostoc*, *Pseudanabaena* (2 OTUs each), *Chroococcidiopsis*, and *Microcoleus* (1 OTU each).

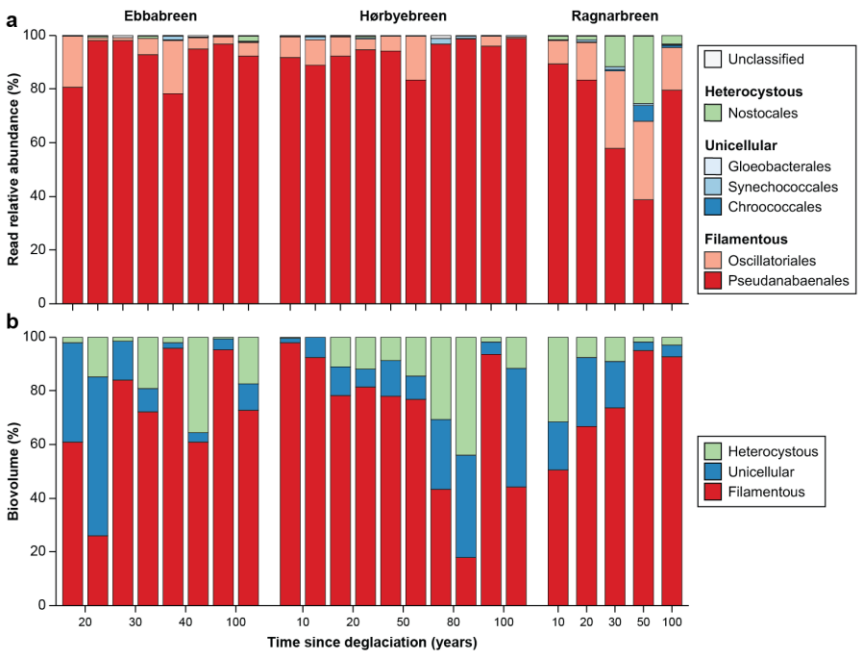

Fig. 2. Cyanobacterial community structure observed by (a) 454 pyrosequencing and (b) epifluorescence microscopy. Suprageneric classification of phylotypes was carried out following the taxonomic system of Hoffmann et al [37].

In Ebba- and Hørbyebreen, communities were largely dominated by sequences from filamentous cyanobacteria (Pseudanabaenales and Oscillatoriales; sensu Hoffmann et al [37]), which comprised together 96.8–99.9% of the quality-filtered reads in each sample (Fig. 2a). Unicellular and heterocystous cyanobacteria accounted for only a minor part of the communities (0.1–2.2% and 0.1–1.7% of the reads, respectively). Community structure differed slightly in Ragnarbreen, where unicellular and heterocystous cyanobacteria reached higher relative abundances in some samples (up to 6.5 and 25.2% of the reads, respectively).

In contrast to the pyrosequencing analysis, biovolume estimations evidenced a higher proportion of unicellular and heterocystous cyanobacteria across all samples, where they comprised up to 59.4 and 43.9% of the total cyanobacterial biovolume, respectively (Fig. 2b).

Nutrients and cyanobacterial abundance increase with time since deglaciation

An overall increase in nutrient content was observed along the deglaciation gradient, i.e. with increasing time since deglaciation (Suppl. Fig. S2). SOC content fluctuated considerably but showed a significant increase from 16.5–45.3 g kg^{-1} in early (10–20 years since deglaciation) to 28.4–52.0 g kg^{-1} in late (80–100 years) samples ($r = 0.29$, $p = 0.02$). SMN content increased steadily from 0.8–3.5 to 2.0–4.3 mg kg^{-1} in early and late samples, respectively ($r = 0.58$, $p = 0.01$). Significant increases in moisture, pH, K, Na, and Mg contents were also observed, while conductivity and Ca showed no linear relationship along the deglaciation gradient (Suppl. Fig. S2). Finally, total cyanobacterial biovolume fluctuated substantially but also followed a significant increasing trend (Fig. 3a).

Cyanobacterial community structure shifts in relation to environmental and spatio-temporal factors

Cyanobacterial phylotype richness decreased along the deglaciation gradient, from 33–49 OTUs in early (10–20 years since deglaciation) to 22–42 OTUs in late (80–100 years) samples (Fig. 3b). Further β-diversity analyses discriminated between three general community types according to time since deglaciation within each forefield (Fig. 4). Early (10–20

years), mid (30–50 years), and late communities (80–100 years) formed well-defined groups and were significantly distinct from each other (PERMANOVA; pseudo-F = 1.84, p = 0.02). This was also confirmed by the CAP procedure, which was carried out taking both forefields together (Fig. 5). The canonical correlations for the two produced axes was high (δ1 = 0.98 and δ2 = 0.74), with a significant separation between community types along the first axis (δ12 = 0.90, p = 0.05).

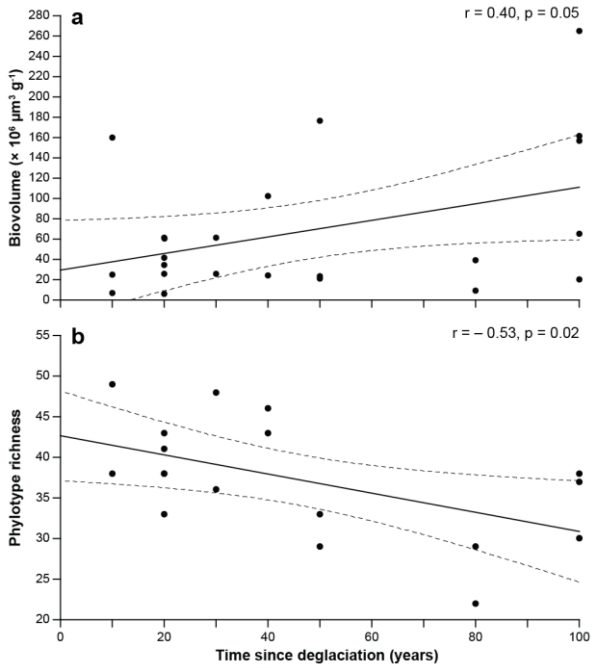

Fig. 3. Shifts in (a) total cyanobacterial biovolume and (b) phylotype richness along the deglaciation gradient. Solid lines correspond to the fitted curves obtained by simple linear regression and dashed lines represent the 95% confidence intervals.

Among environmental factors, moisture, SOC, SMN, K, and Na were the best predictors of community structure, accounting together for 35.8% of the variation (distLM; pseudo-F = 1.45, p = 0.04). When considered alone, time since deglaciation explained 14.1% of the variation (pseudo-F = 2.80, p < 0.01). The complete model, computed using variation partitioning analysis with both sets of environmental and spatio-temporal factors, accounted for 46.9% of the variation. Of these, 32.8% was explained by the selected environmental variables (pseudo-F = 1.48, p = 0.03) and 11.1% by time since deglaciation (pseudo-F = 2.49, p = 0.01). The remaining variation (3.0%) was explained by the combination of both environmental and spatio-temporal factors.

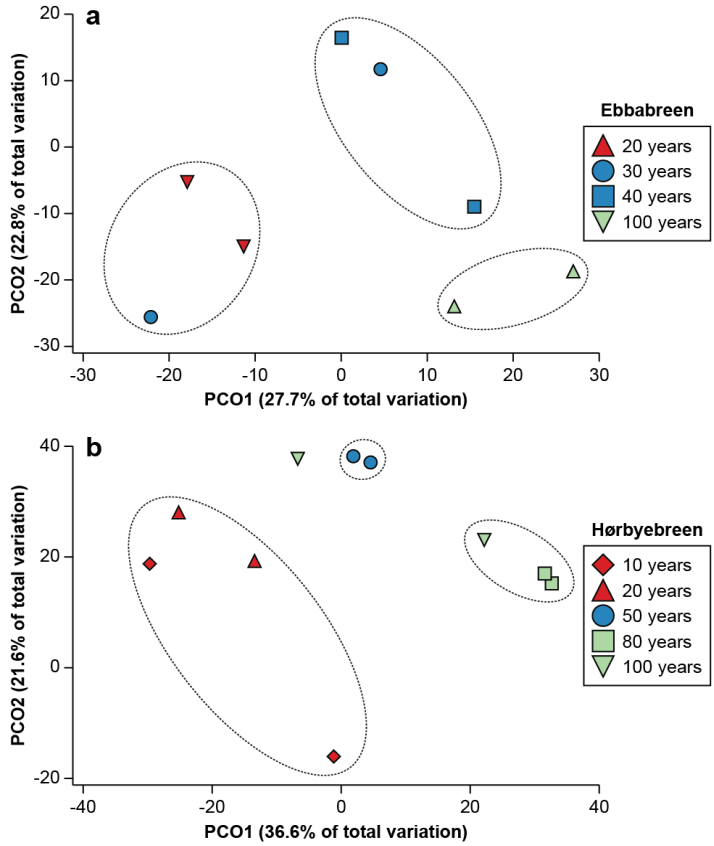

Fig. 4. Principal coordinates analysis (PCO) of 16S rRNA gene assemblages in (a) Ebba- and (b) Hørbyebreen. Dashed circles represent UPGMA groups (see Suppl. Fig. S3).

Early colonizers are mainly related to novel and polar/alpine cyanobacteria

Pearson correlation between OTU distribution data and the CAP axes identified 24 indicator phylotypes, i.e. OTUs associated with a specific community group (Fig. 5). Among the 16 OTUs associated with early and mid communities, 10 OTUs had no closely related (≥ 97.5% similarity) sequences in GenBank and thus likely represent novel cyanobacterial lineages (OTUs 58, 79, 83, 114, 115, 117, 150, 165, 179, and 211; Fig. 6). Remaining early/mid phylotypes were most closely related to *Phormidesmis priestleyi* ANT.LG2.4 (OTU7, 100.0% similarity), *P. priestleyi* ANT.L66.1 (OTU126 and OTU222, 97.5–97.8% similarity), and *Phormidium* sp. CYN64 (OTU8, 98.9% similarity), all isolated from

Antarctic lakes [34, 45]. OTU31 and OTU122, associated with mid communities only, were related (97.5–98.9% similarity) to different strains of *Leptolyngbya*, including *Leptolyngbya antarctica* ANT.LAC.1 isolated from Ace Lake (Antarctica) [34].

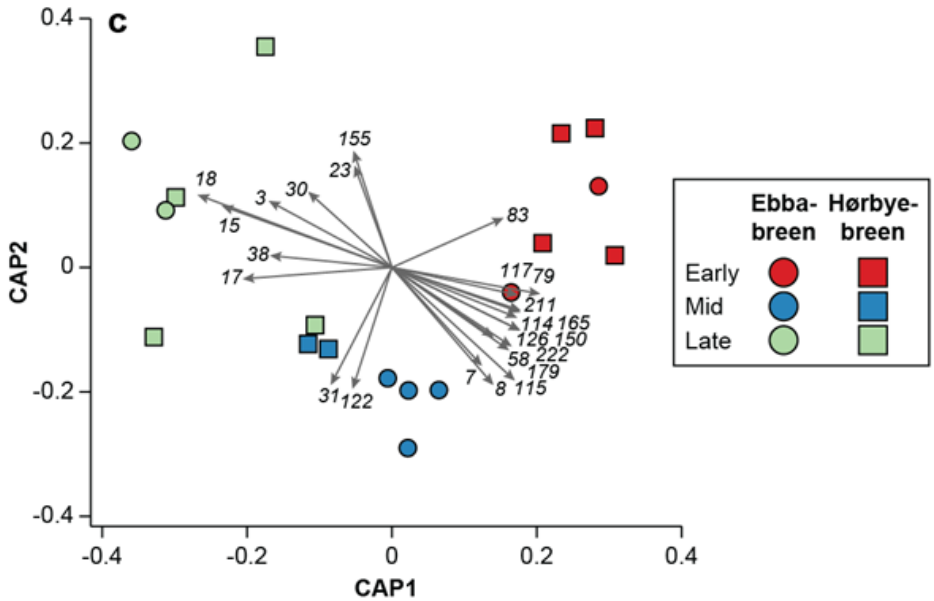

Fig. 5. Canonical analysis of principal coordinates (CAP) ordination of 16S rRNA gene assemblages in Ebba- and Hørbyebreen. Samples were categorized as early (10–20 years since deglaciation), mid (30–50 years), and late (80–100 years) according to Fig. 4. Overlaid arrows represent indicator phylotypes.

BLAST analysis taking into account all closely related (≥ 97.5% similarity) cultured and uncultured sequences from GenBank revealed that phylotypes associated with early/mid communities have restricted biogeographic distributions (Fig. 6). For each phylotype, the vast majority (75.5–100.0%) of BLAST hits consisted of sequences retrieved from polar and alpine regions, including proglacial soil, lacustrine microbial mats, and supraglacial habitats such as glacier snow and cryoconite.

The eight phylotypes associated with late communities, on the other hand, had a much wider geographic distribution. A high proportion (up to 73.1%) of the BLAST hits for these phylotypes consisted of sequences coming from non-polar environments, which, in most cases, also included their closest isolate hit (Fig. 6). For example, OTU30 was most closely related (97.5% similarity) to *Leptolyngbya* sp. FYG isolated from a hot spring in the Yellowstone National Park (USA) [46], and OTU18 was

97.8% similar to *Oscillatoria geminata* SAG 1459-8 isolated from a factory cooling tower in Germany (Friedl et al, unpublished). OTU17 and OTU38 were related (97.8–99.2% similarity) to two *Leptolyngbya* sp. strains (LEGE 07074 and LEGE 07075) isolated from Portuguese estuaries [47]. Finally, OTU155 was related to *Gloeobacter kilaueensis* JS1 isolated from a lava cave in Hawaii [48], although with very low sequence similarity (94.8%).

Discussion

Both molecular and morphological analyses confirmed the role of filamentous cyanobacteria as important components of proglacial soil ecosystems, in agreement with previous investigations of cyanobacterial diversity in polar and alpine glacier forefields [6, 21–22]. *Leptolyngbya*-, *Phormidium*-, and *Microcoleus*-like morphotypes are the main representatives of filamentous non-heterocystous cyanobacteria in BSCs worldwide including the High Arctic [4, 7–8]. They contribute vastly to the stabilization of the soil surface due to their ability to produce high amounts of EPS, which protects them from the physical damages of desiccation and freeze-thaw cycles [7, 9]. Unlike smaller unicellular taxa, large filamentous cyanobacteria such as *Phormidium* and *Microcoleus* have high mobility, which helps them to persist during the earlier successional stages where physical disturbances are frequent [7].

Despite an overall dominance of filamentous cyanobacteria, morphological analysis showed that heterocystous cyanobacteria are also an integral part of the investigated communities (Fig. 2b), as it has been shown for other glacier forefields worldwide [6, 19–22]. They likely play an important role in the accumulation of organic matter in these nutrient-depleted environments due to their ability to fix atmospheric nitrogen [2–4, 49]. Inconsistencies between the community structures observed by pyrosequencing and biovolume estimations were not unanticipated (Fig. 2). The experimental approach underlying each technique differs greatly, and both methods have their own advantages and weaknesses. For instance, the DNA extraction step is an important source of bias in molecular investigations of cyanobacterial assemblages, as high amounts of EPS in the biofilm matrix interfere considerably with cell lysis procedures [50]. Moreover, using data from artificial communities, Pessi et al [26] showed that PCR and sequencing biases shift considerably the observed relative abundances of individual cyanobacterial taxa.

Biovolume estimations, on the other hand, are based on direct counting of cells under epifluorescence microscopy [31], and thus might give a more accurate representation of the abundance of different cyanobacterial groups. Nevertheless, microscopic analyses provide little or no taxonomic information beyond discriminating between major cyanobacterial groups, as the relatively simple morphology of many taxa complicates identification at lower taxonomic levels [51]. Thus, the parallel application of both molecular and microscopy methods seems a valuable approach for assessing cyanobacterial community structure at different taxonomic ranges.

BSC nutrient content was generally low but fell inside the typical values observed in glacier forefields worldwide [3]. Furthermore, in agreement with previous reports for other polar and alpine glacier forefields [14, 16–17, 19–20, 22], a general increase in nutrient content was observed along the spatio-temporal gradient (Suppl. Fig. S1). This was mirrored by an increase in cyanobacterial abundance (Fig. 3a), which is also consistent with previous studies in the Damma and Puca glacier forefields (Swiss Alps and Peruvian Andes, respectively) [17, 20, 22]. There is a wide consensus concerning the importance of cyanobacterial production for organic matter accumulation in proglacial soil ecosystems [2, 4–5, 12]. Nevertheless, in addition to autotrophic production, microbial communities are also maintained by inputs of nutrients from allochtonous sources (e.g. through aerial deposition and runoff from the glacier surface) and ancient organic pools [49]. Even though we are not able to attribute the increase in BSC nutrient status to cyanobacterial production, it is evident that higher nutrient content provides a more favourable environment for microbial growth thus resulting in higher cyanobacterial densities. Larger cyanobacterial populations, in turn, likely lead to increased soil stability and protection against desiccation, freezing, and UV radiation, due to higher amounts of EPS in the biofilm matrix [7].

Despite the rather short spatio-temporal gradient contemplated in this study, frequent disturbance events [2], and the patchy distribution of soil microorganisms [52], our results evidenced marked and consistent shifts in cyanobacterial community structure with time since deglaciation. Cyanobacterial succession was characterized by a decrease in phylotype richness (Fig. 3b) and a marked shift in community structure (Fig. 4, Fig. 5). Decreasing cyanobacterial richness has been observed in other polar and alpine chronosequences [17, 21–22]. This can be explained by the competitive exclusion principle, similarly to what has been proposed for

plant communities in glacier forefields [2]. In recently deglaciated soil close to the glacier front, low nutrient concentrations and high levels of physical disturbance constrain the size of the microbial populations, and thus many taxa are able to coexist. This initial diversity pool is likely enriched by microorganisms transported from supraglacial habitats via glacial runoff [53]. Cryoconite holes, in particular, are hotspots of cyanobacterial diversity and activity in glacier surfaces, and thus may act as an important source of propagules for downstream proglacial habitats during the ablation season [54–56]. As succession progresses, on the other hand, biofilm development and associated changes in the microenvironment (e.g. increased moisture, nutrient status, soil stability, and protection against physical damages) promote higher population densities. Larger populations result in intensified competition for resources, which, in turn, leads to the eventual local extinction of several taxa [2].

Having established that cyanobacterial succession occurred at the spatio-temporal scale considered in this study, we further investigated the possible mechanisms underlying the observed patterns. Time since deglaciation and environmental variation both accounted for different fractions of community structure turnover (11.1 and 32.8%, respectively), with only 3% of the variation being shared by both set of factors. In agreement with previous studies of microbial succession [3], as well as with the general consensus regarding microbial community assembly [52, 57], community turnover was mostly driven by shifts in environmental conditions, mainly BSC moisture, SOC, and SMN content. BSC development and succession are tightly linked to physicochemical properties [7, 9]. Indeed, changes in cyanobacterial composition along a gradient of BSC development in the High Arctic have been attributed to shifts in pH, conductivity, moisture, and SOC content [25].

In addition to the expected effect of environmental parameters, our results showed that changes in cyanobacterial community structure were also partially associated with the spatio-temporal factor. Even though care was taken in order to include the most relevant edaphic parameters, it is possible that the observed relationship between time since deglaciation and community structure might be partially attributed to other unmeasured biotic and abiotic variables. For example, mechanical disturbances and surface instability have a major impact on the composition and succession of BSCs [7, 12 –13]. This is likely more pronounced at earlier stages, as the production of EPS by BSC cyanobacteria leads to increased soil

stability [7]. The observed correlation between community structure and time since deglaciation might also represent the effects of historical processes, as proposed by Freedman and Zak [58] for a long-term chronosequence in the Upper Great Lakes Region (USA). They found that shifts in bacterial community structure were explained by both environmental (53%) and temporal (17%) factors. The authors attributed the latter to lingering effects from historical events (associated with, for example, ecological drift and dispersal limitation), which are known to influence present-day microbial assemblages [52]. In agreement with the study of Freedman and Zak [58], our results support the role of historical events in shaping the successional trajectories of cyanobacterial communities in glacier forefields. We further suggest that dispersal limitation might be an important factor particularly in the earlier stages, as microorganisms inhabiting upstream glacial habitats would have preferential access to the newly formed proglacial environment. Initial colonization might then be readily achieved by local propagules transported from the glacier surface via glacial runoff [53] or through the action of katabatic winds which blow from the glacier plateau towards the valley [28].

It has been proposed that pioneering microorganisms comprise usually cosmopolitan taxa, since they are fundamentally more widely dispersed and thus have higher chances of colonizing remote habitats [57]. Nevertheless, it is likely that the harsh environmental conditions found in recently deglaciated forefield soil preclude the establishment of cosmopolitan, generalist taxa. As our results suggest, cyanobacteria involved in the early colonization of proglacial soil have a marked polar characteristic (Fig. 6). Early phylotypes were either potentially novel (< 97.5% similar to sequences currently available in GenBank) or related to lineages of *P. priestleyi* and *L. antarctica* largely restricted to cold biosphere habitats such as proglacial soil and supraglacial snow and/or cryoconite. Indeed, cyanobacteria make up an important fraction of supraglacial communities [54–56], which provide an important supply of inoculi and nutrients to downstream habitats via glacial runoff [53]. Vonhamme et al [56] showed that filamentous cyanobacteria such as *Phormidium* and *Leptolyngbya* are particularly abundant in cryoconite sediments on Svalbard glaciers, including Hørbye- and Ebbabreen. Mat-forming filamentous cyanobacteria are also among the most abundant cyanobacteria in cryoconite holes worldwide [54]. Moreover, polar cyanobacteria possess several structural and physiological adaptations

which enable them to withstand harsh environmental conditions, as it has been recently shown for *P. priestleyi* strains isolated from a cryoconite hole on the Greenland Ice Sheet and a microbial mat in an Antarctic lake [59–60]. Thus, as our results suggest, and in agreement with recent findings for the Damma glacier forefield (Swiss Alps) [18], cyanobacteria thriving in supraglacial habitats have a pivotal role in the initial colonization of recently deglaciated forefield soil.

Late communities, on the other hand, included phylotypes with a much wider cosmopolitan distribution, being related to sequences retrieved from temperate and even tropical regions (Fig. 6). Thus, it appears that the somewhat milder microenvironment – represented by higher nutrient content and improved structural conditions brought about by the consolidation of BSC communities (e.g. increased soil stability and protection against physical damages) – facilitates the establishment of more widely distributed later in the succession. In this new microenvironment, pioneering, polar cyanobacteria which are favoured in the earlier stages where population densities and competition levels are low, are outcompeted by cosmopolitan taxa since the latter are, by definition, adapted to a wider range of environmental conditions.

In conclusion, our results confirm the role of cyanobacteria as important pioneers and the main phototrophs in recently deglaciated forefield soil. As a result of changes in BSC nutrient status, succession was characterized by an increase in cyanobacterial abundance, a decrease in phylotype richness, and a marked shift in community structure. Early colonization of proglacial soil seems to be accomplished by polar cyanobacteria likely transported from nearby glacial environments, which i) have easy and rapid access to the newly formed habitats, ii) are structurally and physiologically adapted to the severe environment, and iii) benefit from low competition levels arising from low population sizes. Pioneering organisms, in turn, modify the microenvironment by increasing nutrient levels, soil stability, and protection against physical damages, which facilitates the establishment of cosmopolitan taxa later in the succession. The combination of a milder microenvironment and larger population sizes results in increased competition for resources, causing the local extinction of several taxa. Pioneering, polar cyanobacteria are thus replaced by more widely distributed taxa, which are more competitive in this new microenvironment. To the best of our knowledge, this study represents the first in-depth assessment of cyanobacterial succession in High Arctic glacier forefields. It adds to the body of evidence that

microbial communities undergo remarkable succession following glacier retreat, and contributes for a better understanding of the functioning and evolution of polar ecosystems.

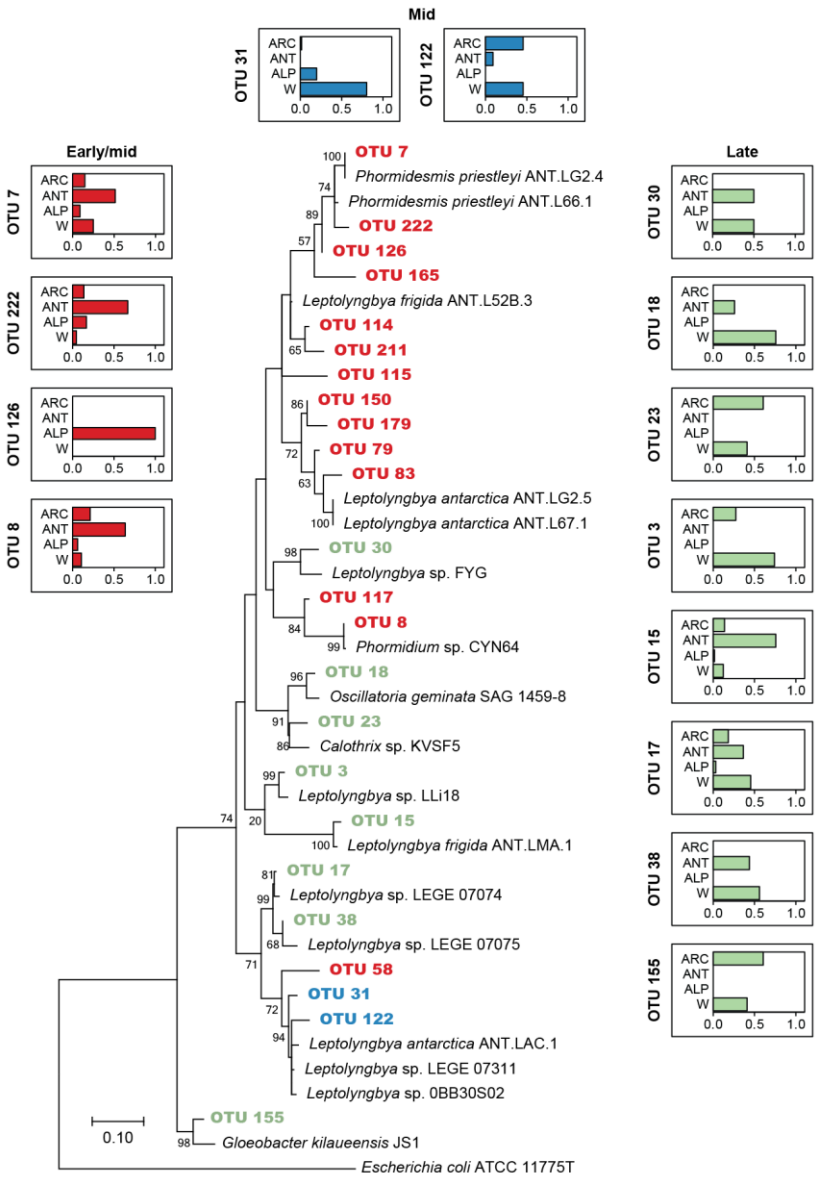

Fig. 6. Phylogenetic and biogeographic analyses of indicator phylotypes associated with early/mid communities (red), mid communities only (blue), and late communities (green). Maximum Likelihood tree of indicator phylotypes and their most closely related isolate sequence in GenBank. Bar plots show the biogeographic distribution of indicator phylotypes based on the geographic origin of all closely related (≥ 97.5% similarity) sequences retrieved from GenBank. ARC: Arctic; ANT: Antarctic; ALP: alpine; W: world.

Acknowledgements

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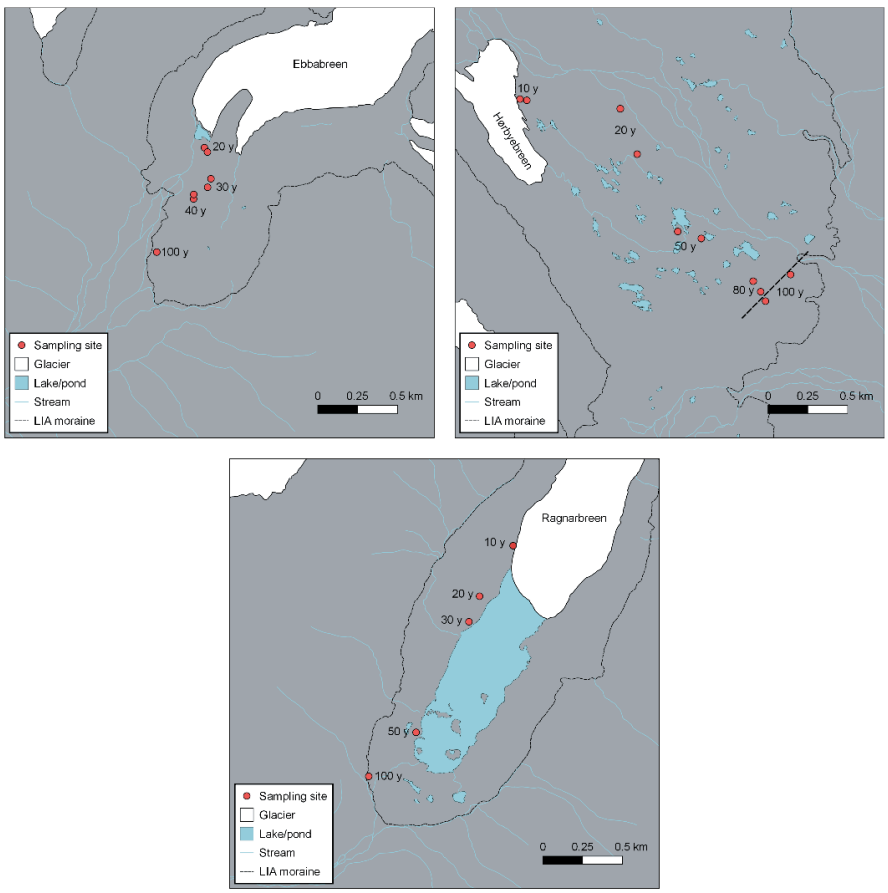
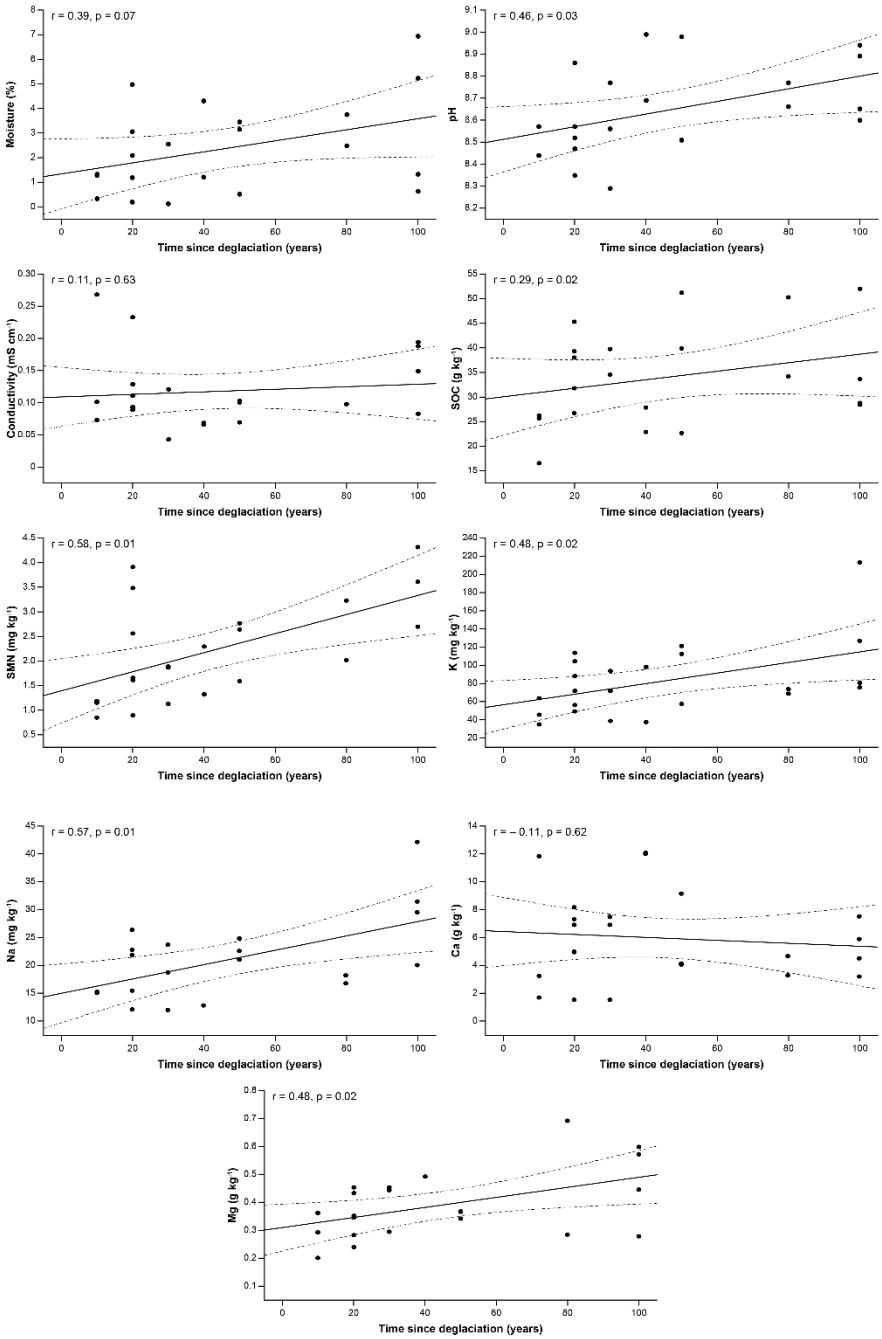
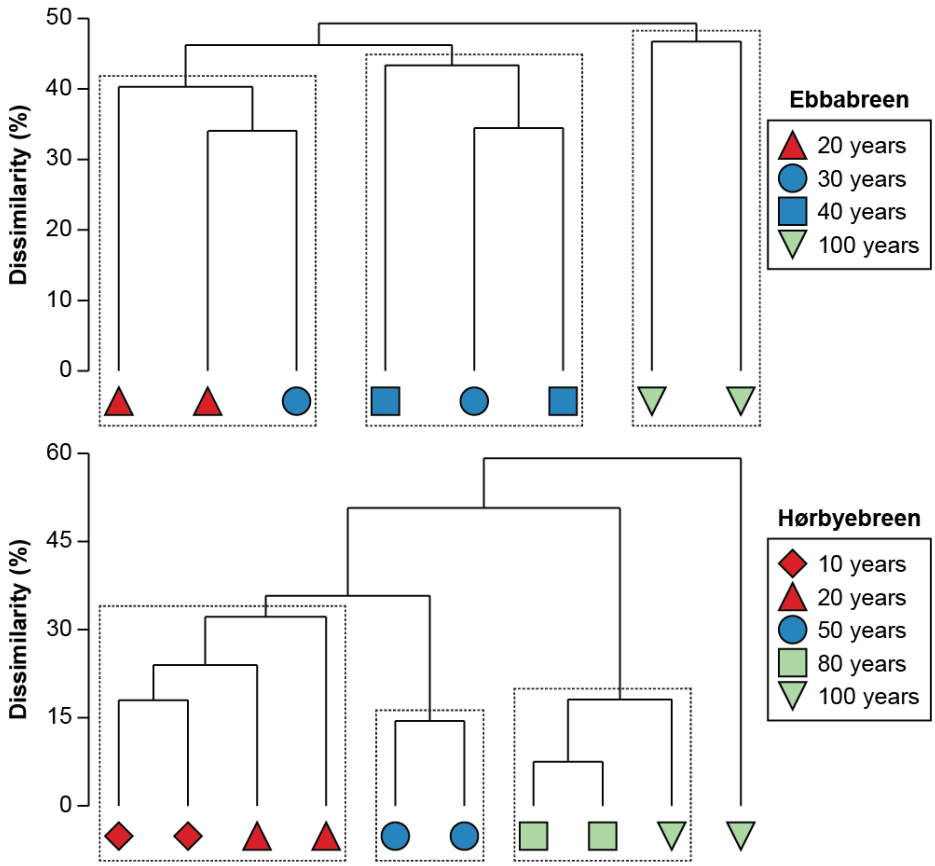
Supplementary material



Suppl. Fig. S1. Detailed overview of the sampling sites. Dashed lines represent the terminal morainescorresponding to the position of the glacier terminus at the end of the "Little Ice Age" (LIA) in the late 19th century. Maps were created using open data from the Norwegian Polar Institute (Tromsø, Norway).



Suppl. Fig. S2. Shifts in BSC physicochemical composition along the deglaciation gradient. Black lines correspond to the fitted curves obtained by simple linear regression and dashed lines represent the 95% confidence intervals.



Suppl. Fig. S3. Unweighted pair group method with arithmetic mean analysis (UPGMA) of 16S rRNA gene assemblages in (a) Ebba- and (b) Hørbyebreen.

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Cyanobacteria inhabiting biological soil crusts of a polar desert: Sør Rondane Mountains, Antarctica

Ekaterina Pushkareva [a*], Igor S Pessi [b], Zorigto Namsaraev [c], Marie-Jose Mano [b], Josef Elster [a,d], Annick Wilmotte [b]

[a]*Centre for Polar Ecology, University of South Bohemia, České Budějovice, Czech Republic*
[b]*InBioS – Centre for Protein Engineering, University of Liège, Liège, Belgium*
[c]*NRC "Kurchatov Institute", Moscow, Russia*
[d]*Institute of Botany, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic*

***Corresponding author:** E. Pushkareva <puekse@gmail.com>

Abstract

Molecular and morphological methods were applied to study cyanobacterial community composition in biological soil crusts (BSCs) from four areas (two nunataks and two ridges) in the Sør Rondane Mountains, Antarctica. The sampling sites serve as control areas for open top chambers (OTCs) which were placed in 2010 at the time of sample collection and will be compared with BSC samples taken from the OTCs in future. Cyanobacterial biovolume was estimated using epifluorescence microscopy, which revealed the dominance of filamentous cyanobacteria in all studied sites except the Utsteinen ridge, where unicellular cyanobacteria were the most abundant. Cyanobacterial diversity was studied by a combination of molecular fingerprinting methods based on the 16S rRNA gene (denaturing gradient gel electrophoresis (DGGE) and 454 pyrosequencing) using cyanobacteria specific primers. The number of DGGE sequences obtained per site was variable and, therefore, a high-throughput method was later employed to improve the diversity coverage. Consistent with previous surveys in Antarctica, both methods showed that filamentous cyanobacteria such as *Leptolyngbya* sp., *Phormidium* sp. and *Microcoleus* sp. were dominant in the studied sites.

In addition, the studied localities differed in substrate type, climatic conditions and soil parameters, which likely resulted in differences in

cyanobacterial community composition. Furthermore, the BSC growing on gneiss pebbles had lower cyanobacterial abundances than BSCs associated to granitic substrates.

Key words: BSC, cyanobacteria, Antarctica, DGGE, 454 pyrosequencing, biovolume

Introduction

Antarctica is the most isolated, coldest, windiest and driest continent on the planet [16]. Ice-free areas cover 0.34% of its total landmass [10] and include nunataks, mountain peaks and other rock formations that are exposed above a glacier or ice sheet [28]. Biological soil crusts (BSCs), which consist of soil aggregates held together by communities of living organisms on the soil surface [3], are common in this type of environment. The active growing season in Antarctica (when the soil surface temperature is above 0°C and liquid water is available) lasts 25-75 days [8] resulting in limited diversity and abundance of photoautotrophic organisms, and low carbon and nitrogen concentrations in Antarctic soil environment.

Cyanobacteria are usually the dominant components of the soil photoautotrophic community in Antarctic BSCs and the primary colonizers of poor Antarctic soils [44,51]. They are involved in important processes such as nitrogen fixation, moisture retention, soil stabilization and organic carbon accumulation [8]. Cyanobacteria are particularly well adapted to severe and variable conditions, having developed a wide range of strategies which allow them to minimize or avoid the harmful effects of harsh environments. Their ecological success can be partially attributed to the production of extracellular polymeric substances (EPS), which protect them from desiccation, and UV-screening pigments such as scytonemin [47].

A recent review on Antarctic soil crusts [5] has provided an inventory of cyanobacteria identified there so far based on morphological descriptions. Nostocales, Chroococcales and Oscillatoriales were present in all presented regions. Microbial community composition in Antarctic soil crusts was intensively studied in Victoria Land [1,14,23,26,51] and the Antarctic Peninsula [31,36,50,52]. Nevertheless, other Antarctic regions have received much less attention and our knowledge on the cyanobacterial diversity of BSCs is still incomplete. Recently, a few

studies have investigated the microbial diversity in different terrestrial habitats in the Sør Rondane Mountains (Dronning Maud Land, East Antarctica), a 250 km-long mountain range that rises up to more than 3000 m a.s.l. Fernández-Carazo et al. [13] and Obbels et al. [19] applied denaturing gradient gel electrophoresis (DGGE) to estimate the cyanobacterial diversity in soil crust and gravel samples from the Utsteinen ridge and nunatak, and a dry valley in the vicinity. In total, 18 operational taxonomic units (OTUs) were observed, of which 11 were present in several samples of the studied areas, which could be explained by an easy dissemination within short distance. In addition, Obbels et al. [33] and Tytgat et al. [49], using high-throughput sequencing to study the bacterial diversity in various terrestrial habitats of the Sør Rondane Mountains, observed that cyanobacteria were abundant there. However, most analyses of the high-throughput data in these studies were carried out at the phylum level only.

Antarctic ecosystems are of particular interest due to the predicted effects of climate change, which are expected to cause changes in the soil microbiota [11]. In-field simulations of climate warming using Open Top Chambers (OTCs) have been increasingly used to study the effects of climate change on the soil microbiome. These passive temperature-enhancing systems also modify several parameters like snow accumulation, moisture and wind [22]. Eight OTCs were installed in 2010 on flat platforms of the Utsteinen and Tanngarden ridges, and the Teltet and Pingvinane nunataks in the Sør Rondane Mountains. These geological formations are isolated and can be considered as oases surrounded by ice-covered terrain. In addition, visual inspections showed that there were more BSC visible on granite substrates than on gneiss ones. Thus, the OTCs were placed on both types of substrates to better understand the role of the soil types. In the present study, we provide a description of the soil parameters, diversity and abundance of BSC cyanobacteria using morphological and molecular tools. A first DGGE survey resulted in unequal coverage and therefore an additional high-throughput sequencing of the same segment of the 16S rRNA gene sequences (V3-V4) was performed to improve the molecular taxonomic data. We hypothesize that the isolated locations and different environmental conditions, including substrate type, result in different cyanobacterial community compositions. Furthermore, the samples were taken in the control areas of OTCs and, therefore, the present assessment constitutes the baseline data for later comparisons with samples collected from inside the OTCs, which will

provide insights on the effects of climate change on Antarctic soil cyanobacterial communities.

Material and methods

Site description and sample collection

The studied sites were located in the western part of the Sør Rondane Mountains within a 30 km radius around the Belgian Princess Elisabeth Station (71°57'S, 23°21'E, 1372 m a.s.l.) (Fig. 1). The Utsteinen ridge stretches from south to north for 700 m and is composed of massive coarse-grained granite. Samples were collected from the southern part of the ridge, approximately 300 m from the main building of the station (71°57'S, 23°21'E). Pingvinane is a range of six granitic nunataks located 20 km west from the station. Samples were taken on the west-southwest slope of the fourth nunatak from the northern end (72°00'S, 22°59'E), on a relatively flat slope composed of granite gravel. Tanngarden ridge is located 30 km west from the station. Soils and crusts developed on granite gravel were sampled in the wind scoop near the northeastern slope (72°01'S, 22°56'E). Teltet is a single nunatak located 8 km south-east from the station. Samples of gravel were collected on the flat surface on the northern ridge of the nunatak (71°59'S, 23°29'E) composed of gneiss pebbles and no developed BSC was observed, but rather a microbial mat. Pictures of studied localities are shown in Suppl. Fig. 1.

In order to obtain microclimatic parameters, temperature and humidity sensors (Maxim i-Buttons®) were installed in each OTC and control sites in 2010. Measurements were carried out in the period from 2010 to 2012 and the data from the sensors were recorded every three hours. Replacement of the sensors was needed each year but could not happen when the OTCs were still under snow cover, what explains the presence of missing data.

Soil crust samples were collected during the austral summer season in January 2010. For the measurements of soil chemistry and cyanobacterial biovolume, 15 representative subsamples including bare soils were collected in each site and mixed together in the field. For the molecular analyses, two to three soil crust samples were taken from each locality (11 samples in total) and analyzed separately. Soil crusts were placed into zip bags and shipped to the laboratory in dry ice. After the sampling, OTCs were installed on each site. Details regarding the implementation and

monitoring of the OTCs will be published elsewhere (Namsaraev et al., in preparation).

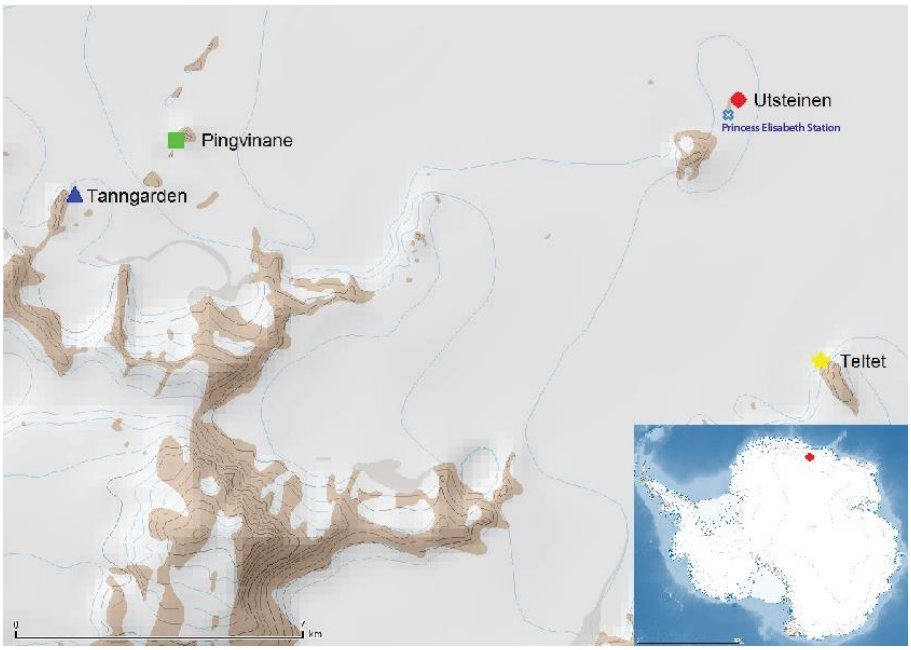

Fig. 1. Map of the sampling sites in Sør Rondane Mountains, Antarctica.

Soil chemistry

Analyses of soil physicochemical parameters were performed according to the methodology described in Czech and European Union standards (ISO 10390, ISO 10523, ČSN EN 27888, ISO 11465, ČSN EN ISO 11732, ČSN EN ISO 13395 and ČSN EN ISO 15681–1). In brief, water content was estimated after drying soils at 105°C for 6 hours. Soil pH was measured in 1M KCl. Conductivity was evaluated in demineralized water. Macroelements (Ca, Mg, K, Na) were analyzed using a ContrAA® atomic absorption spectrometer (Analytik Jena, Jena, Germany). N–NH_4 and N–NO_3 concentrations were measured using a QuikChem® 8500FIA Automated Ion Analyzer (Lachat Instruments, Loveland, USA). Phosphorus was detected as P–PO_4 using ascorbic acid–molybdate and a SHIMADZU UV-1650PC spectrophotometer. The percentage of total organic carbon (TOC) was determined by wet oxidation with acidified dichromate.

Cyanobacterial biovolume

For the estimation of cyanobacterial biovolume, light and epifluorescence microscopy was employed according to Kaštovská et al. [20]. A total of 1 g of soil sample was diluted in 4 ml of distilled water and the solution was transferred to a microscope slide, covered by a coverslip and observed by epifluorescence microscopy with 40x magnification (Olympus BX 51, Japan). Filter cube with green excitation at 510–550 nm (emission 590+ nm) was used to estimate cell abundance. Cyanobacteria were discriminated into three groups according to their cell morphology: unicellular, filamentous and heterocystous. Basic geometric equations for cylinders with hemispherical ends and spheres were applied to calculate the biovolume ($\mu m^3\ g^{-1}$) of soil samples [34].

Molecular analysis of soil crust samples

DNA extraction

A modified protocol based on the method of Zhou et al. [53] was designed and tested to improve the removal of the EPS that might hinder DNA extraction of heavily ensheathed cyanobacteria, as described in Obbels et al. [13]. Collected soil crust samples were crushed several times using a micropestle in 250 µL of 0.5 M EDTA with 0.25 g of glass beads (0.17-0.18 mm diameter; Braun Biotech, Melsungen, Germany). After grinding, 500 µL of 0.5 M EDTA was added and the tubes were vortexed horizontally for 20 min and centrifuged for 3 min at 16000 g. The resultant pellet was resuspended in 100 µL of sterile water and 200 µL of a 0.2 M NaOH/1% SDS solution. Tubes were gently mixed by inversion and incubated for 15 min at 70°C. Subsequently, 150 µL of a cold potassium acetate solution (pH 5.5; 3 M) was added and tubes were gently mixed and placed on ice for 5 min. After centrifugation at 16000 g, the DNA was precipitated from the supernatant by adding 800 µL of 95% ethanol. Tubes were left for 2 min at room temperature and subsequently centrifuged at 16000 g. The pellet was washed with 300 µL of 70% ethanol and air-dried. In order to maximize yield, DNA was further extracted from the pellet as described by Zhou et al. [53]. The DNA extracted from the pellet and from the supernatant were pooled, purified with the Wizard DNA clean-up system (Promega, Madison WI) and eluted by adding 50 µL of TE-4 (10 mM TRIS-HCl pH 8; 0.1 Mm EDTA pH 8).

DGGE

The extracted genomic DNA was submitted to a denaturing gradient gel electrophoresis (DGGE) based on the V3-V4 region of the 16S rRNA gene according to Boutte et al. [4] using the cyanobacteria-specific primers 16S378F and either 16S781RaGC or 16S781RbGC [32]. DGGE was carried out twice for each sample as described in Fernandez-Carazo et al. [13]. Bands were excised from the gels, re-amplified by PCR using the 16S378F/16S781R primers and sequenced with the 16S378F primer on an ABI 3730 xls DNA analyzer (Applied Biosystems, Foster City, USA) at the GIGA Genomic facility (Liège, Belgium).

454 pyrosequencing

The extracted genomic DNA was later used for 454 pyrosequencing analysis. The cyanobacteria-specific primers 359F and 781Ra/781Rb [32] were used to amplify the V3-V4 variable region of the 16S rRNA gene as was performed for the DGGE, but the primers contained a 10-bp sample-specific barcode tag at the 5' end. A separate reaction for each reverse primer was done according to Boutte et al. [4]. PCR reactions and subsequent purification of PCR products were performed using the protocol described by Pessi et al. [35]. Amplification of DNA from the three samples from Teltet nunatak did not produce any bands, and thus these samples were excluded from further analyses. Triplicates were amplified for each sample with each reverse primer, pooled, purified using the GeneJet PCR Purification Kit (Thermo Scientific, Waltham, MA, USA) and quantified using the Quant-iTPicoGreen dsDNA Assay Kit (Life Technologies, Carlsbad, CA, USA). Libraries were pooled in equimolar concentrations and sent to Beckman Coulter Genomics (Danvers, MA, USA) where sequencing adaptors were ligated to the amplicons and sequences were obtained using the 454 GS FLX+ Titanium platform (454 Life Sciences, Branford, CT, USA).

Molecular data processing

Quality filtering of obtained sequences by pyrosequencing was performed using UPARSE [12] according to Pessi et al. [35]. Reads were cut to a length of 370 bp and sequences with more than 0.5 expected errors were discarded. Quality-filtered sequences were clustered into OTUs at

97.5% similarity, according to Taton et al. [48], and classified using CREST [25] based on the Greengenes database [29]. Non-cyanobacterial OTUs were removed from the datasets.

To compare the cyanobacterial community composition between the different sites, the dataset was subsampled to 459 sequences per sample for normalization. Alpha diversity indices (richness, Good's coverage, Chao1 and Shannon's diversity index) were calculated using QIIME [7] and differences between samples were determined using one-way ANOVA followed by Tukey's pairwise post-hoc tests in R software [17].

The five most closely related isolate sequences to the OTUs obtained by both pyrosequencing and DGGE were retrieved using the SeqMatch tool from RDP [9] and all sequences were aligned using MAFFT [22]. A phylogenetic tree comprising the cyanobacterial OTUs and their best SeqMatch hits was constructed using the Maximum Likelihood method based on the Jukes-Cantor model in MEGA6 [46]. Representative sequences of OTUs obtained by both DGGE and pyrosequencing have been deposited in GenBank under the accession numbers MF468222 – MF468273.

Pairwise community dissimilarities were computed using unweighted UniFrac distances [27] based on a minimum evolution tree obtained with the FastTree algorithm [37] implemented in QIIME, followed by unweighted pair group method with arithmetic mean (UPGMA) analysis in PRIMER7 (Primer-E, Plymouth, UK). Differences in community structure between localities were assessed using a permutational ANOVA routine (PERMANOVA) with 1000 permutations [2].

Results

Microclimatic parameters

The measurements from data loggers showed large seasonal variations in soil surface temperature and air humidity as well as differences between sites (Suppl. Table 1; Fig. 2). The mean soil surface temperatures varied between -25.7 and -36.7 ^{o}C in August, and -2 and -6.9 ^{o}C in January. Mean soil surface temperature in Pingvinane was lower than in Utsteinen and Teltet. The extreme values of the soil surface temperature fluctuated from -40 to -17 °C in August and from -15 to +17 ^{o}C in January (minimum and maximum in the four studied sites, respectively) (Suppl. Table 1) (Fig. 2). The maximal values were positive, showing that the soil surface was able

to accumulate heat from the solar radiation during sunlit periods in summer, even if the air temperature was still negative. The air relative humidity (RH) varied seasonally in the range from 12 to 107% in January and from 62 to 101% in August (Suppl. Table 1). RH exceeding 100% (maximum RH was 107%) indicated a supersaturation when the air contained more water vapor than is needed to cause saturation. The variable RH values in austral summer were probably influenced by the meteorological variations that cause either a solar drying or a wetting by snow storms. RH values in austral summer for Teltet were recorded only in 2012 and had the lowest mean, as could be expected from loose gneiss pebbles where any water would quickly drain. However, RH values for Tanngarden were not available, precluding a comparison.

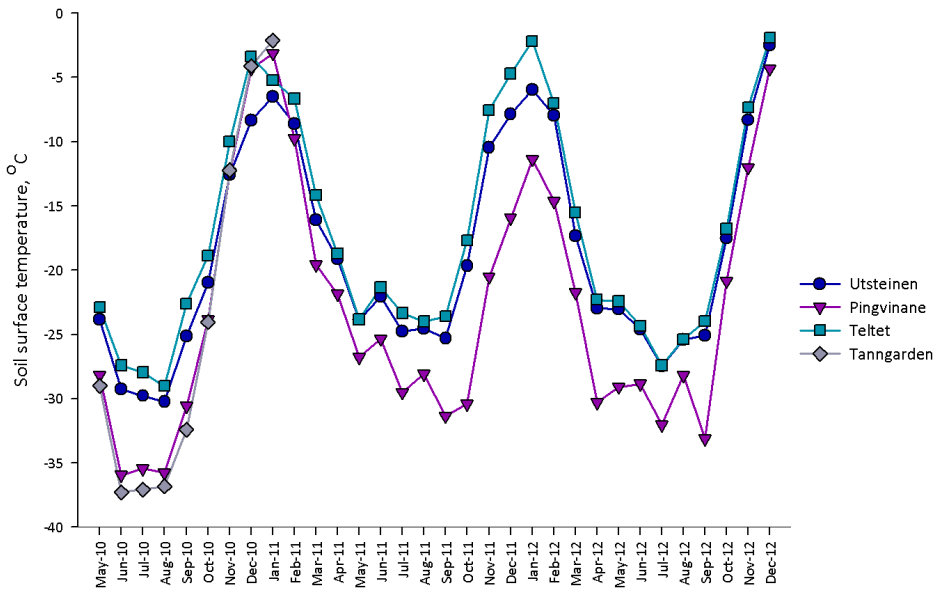

Fig. 2. Soil surface temperature measured in four studied localities in the period from May 2010 to December 2012. Note that data from Tanngarden are available only until January 2011.

Soil parameters

Soil parameters differed between the four studied sites (Table 1). Soils from Utsteinen and Pingvinane were slightly acidic (pH 5.58 and 5.65, respectively). However, they differed in $N–NH_4$, $N–NO_3$ and TOC concentrations, which were higher in soil samples from the Utsteinen

ridge. The soils of Tanngarden had a neutral pH (7.33) and intermediate nutrient concentrations. Finally, the soil samples from the gneiss substrate of Teltet were slightly alkaline (pH 8.31) with a higher conductivity and lower water content than the other sites. This substrate mostly consisted of loose pebbles of small size and this may have enhanced the drainage of water and concentration of salts.

Table 1. Soil chemistry of the sampling sites.

	Utsteinen	**Pingvinane**	**Tanngarden**	**Teltet**
pH	5.58	5.65	7.33	8.31
Conductivity, uS/cm	191.35	21.20	153.20	655.00
N-NH4, mg/kg	22.03	1.54	12.13	1.89
N-NO3, mg/kg	1.35	0.43	5.67	11.69
P-PO4, mg/kg	0.31	0.28	0.72	0.30
Water content, %	5.24	6.78	4.02	3.32
TOC, %	1.81	0.90	1.29	2.20
Ca, g/kg	3.60	3.65	6.87	16.68
Mg, g/kg	2.81	1.29	1.41	12.12
K, g/kg	3.47	2.70	1.49	4.01
Na, g/kg	0.60	0.40	0.35	1.02

Cyanobacterial biovolume

Cyanobacterial biovolume obtained by epifluorescence and light microscopy varied among the studied sites (Fig. 3, 4a). Total cyanobacterial biovolume was highest in Pingvinane (799 x 10^6 μm^3 g^{-1}) and lowest in Teltet (12 x 10^6 μm^3 g^{-1}) soil crusts. Unicellular cyanobacteria were not found in Teltet and Tanngarden, and were rare in Pingvinane, but were the dominant cyanobacteria in Utsteinen (78.0% of total biovolume) soil crusts. The highest biovolume of filamentous cyanobacteria was recorded in Pingvinane and Tanngarden where they were also dominant (52.4 and 51.5% of total biovolume, respectively). Heterocystous cyanobacteria were detected in all sites.

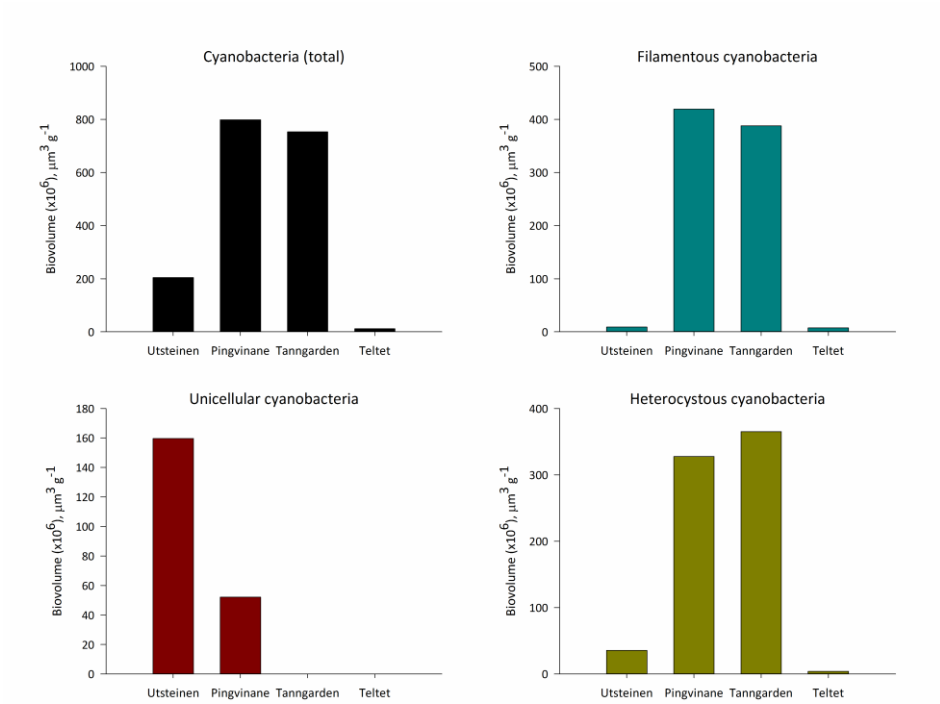

Fig. 3. Cyanobacterial biovolume in soil crusts from the four localities. Note the different scales of the *y* axis in each graph.

Taxonomic composition of cyanobacterial community

DGGE was used for a first comparison of the cyanobacterial community compositions between the samples, and was later complemented by a more in-depth pyrosequencing analysis. Three subsamples were used for each locality except Utsteinen where only one sample was studied. A total of 72 cyanobacterial sequences obtained by DGGE were grouped into 16 OTUs (DGGE_OTUs; Table 2; Suppl. Fig. 2). The majority of the DGGE_OTUs was recorded in Pingvinane (10 DGGE_OTUs) followed by Teltet (7 DGGE_OTUs) and Tanngarden (6 DGGE_OTUs). The only sample from Utsteinen yielded 1 DGGE_OTU that was also present in the two other granitic sites. This is likely related to the number of bands sequenced from the samples in each site (Pingvinane: 31; Teltet: 21; Tanngarden: 13; Utsteinen: 2). DNA fingerprinting of Teltet samples was only possible using the DGGE technique. Moreover, three and two DGGE_OTUs found in Teltet samples were also found in Pingvinane and in Tanngarden, respectively. In addition, in the phylogenetic tree (Suppl.

Fig. 2) built with OTUs obtained by both techniques, 9 lineages comprised both OTUs and DGGE_OTUs. However, five DGGE_OTUs were not associated with pyrosequencing OTUs. DGGE_OTUs 9, 10 and 11 in the *Nostoc* lineage found in the samples from Tanngarden and Teltet were not observed in the pyrosequencing data. DGGE_OTU 2 found in Pingvinane and DGGE_OTU 7 recorded in Tanngarden and Utsteinen were related to Antarctic Oscillatoriales strains (*Phormidesmis priestleyi* ANT.L52.4 and *Phormidium pseudopriestleyi* ANT.LACV5.3, respectively) and were also absent from the pyrosequencing data.

The pyrosequencing analysis produced a total of 47462 16S rRNA gene reads from eight soil crust samples (3 samples from Tanngarden, 3 samples from Pingvinane and 2 samples from Utsteinen). PCR amplification of the samples from Teltet was unsuccessful, probably due to the very low abundance of cyanobacteria there (as shown by microscopy), and thus the low DNA extraction yield that could be aggravated by a possible degradation during storage prior to pyrosequencing. After quality filtering, 25419 sequences remained. Of these, 1665 sequences (6.5%) belonged to the bacterial phyla Acidobacteria, Chloroflexi and TM7, and 1133 sequences (4.5%) were assigned to the plastid of eukaryotic phototrophs from the groups Chlorophyta and Stramenopiles (Heterokonta). As the algal plastids and bacteria other than cyanobacteria were not the focus of this study, the information on their diversity and taxonomic affiliations are given in Suppl. Table 2. After removal of non-cyanobacterial sequences, 22621 sequences (89.0%) remained. Good's coverage estimates varied from 99.3% to 100.0%, which indicates that the majority of the cyanobacterial diversity was captured in the analysis (Suppl. Table 3). After resampling the dataset to 459 sequences per sample, the richness was lowest in Tanngarden (average of 5 OTUs) and did not differ significantly in Utsteinen and Pingvinane (average of 10 OTUs each) (Suppl. Table 3).

Cyanobacterial sequences were clustered into 19 OTUs at 97.5% similarity (Table 3; Suppl. Fig. 2). OTUs belonged to the orders Synechococcales (10 OTUs), Oscillatoriales (2 OTUs), Nostocales (2 OTUs) and Chroococcales (3 OTUs) based on the Greengenes database. Two OTUs (OTUs 14 and 19) could not be assigned to a cyanobacterial order by the classification algorithm, but further BLAST analyses indicated that they likely belonged to Chroococcales and Oscillatoriales, respectively. The majority of sequences in each site (from 85.7 to 94.3%) corresponded to filamentous cyanobacteria (Fig.4b). OTU 1 (97.4%

similar to *Leptolyngbya* sp. ANT.RI8.1) was the most abundant OTU in Utsteinen and Tanngarden (Table 3). OTU 2 (100% similar to *Phormidium autumnale* CCALA 697) was dominant in Pingvinane and was also found by DGGE. Two related OTUs (OTUs 3 and 25; 99.2 and 96.4% similar to *Leptolyngbya compacta*, respectively) were abundant in Pingvinane and Utsteinen. They were also represented by DGGE_OTUs in Pingvinane and Teltet. OTUs 14 and 21 (99.7% and 95.6% similar to *Cyanothece aeruginosa* NIVA-CYA 258/2, respectively) were detected only in Pingvinane, but were not found in other localities. Only two OTUs (OTUs 4 and 9) belonged to Nostocales. Moreover, OTU 4 (100% similar to *Nostoc indistinguendum* CM1-VF10) corresponded to DGGE_OTU 8.

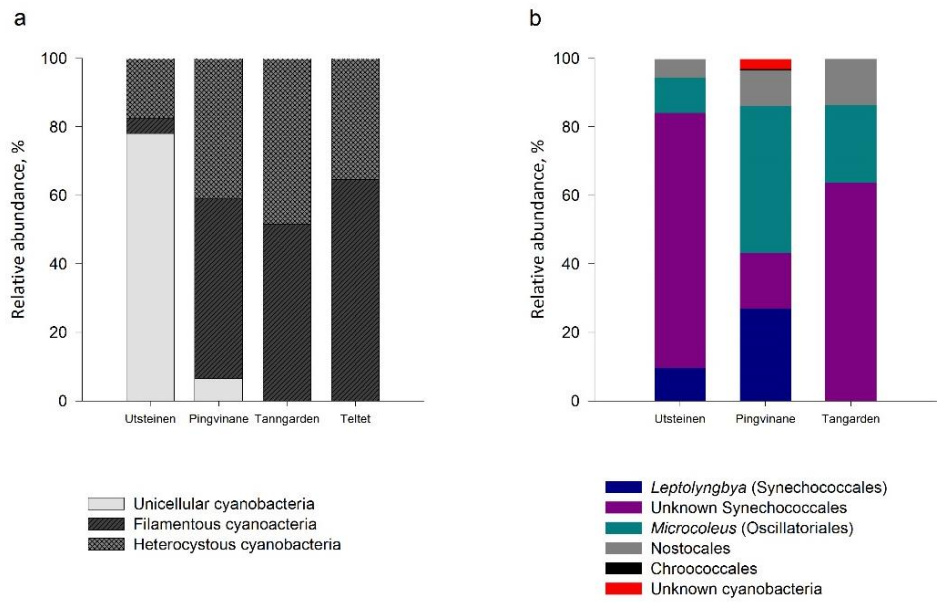

Fig. 4. Relative abundance of cyanobacterial taxa estimated by (a) epifluorescence microscopy and (b) 454 pyrosequencing

Beta diversity analysis showed that the community structures obtained by DGGE were clustered irrespectively of sampling sites (Suppl. Fig. 3a). On the other hand, pyrosequencing data clearly showed that BSCs communities from Tanngarden were distinct from the other two localities (PERMANOVA, pseudo-F = 8.03, p = 0.006) (Suppl. Fig. 3b). Communities from Pingvinane and Utsteinen did not differ significantly ($p > 0.05$), although samples from Pingvinane formed a well-defined cluster.

Table 2. List of OTUs found in soil crust samples from the Sør Rondane Mountains by DGGE.

OTU	Representative sequence	Accession number	Number of sequences				Best SeqMatch isolate hit (ID %)	Best SeqMatch uncultured hit (ID %)
			Utst	Pingv	Tanng	Telt		
DGGE_ OTU 1	10TN45a01C	MF468232	0	0	0	7	*Hormoscilla pringsheimii* SAG 1407-1; KC572078 (98.6%)	uncultured bacterium; SPIT_D12; GQ306044 (99.3%)
DGGE_ OTU 2	10PI43b03C	MF468229	0	2	0	0	*Phormidesmis priestleyi* ANT.L52.4; AY493578 (98.2%)	uncultured cyanobacterium; H-C07; DQ181722 (99.1%)
DGGE_ OTU 3	10PI43a01S	MF468230	0	10	0	2	*Microcoleus antarcticus* UTCC 474; AF218373 (100%)	uncultured cyanobacterium; 89-23; JN814349 (100%)
DGGE_ OTU 4	10TA32a01C	MF468234	2	1	2	0	*Leptolyngbya* sp. ANT.L52.1; AY493584 (97.2%)	uncultured Oscillatoriales cyanobacterium; H_21; FJ490341 (98.9%)
DGGE_ OTU 5	10TN47b04C	MF468226	0	0	0	1	*Hormoscilla pringsheimii* SAG 1407-1; KC572078 (92.7%)	uncultured bacterium; Bact_SIP_153; JX204373 (93.3%)
DGGE_ OTU 6	10PI43a01C	MF468233	0	1	0	1	cyanobacterium OU_20; GQ162224 (95.8%)	uncultured Nostocales cyanobacterium; C_4; FJ490241 (99.4%)
DGGE_ OTU 7	10PI42a01C	MF468231	0	3	0	0	*Phormidium pseudopriestleyi* ANT.LACV5.3; AY493600 (99.7%)	uncultured Antarctic cyanobacterium; SalP10; AY541536 (99.7%)
DGGE_ OTU 8	10TN46b03S	MF468222	0	0	4	5	*Nostoc* sp. ANT.LG2.6; AY493595 (99.1%)	uncultured bacterium; abscm03.1.110; JX255112 (99.1%)
DGGE_ OTU 9	10TA39a02C	MF468223	0	0	4	0	*Nostoc* sp. PCC 9305; AY742453 (99.4%)	uncultured Nostoc sp.; MVMG1; EU359045 (99.4%)
DGGE_ OTU 10	10TA40a04S	MF468224	0	0	1	0	*Nostoc* sp. CCAP 1453/31; HE974996 (98.3%)	uncultured Nostoc sp.; B9_47; AM940880 (98.3%)
DGGE_ OTU 11	10TN47a04C	MF468225	0	0	1	3	*Nostoc* sp. 9.4.22; AY328896 (98.8%)	uncultured cyanobacterium; B6_63; AM940825 (98.8%)
DGGE_ OTU 12	10PI44b03C	MF468235	0	1	0	0	*Leptolyngbya* sp. Greenland_7; DQ431002 (93.1%)	uncultured cyanobacterium; BksYy25500; KC463305 (97.0%)
DGGE_ OTU 13	10PI44b04C	MF468237	0	8	0	2	*Phormidium* sp. CYN64; JQ687330 (97.2%)	uncultured Antarctic cyanobacterium; Fr396; AY151763 (97.5%)
DGGE_ OTU 14	10PI44b01C	MF468236	0	1	0	0	*Phormidium* sp. CYN64; JQ687330 (97.5%)	uncultured Antarctic cyanobacterium; Fr396; AY151763 (97.5%)
DGGE_ OTU 15	10PI44b03C	MF468228	0	2	0	0	*Phormidesmis priestleyi* ANT.L66.1; AY493581 (99.0%)	uncultured Phormidium sp.; 5f-12; JF832301 (100.0%)
DGGE_ OTU 16	10PI43b04S	MF468227	0	2	1	0	*Cyanothece aeruginosa; NIVA-CYA 258/2; Z82775* (93.7%)	uncultured bacterium; PB17018-1_F01; JX172409 (95.4%)

Table 3. List of OTUs found in soil crust samples from the Sør Rondane Mountains using 454 pyrosequencing. Number of sequences and relative abundances were calculated after rarefying datasets to 459 sequences per sample.

OTU	Accession number	Relative abundance, %			Best SeqMatch isolate hit (ID %)	Best SeqMatch uncultured hit (ID %)
		Utst	Pingv	Tanng		
OTU 1	MF468238	73.64	15.98	55.70	*Leptolyngbya* sp. ANT.RI8.1; AY493619 (97.4%)	uncultured Oscillatoriales cyanobacterium; H_21; FJ490341 (99.2%)
OTU 2	MF468252	10.35	42.85	22.73	*Phormidium autumnale* CCALA 697; AM778710 (100.0%)	uncultured cyanobacterium; 89-23; JN814349 (100.0%)
OTU 3	MF468259	0.11	23.97	0.00	*Leptolyngbya compacta* GSE-PSE28-08A; HQ132933 (99.2%)	uncultured cyanobacterium; LVG1348; KC936989 (98.9%)
OTU 4	MF468244	5.34	7.12	13.22	*Nostoc indistinguendum* CM1-VF10; AY577538 (100.0%)	uncultured bacterium; bscm1.1.294; JX255063 (100.0%)
OTU 7	MF468239	0.76	0.00	7.92	*Leptolyngbya* cf. *foveolarum* TM2ULC129; EU852496 (99.7%)	uncultured cyanobacterium; GL2-21; EF215735 (98.6%)
OTU 8	MF468254	0.33	2.76	0.00	*Phormidesmis priestleyi* ANT.LMA.2; AY493613 (98.9%)	uncultured cyanobacterium; B9_81; AM940913 (100.0%)
OTU 9	MF468245	0.00	3.20	0.00	*Godleya alpine* LCR-CYTOL; HQ012539 (100.0%)	uncultured cyanobacterium; S282; JQ776464 (100.0%)
OTU 13	MF468255	0.98	0.15	0.00	*Leptolyngbya frigida* ANT.LJA.1; AY493614 (100.0%)	cyanobacterium enrichment culture clone 4_4_4.3.1_F08-T7; JQ310380 (99.7%)
OTU 14	MF468240	0.00	2.03	0.00	*Cyanothece aeruginosa* NIVA-CYA 258/2; Z82775 (99.7%)	uncultured bacterium; PB17018-1_A08; JX172360 (95.3%)
OTU 15	MF468261	0.11	0.36	0.07	*Leptolyngbya* sp. Greenland_6; DQ431001 (92.3%)	uncultured cyanobacterium; CW1_P2_3B; KC110372 (97.0%)
OTU 17	MF468242	0.22	0.22	0.29	cyanobacterium cCLB-9; HQ230230 (100.0%)	Uncultured cyanobacterium; MB5-20; HM104593 (100.0%)
OTU 19	MF468251	0.00	1.09	0.00	*Hormoscilla pringsheimii* SAG 1407-1; KJ140096 (98.1%)	uncultured bacterium; SPIT_D12; GQ306044 (99.6%)
OTU 21	MF468241	0.00	0.22	0.00	*Cyanothece aeruginosa* NIVA-CYA 258/2; Z82775 (95.6%)	uncultured bacterium; PB17018-1_D07; JX172394 (100.0%)
OTU 25	MF468260	6.54	0.00	0.00	*Leptolyngbya compacta* GSE-PSE28-08A; HQ132933 (96.4%)	uncultured cyanobacterium; A9_71; AM940668 (99.2%)
OTU 28	MF468257	0.54	0.00	0.00	cyanobacterium cCLA-17; HQ230228 (98.1%)	uncultured cyanobacterium; PA122; FJ977099 (99.2%)
OTU 29	MF468258	0.44	0.00	0.00	cyanobacterium cCLA-17; HQ230228 (98.1%)	uncultured bacterium; 8R_plate2e09.b3; HM356205 (99.2%)
OTU 30	MF468256	0.65	0.00	0.00	*Leptolyngbya frigida* ANT.L53B.1; AY493608 (97.2%)	uncultured cyanobacterium; A9_42; AM940642 (100.0%)
OTU 31	MF468243	0.00	0.07	0.07	cyanobacterium cCLB-12; HQ230231 (98.4%)	uncultured cyanobacterium; CNY_02997; JQ402596 (98.9%)
OTU 36	MF468253	excluded*			*Microcoleus vaginatus;* 127-1; AJ871222 (93.8%)	Uncultured cyanobacterium; CNY_02239; JQ401988 (96.3%)

* After resampling the dataset to 459 sequences per sample for normalization, OTU 36 was excluded from the estimation of relative abundance due to low number of sequences (2 sequences in one subsample from Utsteinen).

Discussion

Cyanobacterial community composition in BSCs from two ridges (Utsteinen and Tanngarden) and two nunataks (Pingvinane and Teltet) estimated by morphological and molecular techniques showed differences between the studied sites (Tables 2, 3; Figs. 3, 4). Based on a microscopical analysis, the cyanobacterial biovolume was the highest in soil samples from Pingvinane and Tanngarden with a dominance of

filamentous cyanobacteria while the lowest biovolume of cyanobacteria was recorded in Teltet (Fig. 3, 4a). These dissimilarities could be related to the different type of surface, soil parameters and microclimatic conditions. Indeed, the substrate in Teltet consisted of loose pebbles of gneiss rocks whereas in three other localities it was comprised of big and solid granite boulders and rocks. In contrast to the results obtained by molecular methods, unicellular cyanobacteria were detected by epifluorescence microscopy in Tanngarden and Utsteinen, and were even the most abundant cyanobacteria in the Utsteinen soil samples. A similar observation was made with Arctic BSC samples, for which the abundance of unicellular cyanobacteria measured by epifluorescence microscopy was higher than other cyanobacteria while their relative abundance obtained by 454 pyrosequencing was very low [39,40]. In the present study, the sampling strategy could have played a role in the discrepancy because the subsamples for soil analysis and biovolume determination included BSC, but also bare soil, to represent the whole area. Unicellular cyanobacteria might have been present in the bare soil samples which were not studied by molecular methods. Previous studies of the Utsteinen ridge and nunatak have shown the presence of unicellular taxa. For example, *Asterocapsa* sp., *Chroococcus* sp. and *Cyanothece aeruginosa* were observed in BSC samples (though the samples were named as "microbial mats" and not as "BSC") using microscopy and DGGE [13]. Moreover, Chroococcales and Synechococcales sequences were detected by 454 pyrosequencing with bacterial PCR primers in samples from the Utsteinen ridge [33]. In addition, at the molecular methodological level, it is known that several unicellular cyanobacteria form colonies surrounded by a thick polysaccharidic sheath that might hinder DNA extraction, whereas this problem is absent for thin filamentous taxa. However, an alternative DNA extraction method had been designed to address this problem and the sequences of Nostocales living in sheathed colonies were obtained [19].

The same methods to measure cyanobacterial biovolume have been used for BSCs from the Arctic [20,21,39] and Himalaya [6,19,41], which allowed us to compare different regions with a severe climate. For example, the total cyanobacterial biovolume in soil crusts from Himalaya ranged from 789 to 1110 x 10^6 μm^3 g^{-1} [6,19] while in the Arctic, it varied between 13 and 233 x 10^6 μm^3 g^{-1} depending on the stage of soil crust development [39]. The cyanobacterial biovolumes of soil crusts from Teltet and Utsteinen (12 x 10^6 and 205 x 10^6 μm^3 g^{-1}, respectively) were comparable to those of Arctic soil crusts. However, the values for

Tanngarden and Pingvinane (753 and 799 x 10^6 μm^3 g^{-1}, respectively) were closer to the Himalayan ones. Interestingly, the cyanobacterial biovolume in Antarctic soil crusts was higher than in Arctic which might be explained by less competition with vascular plants for light and space or lower grazing pressure in the south polar area. However, this observation should be tested with BSC samples from more localities in polar and alpine environment.

At the molecular level, DGGE and 454 pyrosequencing revealed similar cyanobacterial richness (16 and 19 OTUs, respectively). Even though the representative sequences of the majority of OTUs obtained by both techniques were more than 98.2% similar to each other, five OTUs obtained by DGGE (DGGE_OTUs) differed from OTUs found by pyrosequencing. Based on the limitations of the band separation efficiency and the fact that not all bands can be extracted and sequenced, DGGE only allows an assessment of the dominant members of microbial communities in natural systems. In contrast, 454 pyrosequencing can provide a diversity analysis with much higher throughput. Therefore, it was surprising to observe that DGGE could still be a useful complement to the next-generation sequencing and provided additional information on the community structure. The same DNA was used for both techniques at five years interval, but it was freshly extracted for the DGGE. Moreover, some protocol steps, like the use of extensions with various lengths and GC% at the 5' end of one or both cyanobacterial primers, may play a role by producing different biases during PCR and maybe selective amplification.

Using 454 pyrosequencing, the three studied sites were dominated by cyanobacteria from the order Synechococcales and Oscillatoriales (86-94% of sequences) (Fig. 4b; Table 3). These filamentous cyanobacteria are common in polar freshwater and terrestrial habitats [6,15,24,35,38] and often inhabit coarse and unstable soils [40]. Seven OTUs (92.3-100.0% similar to *Leptolyngbya* spp.) composed the majority of sequences in Utsteinen (82.79%) and Tanngarden (63.69%). OTU 2 (100% similar to *Phormidium autumnale* CCALA 697) was the dominant OTU in soil crust from Pingvinane, comprising 42.85% of all sequences there. The cyanobacterial genera Leptolyngbya and Phormidium are very common in soil crusts in Antarctica [15,30] as well as in other parts of the world [6,38,42]. Heterocystous cyanobacteria from the order Nostocales, which are the main source of fixed nitrogen in soil crusts [45], were also recorded in the studied samples. Two OTUs affiliated to the genera *Nostoc* (OTU 4; 100% similarity) and *Godleya* (OTU 9; 100% similarity) were detected

by pyrosequencing. Sequences similar to those of OTU 4 and OTU 9 have already been found in Antarctic aquatic mats [35] and Antarctic soils [43], respectively. In addition, the representative sequence of OTU 9 was 99.1% similar to sequences that were very abundant in samples from the Utsteinen nunatak [49]. Interestingly, few OTUs (17, 28, 29 and 31) corresponded to non-uncultured cyanobacteria (98.1-100.0% similarity) previously found in Arctic snow [18]. Moreover, five OTUs had sequences that were < 97.5% similarity to known sequences in RDP and thus likely represent yet uncharacterized lineages.

UPGMA analysis based on DGGE data indicated no clear clustering between the studied subsamples while the pyrosequencing data showed that the subsamples were well grouped by their site of origin, and that Utsteinen and Pingvinane shared more OTUs between each other than with Tanngarden. Most probably, it could be correlated with the physicochemical conditions of the environment [26]. It has been already shown that pH, water content and carbon concentration influence soil crust cyanobacteria in the Arctic [40]. Indeed, soils from Utsteinen and Pingvinane had more similar soil pH (5.58 and 5.65, respectively) and water content (5.24 and 6.78%, respectively) than other localities. Furthermore, Tytgat et al. [49] proposed that the structure of soil microbial communities in the SørRondane Mountains is mainly controlled by TOC concentration, pH, bedrock type and the availability of moisture. They found differences between microbial diversity on granitic or gneiss substrates, and a number of bacterial phyla, including cyanobacteria, dominated the soil samples with a high TOC concentration that were mainly of granitic origin. Therefore, the lowest cyanobacterial biovolume in soil crusts from Teltet nunatak observed in our study might be explained by the bedrock type (gneiss), low water content and high pH. Negative correlation between conductivity and soil microbiota has previously been observed in the Mc Murdo Dry Valleys [26]. Thus, the high conductivity measured in soil from Teltet could negatively influence the cyanobacterial community there as well. However, the sampling design was not initially planned for replication and no statistical analysis could be performed.

Conclusions

In this study, we provided a description of the cyanobacterial diversity in soil crusts in four sites from the Sør Rondane Mountains, Antarctica using molecular and morphological methods. DGGE and 454

pyrosequencing gave complementary data to describe the cyanobacterial community compositions. In agreement with previous studies, at the taxonomic level, the soil crust samples were dominated by filamentous and heterocystous cyanobacteria (Synechococcales, Oscillatoriales and Nostocales). In addition, OTUs that were more than 97.5% similar to Leptolyngbya sp. and Microcoleus antarcticus were dominant in the studied BSCs. Likewise, the biovolume of filamentous cyanobacteria was the highest in all samples, except those from Utsteinen ridge.

The obtained results revealed differences in cyanobacterial community composition which most probably was correlated to the substrate type and physicochemical soil properties. Moreover, a role of geographic separation might be an important factor in community structure, since each studied area was isolated and acted as an island with a distinct cyanobacterial community on the basis of the pyrosequencing data. It highlights the great spatial variation of microbial communities in this environment, which might be of special relevance for conservation measures of Antarctic ecosystems in light of climate change.

Furthermore, these data will be later used to test the effect of climate manipulation on cyanobacterial communities inside the OTCs during a period of several years. Knowing the effect of such a change would help to make predictions on the evolution of the cyanobacterial communities in the terrestrial biotopes of the Sor Rondane Mountains.

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Supplementary material

Suppl. Table 1. Soil surface temperature and air humidity of studied soil crusts measured by data loggers.

	Temperature, °C							Relative humidity, %						
	2010	2011		2012		Mean		2010	2011		2012		Mean	
	Aug	Jan	Aug	Jan	Aug	Jan	Aug	Aug	Jan	Aug	Jan	Aug	Jan	Aug
Utsteinen														
Mean	-30	NA	-24.2	-5.4	-25.1	-5.4	-26.4	93.7	78.2	96	68	81.8	73.1	90.5
Max	-23	NA	-18	9.5	-16	9.5	-19	96	103	98	106.5	99.5	104.8	97.8
Min	-37	NA	-32.5	-15	-36.5	-15	-35.3	91	22	93.5	23	62.5	22.5	82.3
Pingvinane														
Mean	-35.6	-2.9	-27.7	-10.8	-27.7	-6.9	-30.4	NA	NA	97.1	103.8	90.3	103.8	93.7
Max	-19	14	-23	-2	-16	6	-19.3	NA	NA	99	107	97	107	98
Min	-40	-11	-37	-18	-41	-14.5	-39.3	NA	NA	92	88	62	88	77
Teltet														
Mean	-28.6	NA	-23.6	-2	-25	-2	-25.7	NA	NA	96.6	54	96.1	54	96.4
Max	-20	NA	-14	17	-17	17	-17	NA	NA	101	107	99	107	100
Min	-38	NA	-36	-13	-37	-13	-37	NA	NA	93	12	91	12	92
Tanngarden														
Mean	-36.7	-2	NA	NA	NA	-2	-36.7							
Max	-21	12	NA	NA	NA	12	-21				NA			
Min	-40	-10	NA	NA	NA	-10	-40							

Suppl. Table 2. List of OTUs other than cyanobacteria found in soil crust samples from Dronning Maud Land using 454 pyrosequencing.

OTU	Accession number	Greengenes assigment
OTU 5	MF468246	Chloroplast, Chlorophyta
OTU 6	MF468263	Bacteria, Chloroflexi, Thermomicrobia, HN1-15
OTU 10	MF468247	Chloroplast, Chlorophyta
OTU 11	MF468264	Bacteria, Chloroflexi, Thermomicrobia, HN1-15
OTU 12	MF468272	Bacteria, TM7, TM7-1
OTU 16	MF468266	Bacteria, Chloroflexi, Thermomicrobia, HN1-15
OTU 18	MF468268	Bacteria, Chloroflexi
OTU 20	MF468267	Bacteria, Chloroflexi, Thermomicrobia, HN1-15
OTU 22	MF468262	Chloroplast, Stramenopiles
OTU 23	MF468270	Bacteria, Chloroflexi, Thermomicrobia
OTU 24	MF468271	Bacteria, Chloroflexi, Thermomicrobia
OTU 26	MF468265	Bacteria, Chloroflexi, Thermomicrobia, HN1-15
OTU 27	MF468248	Chloroplast, Chlorophyta
OTU 32	MF468249	Chloroplast, Chlorophyta
OTU 33	MF468250	Chloroplast, Chlorophyta
OTU 34	MF468269	Bacteria, Chloroflexi, Thermomicrobia
OTU 35	MF468273	Bacteria, Acidobacteria, Chloracidobacteria

Suppl. Table 3. Cyanobacterial richness and diversity indices obtained by pyrosequencing 454 in studied soil crusts.

	Observed species	*Good's coverage estimator (%)*	*Chao 1*	*Shannon's index*
Utsteinen				
PE1081	8	99.6	9.00	1.86
PE1082	11	99.3	12.50	0.58
mean	10^{a}	99.5^{a}	10.75^{a}	1.22^{a}
Pingvinane				
PE1042	10	99.6	10.33	2.21
PE1043	10	99.6	11.00	2.19
PE1044	11	99.3	12.50	1.94
mean	10^{a}	99.5^{a}	11.28^{a}	2.11^{a}
Tanngarden				
PE1039	5	99.8	5.00	1.04
PE1040	5	100	5.00	1.07
PE1041	6	99.8	6.00	1.51
mean	5^{b}	99.9^{a}	5.33^{b}	1.21^{a}

Same letters indicate no statistical difference according to one-way ANOVA followed by Tukey's pairwise posthoc tests ($P < 0.05$).

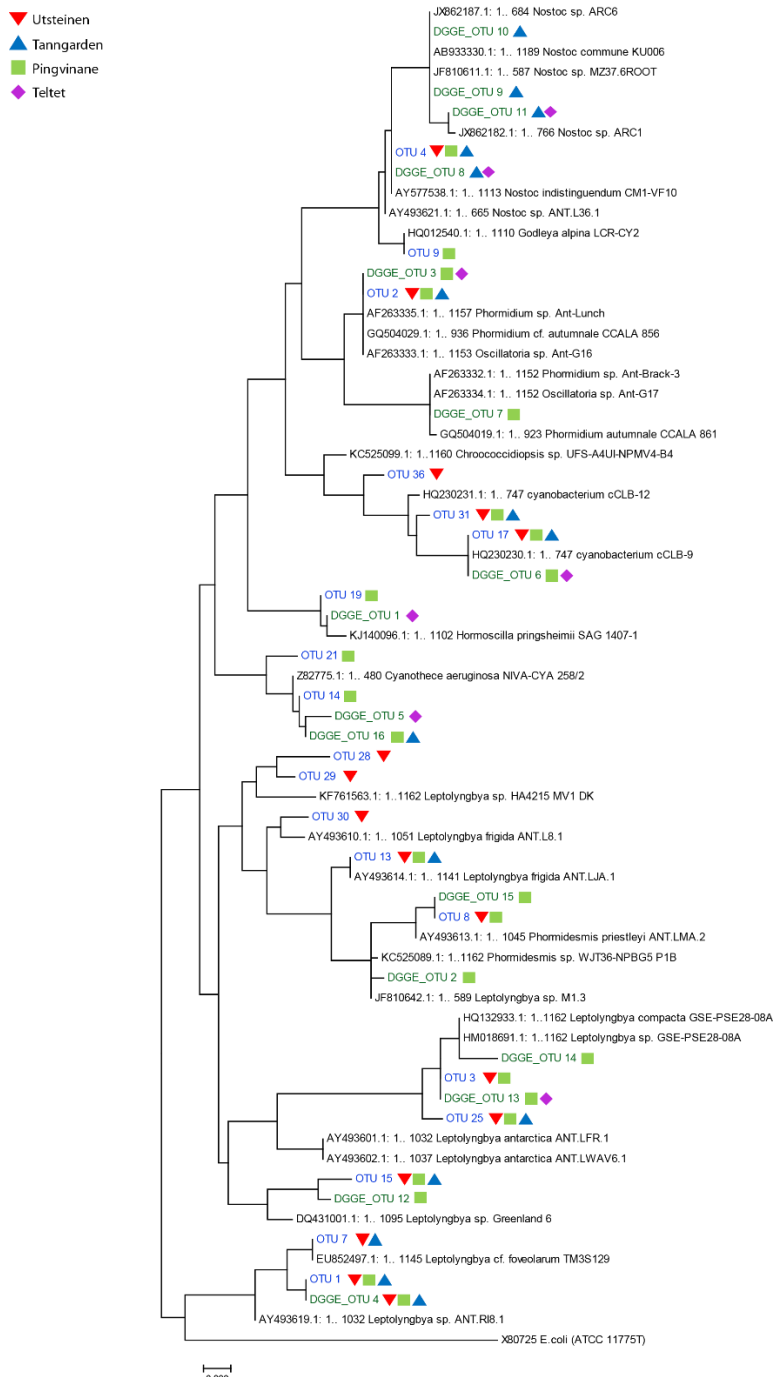
Utsteinen

Tanngarden

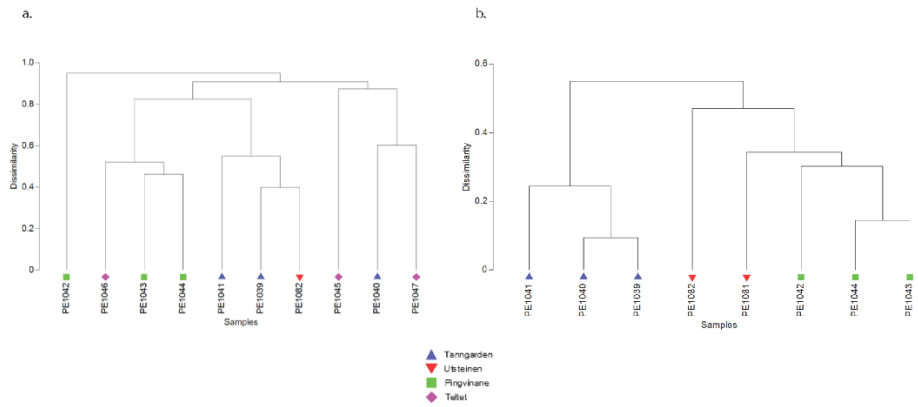
Teltet

Pingvinane

Suppl. Fig. 1. Pictures of the studied localities in Sør Rondane Mountains, Antarctica.



Suppl. Fig. 2. Maximum-likelihood tree based on analysis of 16S rRNA gene comprising the cyanobacterial OTUs obtained by DGGE (DGGE_OTUs) and pyrosequencing 454 (OTUs) and their best SeqMatch hits. Shapes next to the OTUs indicate the locality where the sequences were found.



Suppl. Fig. 3. Cyanobacterial community composition in studied soil crusts analyzed by different molecular tools (a – DGGE; b – pyrosequencing 454) indicated by UPGMA analyses of pairwise unweighted UniFrac distances.

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Ekaterina Pushkareva
puekse@gmail.com

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University of South Bohemia in České Budějovice
Faculty of Science
Branišovská 1760
CZ-37005 České Budějovice, Czech Republic

Phone: +420 387 776 201
www.prf.jcu.cz, e-mail: sekret-fpr@prf.jcu.cz