University of South Bohemia

Faculty of Science

Marek Stibal

Photoautotrophic microorganisms in the glacial ecosystem of Svalbard, high Arctic

Ph.D. Thesis

Supervised by Dr Josef Elster, Institute of Botany, Czech Academy of Sciences, Třeboň

České Budějovice 2009

Stibal M (2009) Photoautotrophic microorganisms in the glacial ecosystem of Svalbard, high Arctic. PhD thesis, University of South Bohemia, Czech Republic.

Annotation: Photoautotrophic microorganisms, i.e. cyanobacteria and microalgae, are ubiquitous in the glacial ecosystem of the high Arctic archipelago of Svalbard. Their communities play significant roles in the ecosystem, including organic carbon production on the glacier surface and its supply to downstream environments, initiating microbial colonisation after glacier retreat and preparing proglacial substrata for further succession.

I declare that the work in this dissertation is original, except where indicated by special reference in the text. Any views expressed in the dissertation are those of the author.

I declare that in compliance with law 111/1998 §47b I consent to electronic publication of the full version of this dissertation in the public section of the University of South Bohemia STAG database.

SIGNED: ..... DATE: .....

I certify that the author of this thesis contributed considerably to designing, conducting and interpretation of research published in Papers 1, 2 and 5 of which I am the first author, and I agree that they are included in the thesis.

SIGNED: ..... DATE: .....

Contents

1. Introduction

1.1. The cryosphere and glacial ecosystems

A great portion of water present on the Earth's surface is in the solid phase – as ice and snow. The part of our planet where water is predominantly frozen is collectively termed the cryosphere, and consists of a variety of environments, including sea-ice, freshwater ice on lakes and rivers, the snow cover, permafrost, and of course glaciers and ice sheets (Priscu and Christner 2004).

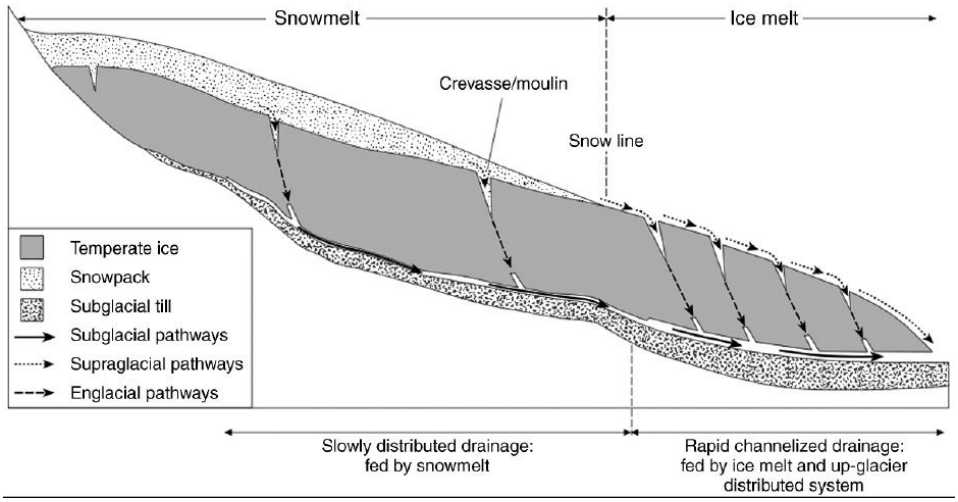

Figure 1.1. Schematic of the principal environments of a valley glacier (from Hodson et al. 2008). Snowpack and the surface of the ice represent the supraglacial environment. Englacial environments are contained within the ice, including the walls of crevasses and moulins. The subglacial environment is represented by the subglacial till layer and its contact with the glacial ice.

Glaciers and ice sheets cover approximately 16,000,000 km^2, or ca 10% of the Earth's land surface, and contain ca 33,000,000 km^3, or ca 70%, of freshwater (Benn and Evans

1998). Most of the ice is contained within the Antarctic (85% of area, >90% of volume) and Greenland (12% of area, 7% of volume) ice sheets, while the rest exists as high-latitude and high-altitude ice caps and glaciers (Benn and Evans 1998). Glaciers were formerly thought to be virtually lifeless and unimportant for global biogeochemical cycles. However, in recent years they have been established as highly dynamic ecosystems in their own right whose physical and chemical conditions support thriving microbial communities (Hodson et al. 2008). There are several principal glacial environments in the cryosphere: the subglacial environment under glaciers at the ice-bed interface, the supraglacial environment on the surface of glaciers, the proglacial environment exposed after glacier retreat, and the temporary snow environment. A schematic of the principal glacial environments of a small valley glacier is shown in Figure 1.1.

1.2. The subglacial environment

The interface between the glacier ice and the bedrock (Figure 1.2) has been shown to be inhabited by active microbial communities under glaciers in the Alps (Sharp et al. 1999), Canadian Arctic (Skidmore et al. 2000, 2005, Bhatia et al. 2006), Svalbard (Wadham et al. 2004), Antarctic Dry Valleys (Mikucki et al. 2004) and New Zealand (Foght et al. 2004). Microbial processes in subglacial environments are likely to be affected by the hydraulic conditions under the glacier, ranging from aerated environments close to drainage channels to anaerobic sediments (Tranter et al. 2005). The microbial communities of these sites are primarily associated with sediments and basal ice derived from the glacier bed. Basal ice typically has a higher solute and sediment content, as it is formed by processes at the glacier bed, such as freezing of subglacial waters and entrainment of subglacial sediment and debris. Due to the lack of light in subglacial environments, a variety of microbial metabolisms is present at the glacier bed, including denitrification, sulphide oxidation, sulphate reduction and methanogenesis (Skidmore et al. 2000, Bottrell and Tranter 2002, Wadham et al. 2004), and microscopical, biochemical and isotopic evidence has proved that during

the growth period, when there are sufficient liquid water and nutrient supplies, the subglacial microbes may influence the chemistry of glacial meltwater (Foght et al. 2004, Wadham et al. 2004). The source of organic carbon in subglacial sediments is permafrost soils overridden by the advancing glacier and then finally ground by subglacial abrasion processes, and material brought to the glacier bed via moulins and crevasses. The organic material consists of cyanobacterial and algal mats, and plant residues. These types of carbonaceous material are easily biodegradable by microbes (Skidmore et al. 2000). Active subglacial microbes may achieve relatively high abundances (10^6-10^7 cells g^{-1} or 10^2-10^4 cells ml^{-1}; Skidmore et al. 2005, Hodson et al. 2008) and may become limited by organic carbon (Wadham et al. 2004, Bhatia et al. 2006).

Figure 1.2. The subglacial environment of Svalbard: a subglacial drainage channel at the bed of Werenskioldbreen (photo: Stanislav Řehák).

Despite the lack of light, photoautotrophic microbes are not sentenced to death in these systems. Viable cyanobacteria and algae may be able to survive in subglacial environments in a dormant state. Except for a passive role of becoming a source of organic carbon for autochthonous microbial populations, they may have an active role: after glacier retreat and an exposure of the subglacial sediments to solar irradiation, they may take their part in microbial recolonisation of the new proglacial areas. The source of cyanobacterial and algal propagules is most likely the nearby growth-supporting habitats such as shallow wetlands and soils. In early autumn, when desiccation occurs, a great number of cyanobacterial and algal cells are transported by wind and deposited on glacier surfaces, and washed into the subglacial system by meltwater. Species that are able to survive in snow and/or ice provide a pool for subglacial survivors and hence potential colonists (Marshall and Chalmers 1997).

1.3. The supraglacial environment

The environment of the glacier surface (Figure 1.3) is of great importance for the entire glacial ecosystem. It receives solar radiation and is often the locus of melt water production. Liquid water, essential for virtually all biological processes (Kennedy 1993), is often readily available on many temperate and sub-polar glacier surfaces over the course of ablation season (Tranter et al. 1997, 2002, Wadham et al. 1998, Hodson et al. 2005, 2008).

Figure 1.3. The supraglacial environment of Svalbard: Vestre Torellbreen (photo: Stanislav Řehák).

Further, wind-borne debris and aerosol from the atmosphere are deposited onto the surface (Wharton et al. 1985, Takeuchi et al. 2001, Fountain et al. 2004), often providing an essential source of nutrients, including nitrogen and phosphorus (Tranter et al. 2004, Fortner et al. 2005, Hodson et al. 2005, 2008). Once deposited, the supraglacial material is transported down glacier, sorted, and reworked into different forms. Fine sediment on glacier surfaces is usually concentrated into cryoconite holes, vertical cylinder-shaped depressions in the ice surface formed in ablation zones by preferential melting as a result of relatively lower albedo of the dark sediment. They vary in diameter from 1 cm to 1 m, and are up to several decimetres deep (Fountain et al. 2004, Mueller and Pollard 2004). They may cover several percent of glacial surface and be interconnected with shallow surface channels.

Airborne microbial propagules also land on glaciers, and give rise to microbial communities (Wharton et al. 1985, Takeuchi et al. 2001), which consist of

photoautotrophic and heterotrophic microbes, fungi and low numbers of ciliates, rotifers, tardigrades and nematodes (Säwström et al. 2002, Christner et al. 2003, Mueller and Pollard 2004, Porazinska et al. 2004). Photoautotrophic microorganisms that thrive here can serve as the primary source of organic carbon at the base of foodwebs on glacier surfaces, and they are potentially important contributors to the primary production of the entire terrestrial polar ecosystem (Säwström et al. 2002, Hodson et al. 2007). The supraglacial ecosystem may, therefore, be significant for local, regional and even global carbon cycling (Hodson et al. 2007, 2008).

1.4. The proglacial environment

The ongoing glacier retreat exposes large areas of glacial till every year. The exposed till, formed by crushing of bedrock by the glacier, thus becomes proglacial soil (Figure 1.4). New proglacial soil is poorly developed and mostly consists of coarse mineral substrate with a low content and a patchy distribution of organic matter and nutrients (Bardgett et al. 2007). The development following glacial regression in the high Arctic is not fully documented and understood (i.e. Hodkinson et al. 2003); however, it is clear that microbial and plant succession is highly constrained by the low temperature, short growing seasons, limited water and nutrient availability, cryoturbation of soils and the effects of permafrost in restricting developing soil depth (Coulson et al. 1993, Ohtonen et al. 1999, Hodkinson et al. 2003).

Figure 1.4. The proglacial environment of Svalbard: the forefield of Werenskioldbreen

Airborne microbial propagules are deposited in proglacial soils, and, together with subglacial survivors, may give rise to a soil microbial community. Photoautotrophic microorganisms are likely to be very important here since they produce new organic carbon necessary for the further development of the ecosystem.

1.5. The snow environment

Large areas in glaciated areas are seasonally or perennially covered in snow. The snow surface is a typical low-temperature and high irradiation environment. The temperature is rather stable around the freezing point, and the intensities of light may reach very high values (Gorton et al. 2001). Microbial assemblages have been found in various snow environments around the world (Hoham and Duval 2001, and references

therein). Snow algal populations living within liquid water retained among snow crystals are the most prominent microorganisms living in such cold environments.

1.6. Photoautotrophic microbes in glacial environments

High Arctic glacial environments are simple ecosystems dominated by microorganisms due to their greater resilience and resistance to environmental stresses compared with higher plants and metazoans (Vincent 2000, Elster 2002). Great attention has been paid to the process of microbial colonisation of recently deglaciated environments (Ohtonen et al. 1999, Sigler et al. 2002, Hodkinson et al. 2003). Glaciers themselves are now considered dynamic ecosystems in their own right, with an abundant and active microbial component (Hodson et al. 2008).

The photoautotrophic component of the microbial assemblages, i.e. cyanobacteria and microalgae, plays significant roles in Arctic and alpine terrestrial ecosystems. Cyanobacteria and microalgae photosynthesise and thus are the principal source of new organic carbon, which may partly sustain heterotrophic communities in the ecosystem (Tscherko et al. 2003, Bardgett et al. 2007). Cyanobacteria can also be important in nitrogen cycling in glacial environments due to their ability to fix atmospheric nitrogen (Liengen and Olsen 1997, Duc et al. 2009). Some photoautotrophs, mainly filamentous cyanobacteria, may be essential for the stabilisation of the substrate in recently deglaciated areas by forming mats and crusts, thus enabling further colonisation by more demanding organisms such as mosses and higher plants (Belnap and Lange 2001, Hodkinson et al. 2003, Breen and Lévesque 2008). However, microbial colonisation of recently deglaciated areas is likely to be only a part of a little known process of circulation of microbial cells and/or communities throughout catchments, or beyond them, via wind and water pathways. Viable cells of photoautotrophic microbes have been found in wind-borne debris and aerosols (Marshall and Chalmers 1997) and in cryoconite holes on glaciers (Säwström et al. 2002, Mueller and Pollard 2004). It is evident that microbial cells, including

cyanobacteria and microalgae, are passively transported through various types of Arctic terrestrial environments, and their ability to colonise the substrate may be important for the development of these environments.

Glacial environments may also play the role of a pool of propagules for microbial colonisation after glacier retreat, which is supported by the fact that microbial assemblages in ice and soil habitats are relatively similar (Wynn-Williams 1990). However, the proportion of the ice survivors on the colonisation processes to wind-borne spores is still unclear. Also, the role of physico-chemical factors of the soil in recolonisation may be of great importance. Much attention has been paid to the reinvasion and establishment of plant and animal life after retreat of glaciers and to the effects of climate warming (e.g. Chapin et al. 1992, Coulson et al. 1993); however, little emphasis has been placed on the primary colonisers although higher plant invasion and subsequent plant community development is dependent on them.

One of the most conspicuous microbes in glacial environments are snow algae, which often reach high abundances and cause the well-known red colouration of snow. Snow algae possess some specific ecological and physiological adaptations to the harsh environment of the snow. Most of the 'true' snow algae, defined as those that grow and reproduce entirely within the water during snowmelt, are green algae of the order Chlamydomonadales (Chlorophyceae); *Chlamydomonas nivalis* is the most common inhabitant of snowfields on Svalbard (Kol and Eurola 1974, Newton 1982, Müller et al. 1998, 2001). Snow algae may play an important role as primary producers, and so comprise the base of the food web in snow (Hoham and Duval 2001). They can also be a considerable source of organic matter for downstream ecosystems. Despite that, little attention has been paid to photosynthesis and primary production in Arctic snow environments, where snow and its inhabitants play a much greater role in the whole ecosystem than in the better studied high-altitude environments.

2. Objectives

The present thesis is compiled of papers focussed on the community structure, abundance and activity of photoautotrophic microbes – cyanobacteria and algae – in various types of glacial environments on Svalbard in the high Arctic. The underlying objective of all these papers was to gain a deeper insight into the role of photoautotrophic microorganisms in an Arctic terrestrial ecosystem.

Paper 1 (**Kaštovská et al. 2005**) is a pioneering study of the presence and role of photoautotrophs in a Svalbard glacial ecosystem. It was primarily focussed on the community structure, abundance of bacteria, cyanobacteria and algae in different types of proglacial and subglacial sediments using microscopy and culturing techniques. Multivariate ecological analyses were used to assess the relationship between the microbial component and the physical and chemical parameters of the environments. We also discuss the potential ability of algae and cyanobacteria to take part in the recolonisation process of barren proglacial soils.

Paper 2 (**Kaštovská et al. 2007**) concentrates on the microbial community found at the bed of two polythermal glaciers on Svalbard. We investigated the microbial community structure and abundance using epifluorescence microscopy, phenotypic fingerprinting techniques (PLFA), and traditional culturing methods. Multivariate ecological analyses were also used here to assess the relationship between the microbial component and the physical and chemical parameters of the environments. We discuss the role of subglacial environments as a potential reservoir for phototrophic microbes in the overall cell circulation within the glacial ecosystem.

Paper 3 (**Stibal et al. 2006**) focuses on the microbial communities occurring in supraglacial environments at several Svalbard glaciers, whose subglacial and proglacial soil microbial populations have been described previously, and whose hydrology and water chemistry have been monitored for more than 10 years. Particular emphasis is laid on the distinctions in chemical and physical properties of the supraglacial microhabitats and their relations to the microbial communities, which have been largely unknown. We used a novel approach that applies the multivariate ecological analyses on the evaluations of these relationships. The fate of a microbial cell deposited on the glacier surface is discussed. This is also the first study to bring information on microbial life associated with supraglacial moraines and kames.

Paper 4 (**Stibal et al. 2007**) aimed to monitor a snow algal population dominated by *Chlamydomonas nivalis* in an Arctic snowfield on Svalbard, and to study the physiological state and photosynthetic activity of this organism over an entire growing season using microscopy and pulse-amplitude modulation fluorometry. We discuss the diurnal and seasonal dynamics of photosynthetic activity of the algae and their response to varied light conditions and other environmental factors.

Paper 5 (**Řeháková, Stibal et al. submitted**) is the first study to determine the survival and colonisation potential of photoautotrophic microbes in different soil/sediment environments in a high Arctic glacierised catchment, and is largely based on the previous studies (Papers 1,2,3). We conducted one-year reciprocal transplant incubations of photoautotrophic microbial communities from three principal terrestrial glacial environments in the catchment of a well-researched Svalbard glacier: old vegetation-covered soil, recently deglaciated barren soil and subglacial sediments that may become proglacial in ~10-50 years given the current glacier retreat rate. We determined the abundance and community structure of photoautotrophic microbes and their changes over time and between soil/sediment types.

3. Published results

(1) Kaštovská K., Elster J., **Stibal M.**, Šantrůčková H. (2005) Microbial assemblages in soil microbial succession after glacial retreat in Svalbard (High Arctic). *Microbial Ecology* **50**: 396-407

Microbial community composition was studied in subglacial and proglacial habitats on five glaciers near Ny-Ålesund, Svalbard (79°N). Soil microbial communities from nonvegetated sites (subglacial, recently deglaciated, and cryoconite sediments) and sites with plant cover (deglaciated some hundreds of years ago) were analysed. Physico-chemical analyses (pH, texture, water content, organic matter, total C and N content) were also performed on the samples. In total, 57 taxa of 23 genera of cyanobacteria and algae were identified. Algae from the class Chlorophyceae (25 species) and cyanobacteria (23 species) were richest in biodiversity. The numbers of identified species in single habitat types were 23 in subglacial, 39 in barren, 22 in cryoconite, and 24 in vegetated soils. The highest cyanobacterial and algal biovolume and cell numbers, respectively, were present in cryoconite (13×10^4 μm^3 mg^{-1} soil and 508 cells per mg of soil), followed by barren (5.7×10^4 and 188), vegetated (2.6×10^4 and 120) and subglacial (0.1×10^4 and 5) soils. Cyanobacteria prevailed in all soil samples. Algae (mainly green algae) were present only as accessory organisms. The abundance of bacteria showed a slightly different trend to that of the cyanobacterial and algal assemblages. The highest number of bacteria was present in vegetated (mean: $13{,}722\times10^8$ cells per mg of soil dry wt.), followed by cryoconite (3802×10^8), barren (654×10^8) and subglacial (78×10^8) soils. Response of cyanobacteria and algae to physical parameters showed that soil texture and water content are important for biomass development. In addition, it is shown that nitrogen and water content are the main factors affecting bacterial abundance and overall soil respiration. Redundancy analysis (RDA) with forward selection was used to create a model explaining variability in cyanobacterial, algal, and bacterial abundance. Cryoconites accounted for most of the variation in cyanobacteria and algae biovolume, followed by barren soils. Oscillatoriales, desmids and green coccoid algae preferred cryoconites, whereas Nostocales and Chroococcales occurred mostly in barren soils. From the data obtained, it is evident that of the studied habitats cryoconite sediments are the most suitable ones for the development of microbial assemblages. Although subglacial sediments do not provide as good conditions as cryoconites, they support the survival of microbial communities. Both mentioned habitats are potential sources for the microbial recolonisation of freshly deglaciated soil after the glacier retreat.

Author's contribution 40%

(2) Kaštovská K., **Stibal M.**, Šabacká M., Černá B., Šantrůčková H., Elster J. (2007) Microbial community structure and ecology of subglacial sediments in two polythermal Svalbard glaciers characterized by the epifluorescence microscopy and PLFA. *Polar Biology* **30**: 277-287

Biological and physico-chemical characteristics of subglacial sediments were studied in Svalbard. Sediment from close proglacial and supraglacial environments was used for a comparison. Viable bacteria, cyanobacteria and microalgae were detected in subglacial sediments from two polythermal glaciers using epifluorescence microscopy and phospholipid fatty acid (PLFA) analyses. The subglacial samples were generally of higher pH values, coarser texture and lower water content, organic matter, organic carbon, and nitrogen compared to proglacial and supraglacial sediments). Bacterial counts of 1.6×10^{7} cells mg^{-1} OM (organic matter) were found. Cyanobacteria and algae were also of low abundance [4.2 cells mg^{-1} DW (dry weight)]. Cyanobacteria comprised the major proportion of the photoautotrophic assemblages of subglacial soils. Deglaciated soils were similar to subglacial sediment in physico-chemical properties and microbial structure and numbers, unlike soil from vegetated sites or cryoconite sediment. In subglacial and deglaciated soil, relatively low diversity of microorganisms and low substrate availability was detected by PLFA analyses. Good accordance in microbial community structure assessments between epifluorescence microscopy and PLFA analyses was found. Our results suggest that the subglacial microbial populations can be divided into two groups: autochthonous microorganims (chemoheterotrophic bacteria) and allochthonous that retain the ability to proliferate and give rise to active population when conditions become favourable.

Author's contribution 30%

(3) **Stibal M.**, Šabacká M., Kaštovská K. (2006) Microbial communities on glacier surfaces in Svalbard: impact of physical and chemical properties on abundance and structure of cyanobacteria and algae. *Microbial Ecology* **52**: 644-654

Microbial communities occurring in three types of supraglacial habitats - cryoconite holes, medial moraines, and supraglacial kames - at several glaciers in the Arctic archipelago of Svalbard were investigated. Abundance, biovolume, and community structure were evaluated by using epifluorescence microscopy and culturing methods. Particular emphasis was laid on distinctions in the chemical and physical properties of the supraglacial habitats and their relation to the microbial communities, and quantitative multivariate analyses were used to assess potential relationships. Varying pH (4.8 in cryoconite; 8.5 in a moraine) and texture (the proportion of coarse fraction 2% of dry weight in cryoconite; 99% dw in a kame) were found, and rather low concentrations of organic matter (0.3% of dry weight in a kame; 22% dw in cryoconite) and nutrients (nitrogen up to 0.4% dw, phosphorus up to 0.8% dw) were determined in the samples. In cryoconite sediment, the highest numbers of bacteria, cyanobacteria, and algae were found, whereas relatively low microbial abundances were recorded in moraines and kames. Cyanobacterial cells were significantly more abundant than microalgal ones in cryoconite and supraglacial kames. Different species of the cyanobacterial genus *Leptolyngbya* were by far the most represented in all samples, and cyanobacteria of the genera *Phormidium* and *Nostoc* prevailed in cultures isolated from cryoconite samples. These species are considered opportunistic organisms with wide ecological valency and strong colonising potential rather than glacial specialists. Statistical analyses suggest that fine sediment with higher water content is the most suitable condition for bacteria, cyanobacteria, and algae. Also, a positive impact of lower pH on microbial growth was found. The fate of a microbial cell deposited on the glacier surface seems therefore predetermined by the physical and chemical factors such as texture of sediment and water content rather than spatial factors or the origin of sediment.

Author's contribution 80%

(4) **Stibal M.**, Elster J., Šabacká M., Kaštovská K. (2007) Seasonal and diel changes in photosynthetic activity of the snow alga *Chlamydomonas nivalis* (Chlorophyceae) from Svalbard determined by PAM fluorometry. *FEMS Microbiology Ecology* **59**: 265-273

The seasonal and diel dynamics of the physiological state and photosynthetic activity of the snow alga *Chlamydomonas nivalis* were investigated in a snow field in Svalbard. The snow surface represents an environment with very high irradiation intensities along with stable low temperatures close to freezing point. Photosynthetic activity was measured using pulse amplitude modulation fluorometry. Three types of cell (green biflagellate vegetative cells, orange spores clustered by means of mucilaginous sheaths, and purple spores with thick cell walls) were found, all of them photosynthetically active. The pH of snow ranged between 5.0 and 7.5, and the conductivity ranged between 5 and 75 µS cm^{-1}. The temperature of snow was stable (-0.1 to +0.1°C), and the incident radiation values ranged from 11 to 1500 µmol photons m^{-2} s^{-1}. The photosynthetic activity had seasonal and diel dynamics. The F_v/F_m values ranged between 0.4 and 0.7, and generally declined over the course of the season. A dynamic response of F_v/F_m to the irradiance was recorded. According to the saturating photon fluence values E_k, the algae may have obtained saturating light as deep as 3 cm in the snow when there were higher-light conditions, whereas they were undersaturated at prevalent low light even if on the surface.

Author's contribution 90%

4. Results in preparation for publication

(5) Řeháková K., **Stibal M.**, Šabacká M., Řehák J. (submitted) Survival and colonisation potential of photoautotrophic microorganisms within a glacierised catchment on Svalbard, high Arctic. *Polar Biology*.

The survival and colonisation potential of photoautotrophic microbes (cyanobacteria and microalgae) was investigated in three principal terrestrial environments in a glacierised catchment on Svalbard - old vegetation-covered soil, recently deglaciated barren soil and subglacial sediments. One-year reciprocal transplant incubations of photoautotrophic microbial communities from the three soil/sediment environments were conducted in order to reveal the autochthonous or allochthonous origin of the present photoautotrophs. The abundance and community structure of photoautotrophic microbes and their changes over time and between soil/sediment types were determined and physico-chemical analyses were performed on the soil samples. The recovery times by import of cells were between several months in subglacial and vegetated soils and up to 27 years in proglacial soils. No active growth was recorded in subglacial sediments, while positive growth, and so the potential for autochthonous recovery, was found in proglacial and vegetated soils. The most suitable environment for the survival of transplanted microbes was provided in proglacial soil. The proglacial areas will be expanding due to the changes of climate and the ongoing glacial retreat, and we show here that new proglacial substrata can be rapidly and successfully colonised by photoautotrophic microbes from nearby terrestrial environments.

Author's contribution 40%

5. Conclusions

Based on the results obtained in the presented thesis, the following conclusions can be drawn. First, photoautotrophic microorganisms are ubiquitous in the terrestrial ecosystem of the high Arctic archipelago of Svalbard, and second, their communities can be coarsely divided into three principal groups based on their diversity, abundance, activity, the level of adaptation to the glacial environment and their role in it. These three groups are 1) the best adapted, least diverse and rather ephemeral snow algae, 2) the most abundant and active, but spatially limited, cryoconite communities and 3) widespread, but least abundant and active soil/sediment communites in subglacial and proglacial environments.

The snow algal community is composed of one or very few dominant species and confined to the spatially and temporally limited environment of melting snow. Although their photosynthetic activity is not limited to vegetative cells and continues in the conspicuously coloured spores, their overall role in the terrestrial ecosystem of Svalbard is relatively unimportant, and their abundance are very low or undetectable in other glacial environments. However, they are a good example of highly specialised microorganisms that are well adapted to life in Arctic terrestrial environments.

Photoautotrophs in other glacial environments – supraglacial, subglacial and proglacial – are not likely to be specifically adapted to cold Arctic environments. This may have an adverse effect on their activity, but enables them to travel across different environments and, thus, to start microbial succession after glacial retreat, one of the major process in Arctic terrestrial ecosystems. Cyanobacteria are much more important in these groups than algae.

The fate of a microbial cell deposited on the glacier surface is predetermined by the physical and chemical factors such as texture of sediment and water content rather than spatial factors or the origin of sediment. Therefore, photoautotrophs preferentially colonise cryoconite holes, and their high abundance and activity may result in significant carbon fixation. The cells and the produced organic carbon can be

subsequentially exported to downstream environments, including proglacial and subglacial sediments.

The subglacial environment is rather a conduit for photoautotrophic communities than a place of growth and production; however, the supply of viable photautotrophs is relatively high and can serve as a significant resource of nutrients for autochthonous subglacial microbial communities. Cyanobacteria and microalgae are inactive under glaciers, but may be of special importance in newly deglaciated soil. It can be said that the microbial succession of soil starts before the glacier actually recedes from it, since viable photoautotrophic microbes are already present there and so form a base of the community.

Circulation of photoautotrophic microbes in a glacierised catchment is a very important process, particularly with regard to colonisation of new substrata. Given the changes of climate and the ongoing glacial retreat, the proglacial areas will be expanding and the interaction between melt waters and sediments/soils in the glacial forefield will become more significant. New proglacial substrata can be successfully colonised by photoautotrophic microbes. With progressing deglaciation, the pool of microbial cells available for circulation will increase, and the coupling between all the glacial environments is likely to be enhanced. Transport of cells may in some cases exceed *in situ* microbial growth and thus may be important in environments with low productivity.

References

Bardgett RD, Richter A, Bol R, Garnett MH, Bäumler R, Xu XL, Lopez-Capel E, Manning DAC, Hobbs PJ, Hartley IR, Wanek W (2007) Heterotrophic microbial communities use ancient carbon following glacial retreat. Biol Lett 3:487–490

Belnap J, Lange OL (2001) Structure and function of biological soil crusts: synthesis. In: Belnap, J, Lange, OL (eds) Biological Soil Crusts: Structure, Function, and Management. Springer, Berlin, pp 471–480

Benn DI, Evans DJA (1998) Glaciers and glaciation. Arnold, London, 734 pp

Bhatia M, Sharp MJ, Foght J (2006) Distinct bacterial communities exist beneath a high Arctic polythermal glacier. Appl Environ Microbiol 72:5838–5845

Bottrell SH, Tranter M (2002) Sulphide oxidation under partially anoxic conditions at the bed of the Haut Glacier d'Arolla, Switzerland. Hydrol Process 16:2363–2368

Breen K, Lévesque E (2008) The influence of biological soil crusts on soil characteristics along a High Arctic glacier foreland, Nunavut, Canada. Arct Antarct Alp Res 40:287–297

Chapin FS, Jefferies RL, Reynolds JF, Shaver GR, Svoboda J (1992) Arctic ecosystems in a changing climate. An ecophysiological perspective. Academic Press, San Diego, 469 pp

Christner BC, Kvitko BH II, Reeve JN (2003) Molecular identification of Bacteria and Eukarya inhabiting an Antarctic cryoconite hole. Extremophiles 7:177-183

Coulson S, Hodkinson ID, Strathdee A, Bale JS, Block W, Worland MR, Webb NR (1993) Simulated climate change: the interaction between vegetation type and microhabitat temperatures at Ny-Ålesund, Svalbard. Polar Biol 13:67–70

Duc L, Noll M, Meier BE, Bürgmann H, Zeyer J (2009) High diversity of diazotrophs in the forefield of a receding Alpine glacier. Microb Ecol 57:179-190

Elster J (2002) Ecological classification of terrestrial algal communities of polar environment. In: Beyer L, Bölter M (eds) Geoecology of terrestrial oases. Ecological Studies. Springer-Verlag, Berlin, pp 303–319

Foght J, Aislabie J, Turner S, Brown CE, Ryburn J, Saul DJ, Lawson W (2004) Culturable bacteria in subglacial sediments and ice from two southern hemisphere glaciers. Microb Ecol 47:329–340

Fortner SK, Tranter M, Fountain A, Lyons WB, Welch KA (2005) The geochemistry of supraglacial streams of Canada Glacier, Taylor Valley (Antarctica), and their evolution into proglacial waters. Aquat Geochem 11:391-412

Fountain AG, Tranter M, Nylen TH, Lewis KJ, Mueller DR (2004) Evolution of cryoconite holes and their contribution to meltwater runoff from glaciers in the McMurdo Dry Valleys, Antarctica. J Glaciol 50:35-45

Gorton HL, Williams WE, Vogelmann TC (2001) The light environment and cellular optics of the snow alga *Chlamydomonas nivalis* (Bauer) Wille. Photochem Photobiol 73:611–620

Hodkinson ID, Coulson SJ, Webb NR (2003) Community assembly along proglacial chronosequences in the high Arctic: vegetation and soil development in north-west Svalbard. J Ecol 91:651-663

Hodson AJ, Mumford PN, Kohler J, Wynn PM (2005) The High Arctic glacial ecosystem: new insights from nutrient budgets. Biogeochemistry 72:233-256

Hodson A, Anesio AM, Ng F, Watson R, Quirk J, Irvine-Fynn T, Dye A, Clark C, McCloy P, Kohler J, Sattler B (2007) A glacier respires: quantifying the distribution and respiration CO_2 flux of cryoconite across an entire Arctic supraglacial ecosystem. J Geophys Res 112:G04S36

Hodson A, Anesio AM, Tranter M, Fountain AG, Osborn M, Priscu J, Laybourn-Parry J, Sattler B (2008) Glacial ecosystems. Ecol Monogr 78:41-67

Hoham RW, Duval B (2001) Microbial ecology of snow and freshwater ice. In: Jones HG, Pomeroy JW, Walker DA, Hoham RW (eds) Snow Ecology. Cambridge University Press, Cambridge, pp 168–228

Kennedy AD (1993) Water as a limiting factor in the Antarctic terrestrial environment – a biographical synthesis. Arct Alp Res 25:308-315

Kol E, Eurola S (1974) Red snow algae from Spitsbergen. Astarte 7:61–66

Liengen T, Olsen RA (1997) Nitrogen fixation by free-living cyanobacteria from different coastal sites in a high arctic tundra, Spitsbergen. Arct Alp Res 29:470–477

Marshall WA, Chalmers MO (1997) Airborne dispersal of Antarctic algae and cyanobacteria. Ecography 20:585–594

Mikucki JA, Foreman CM, Sattler B, Lyons WB, Priscu JC (2004) Geomicrobiology of Blood Falls: an iron-rich saline discharge at the terminus of the Taylor Glacier, Antarctica. Aquat Geochem 10:199–220

Mueller DR, Pollard WH (2004) Gradient analysis of cryoconite ecosystems from two polar glaciers. Polar Biol 27:66–74

Müller T, Bleiß W, Martin C-D, Rogaschewski S, Fuhr G (1998) Snow algae from northwest Svalbard: their identification, distribution, pigment and nutrient content. Polar Biol 20:14–32

Müller T, Leya T, Fuhr G (2001) Persistent snow algal fields in Spitsbergen: field observations and a hypothesis about the annual cell circulation. Arct Antarct Alp Res 33:42–51

Newton APW (1982) Red-colored snow algae in Svalbard – some environmental factors determining the distribution of *Chlamydomonas nivalis* (Chlorophyta Volvocales). Polar Biol 1:167–172

Ohtonen R, Fritze H, Pennanen T, Jumpponen A, Trappe J (1999) Ecosystem properties and microbial communities changes in primary succession on a glacier forefront. Oecologia 119:239–246

Porazinska DL, Fountain AG, Nylen TH, Tranter M, Virginia RA, Wall DH (2004) The biodiversity and biogeochemistry of cryoconite holes from McMurdo Dry Valley glaciers, Antarctica. Arct Antarct Alp Res 36:84-91

Priscu JC, Christner BC (2004) Earth's icy biosphere. In: Bull A (ed) Microbial diversity and bioprospecting. American Society for Microbiology, Washington, DC, pp 130–145

Säwström C, Mumford P, Marshall W, Hodson A, Laybourn-Parry J (2002) The microbial communities and primary productivity of cryoconite holes in an Arctic glacier (Svalbard, 79°N). Polar Biol 25:591–596

Sharp M, Parkes J, Cragg B, Fairchild IJ, Lamb H, Tranter M (1999) Widespread bacterial populations at glacier beds and their relationship to rock weathering and carbon cycling. Geology 27:107–110

Sigler WV, Crivii S, Zeyer J (2002) Bacterial success in glacial forefield soils characterized by community structure, activity and opportunistic growth dynamics. Microb Ecol 44:306–316

Skidmore ML, Foght JM, Sharp MJ (2000) Microbial life beneath a high Arctic glacier. Appl Environ Microbiol 66:3214–3220

Skidmore M, Anderson SP, Sharp M, Foght J, Lanoil BD (2005) Comparison of microbial community compositions of two subglacial environments reveals a possible role for microbes in chemical weathering processes. Appl Environ Microbiol 71:6986–6997

Takeuchi N, Kohshima S, Seko K (2001) Structure, formation, and darkening process of albedo-reducing material (cryoconite) on a Himalayan glacier: A granular algal mat growing on the glacier. Arct Antarct Alp Res 33:115-122

Tranter M, Sharp MJ, Brown GH, Willis IC, Hubbard BP, Nielsen MK, Smart CC, Gordon S, Tulley M, Lamb HR (1997) Variability in the chemical composition of in situ subglacial meltwaters. Hydrol Process 11:59-77

Tranter M, Sharp MJ, Lamb HR, Brown GH, Hubbard BP, Willis IC (2002) Geochemical weathering at the bed of Haut Glacier d'Arolla, Switzerland - a new model. Hydrol Process 16: 959-993

Tranter M, Skidmore M, Wadham J (2005) Hydrological controls on microbial communities in subglacial environments. Hydrol Process 19:995–998

Tscherko D, Rustemeier J, Richter A, Wanek W, Kandeler E (2003) Functional diversity of the soil microflora in primary succession across two glacier forelands in the Central Alps. Eur J Soil Sci 54:685-696

Vincent WF (2000) Cyanobacterial dominance in the polar regions. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Kluwer, Dordrecht, pp 321-340

Wadham JL, Hodson AJ, Tranter M, Dowdeswell JA (1998) The hydrochemistry of meltwaters draining a polythermal-based, high Arctic glacier, south Svalbard: I. The ablation season. Hydrol Process 12:1825-1849

Wadham JL, Bottrell S, Tranter M, Raiswell R (2004) Stable isotope evidence for microbial sulphate reduction at the bed of a polythermal high Arctic glacier. Earth Planet Sci Lett 219:341–355

Wharton RA Jr, McKay CP, Simmons GM Jr, Parker BC (1985) Cryoconite holes on glaciers. BioScience 35:499-503

Wynn-Williams DD (1990) Ecological aspects of Antarctic microbiology. Adv Microb Ecol 11:71–146

Appendix 1 – Paper 5 in manuscript

Survival and colonisation potential of photoautotrophic microorganisms within a glacierised catchment on Svalbard, high Arctic

Klára Řeháková[1], Marek Stibal[1,2], Marie Šabacká[1,3], Josef Řehák[4]

[1]Institute of Botany, Czech Academy of Sciences, Třeboň, Czechia

[2]Bristol Glaciology Centre, School of Geographical Sciences, University of Bristol, UK

[3]Department of Land Resources & Environmental Sciences, Montana State University, Bozeman, USA

[4]Speleo-Řehák, Semily, Czechia

Running title: Colonisation potential of phototrophs in Svalbard soils

Introduction

High Arctic terrestrial environments, including retreating glaciers and expanding deglaciated areas, are simple ecosystems dominated by microorganisms due to their greater resilience and resistance to environmental stresses compared with higher plants and metazoans (Vincent 2000; Elster 2002). Great attention has been paid to the process of microbial colonisation of recently deglaciated environments (Ohtonen et al. 1999; Sigler et al. 2002; Hodkinson et al. 2003; Kaštovská et al. 2003), and glaciers themselves are now considered dynamic ecosystems in their own right, with an abundant and active microbial component (Hodson et al. 2008).

The photoautotrophic component of the microbial assemblages, i.e. mainly cyanobacteria and microalgae, plays significant roles in Arctic and alpine terrestrial ecosystems. Cyanobacteria and microalgae photosynthesise and thus are a major source of new organic carbon, which may partly sustain heterotrophic communities in the ecosystem (Tscherko et al. 2003; Bargett et al. 2007). Cyanobacteria can also be important in nitrogen cycling in Arctic terrestrial environments due to their ability to fix atmospheric nitrogen (Liengen and Olsen 1997). Some photoautotrophs, mainly filamentous cyanobacteria, may be essential for the stabilisation of the substrate in recently deglaciated areas by forming mats and crusts, thus enabling further colonisation by more demanding organisms such as mosses and higher plants (Belnap and Lange 2001).

However, microbial colonisation of recently deglaciated areas is likely to be only a part of a little known process of circulation of microbial cells and/or communities throughout catchments, or beyond them, via wind and water pathways. Viable cells of photoautotrophic microbes have been found in wind-borne debris and aerosols (Marshall and Chalmer 1997; Pearce et al. 2009). Cryoconite holes on glaciers, which contain a rich microbial community (Säwström et al. 2002; Mueller and Pollard 2004; Stibal et al. 2006), are mostly formed by deposition of wind-borne debris, including living microbes, on glacier surfaces (Wharton et al. 1985). Therefore, it is evident that microbial cells, including cyanobacteria and microalgae, are passively transported

through various types of Arctic terrestrial environments, and their ability to colonise the substrate may be important for the development of these environments.

This is the first study to quantify the survival and colonisation potential of photoautotrophic microbes in different soil/sediment environments in a high Arctic glacierised catchment. In order to do so, we conducted one-year reciprocal transplant incubations of photoautotrophic microbial communities from three principal terrestrial glacial environments in the catchment of a well-researched Svalbard glacier: old vegetation-covered soil, recently deglaciated barren soil and subglacial sediments that may become proglacial in ~10-50 years given the current glacier retreat rate. We determined the abundance and community structure of photoautotrophic microbes and their changes over time and between soil/sediment types. Based on the results, we discuss their ability to survive transport between soil/sediment environments and to take part in the colonisation processes.

Materials and methods

Field site

The catchment of Werenskioldbreen is located in southwest Spitsbergen, Svalbard, at 77°04´N; 15°15´E (Figure 1). Its total area is approximately 32 km^2, with the glacier surface area of 26.4 km^2 in 2006 (Řehák et al. 2007), which means that approximately 80% of the catchment area is glacierised. The catchment includes smaller "hanging" valleys, including Tonedalen, which contains a residual glacier that drains separately to the coastal plain. Mass balance measurements conducted on Werenskioldbreen in years 1993/94 and 1998/99 were negative, –0.36 m w.e. and –0.66 m w.e., respectively, and the glacier terminus retreated by 16 and 25 m per year in 2005 and 2006, respectively (Řehák et al. 2007). This means that 4-6 ha of subglacial sediment is exposed each year in this catchment and becomes proglacial soil. During the ablation

season, ~25% of melt water flows surficially in channels reaching the snout, while the rest is routed through moulins and englacial conduits to the bed (Řehák et al. 2007).

Microbial communities have been found in all studied soil/sediment environments of the Werenskioldbreen catchment, including subglacial sediments (Kaštovská et al. 2007), barren proglacial as well as older vegetated soils (Kaštovská et al. 2007), and the debris on the surface of the glacier (Stibal et al. 2006; Stibal and Tranter 2007). They mainly consist of heterotrophic bacteria and photoautotrophic cyanobacteria and microalgae, with other microorganisms such as fungi and small metazoans present in low amounts. The abundance of heterotrophic bacteria can reach over 1×10^6 cells per mg of the soil dry weight, and that of photoautotrophic microbes dominated by filamentous cyanobacteria up to 1×10^2 cells mg^{-1} of the soil dry weight (Kaštovská et al. 2007).

Sampling procedure

Samples were collected from three soil/sediment environments in the catchment: first, subglacial sediments (4 localities), second, barren proglacial soils that were deglaciated <15 years ago and whose vegetation cover was 15% at maximum (4 localities), and third, older vegetated soils (deglacied 80 and more years ago) with plant cover of more than 60% (only two localities were available within the studied area). Subglacial samples were retrieved from four subglacial systems (Upper and Lower Kvisla, Tone and Sněženka; Figure 1) previously described by Kaštovská et al. 2007. The investigated subglacial environments are part of dranaige systems of polythermal glaciers and contain soil/sediment originating from the ice-bedrock interface or lateral moraines. Samples of barren proglacial soil were collected close to the sampled subglacial environments. Vegetated soil was sampled on the coastal plain Kvartsittsletta (vegetation cover ~95%), connecting the forefield of Werenskioldbreen and the sea coast, and from vegetated sites near the Tone subglacial system in Tonedalen (vegetation cover ~60%). The soil was collected from the space without the vegetation cover. The position of the sampling sites is marked in Figure 1. In 2004, bulked samples of soil/sediment from the surface from the maximal depth of 3 cm

were collected at each site. Two kilograms of soil was collected in the vicinity of transplant experimental sites and mixed immediatelly in a sterile plastic bag. From this bulked sample were randomly filled sterile 100 ml WhirlPak bags (Nasco, Fort Atkinson, USA) using ethanol-bathed and flame-sterilised spatulas. Aliquots of the samples were frozen to -20°C immediately after the return to the field station, and kept in a chest freezer at this temperature for a maximum of 6 months until they were melted at room temperature for 15 min and used for physico-chemical analyses and microscopical enumerations of microbial cells. The rest of samples were used for *in situ* reciprocal transplant experiments (see below). Maximum effort was made to avoid possible cross-contamination during sampling by wearing gloves and sterilising the spatulas between samplings.

Physico-chemical properties of sediment

The sediment samples from all types of environments were analysed for pH, texture, water content and chemical composition in the years 2004 and 2005. The pH values were determined in a soil suspension (soil:water 1:5 w/v) using a WTW-340i pH-meter (WTW, Weilheim, Germany). Water content of the samples was determined gravimetrically after drying at 105°C for 5 hours. Organic matter content was determined gravimetrically after dry combustion at 450°C for 5 hours. Texture of the soil/sediment samples was analysed by wet sieving as a ratio of two fractions: the fine fraction, with grains smaller than 0.5 mm, and the coarse fraction of grains greater than 0.5 mm. Total carbon and nitrogen contents were determined from non-fractionated subsamples using an NC 2100 elemental analyser (ThermoQuest, Rodano, Italy). Subsamples were completely oxidised by combustion, organic nitrogen was converted into elemental nitrogen and carbon into carbon dioxide; the gas mixture was then separated on a gaschromatographic column and measured using a thermoconductivity detector. Carbonates were removed using 7% HCl prior to the analyses.

Enumerations of microbial cells

Epifluorescence microscopy was used for the determination of the abundance of viable cells within the sediment and of the proportional representation of cyanobacteria and algae. A non-staining method using chlorophyll autofluorescence was employed, and the observed cells were identified on the basis of their morphology (Kaštovská et al. 2005). The amounts of cyanobacteria and algae were expressed as biovolume (Hillebrand et al. 1999) or the number of cells per milligram of dry sediment.

Transplant experiments

During *in situ* experiments soil/sediment samples were transferred between different soil/sediment environments as shown in Figure 2. In 2004, soil/sediment from each environment (subglacial, barren proglacial and vegetated) was collected and placed into 16 sterile 70 ml containers (50 g per container). The containers were open non-corrosive metal cups with perforated bottoms, which allowed natural circulation of water and nutrients in the experimental soil block in similar regime as in the surroundings soil. The containers were dug into the soil/sediment so that the rim of the container was aligned with the soil surface in order to minimise the effect on the microclimatic conditions. One set of container (four pieces) was left in place as control; one set was heat-sterilised at 100°C for 30 minutes twice in order to eradicate all microorganisms and left in place. Two sets of containers were transferred to the other two investigated environments. Four replicates were prepared for each treatment. The containers were incubated in their respective sites for one year, from August 2004 to August 2005. After the termination of experiments, soil/sediment from the containers was transferred to sterile WhirlPak bags and transported to the laboratory for analyses of microbial abundance and the physico-chemical parameters.

In order to assess the recovery of the photoautotrophic microbes and their colonisation potential, the following parameters were calculated:

Recovery of a photoautotrophic microbial community after sterilisation (R_{allo}, expressed as percentage of initial abundance)

$$R_{allo} = \frac{A_{2005ster}}{A_{2004}} \times 100$$

where $A_{2005ster}$ is the abundance of photoautotrophs in the previously sterilised sample and A_{2004} is the initial abundance in 2004. It shows import of viable allochthonous photoautotrophic cells to the given environment over the period of one year.

T_{allo} is the reciprocal value of R_{allo}

$$T_{allo} = \frac{A_{2004}}{A_{2005ster}} = \frac{100}{R_{allo}}$$

It is used here as it indicates the number of years potentially needed for the recovery of the initial community by import of cells given the import rate is stable.

Change in the abundance of an autochthonous photoautotrophic community by actual growth and division of cells (R_{auto})

$$R_{auto} = \frac{A_{2005ctrl} - A_{2005ster}}{A_{2004}} \times 100$$

where $A_{2005ctrl}$ is the abundance in the control sample in 2005.

T_{auto} is the reciprocal value of R_{auto}

$$T_{auto} = \frac{A_{2004}}{A_{2005ctrl} - A_{2005ster}} = \frac{100}{R_{auto}}$$

and indicates the number of years potentially needed for the autochthonous recovery of the initial community by growth.

Transplant success of the photoautotrophic community in environment *x* (TS_x, expressed as percentage)

$$TS_x = \frac{A_{2005trans} - A_{2005ster}}{A_{2004}} \times 100$$

where $A_{2005trans}$ is the abundance in the sample transplanted to *x* and $A_{2005ster}$ the abundance of the previously sterilised sample from *x*. A_{2004} is the original abundance at the beginning of the experiment of the transplanted sample. This shows the growth of photoautotrophic cells transferred to another environment over the period of one year.

Statistical analysis

Statistical comparison between samples from different soil/sediment environments in all measured physical and chemical parameters as well as microbial abundance and composition was conducted using one-way ANOVA followed by the Tukey honest significant difference test on the probability level $p<0.01$.

Results

The physico-chemical characteristics of the investigated soils/sediments from the catchment of Werenskioldbreen from the years 2004 and 2005 are given in Table 1. There were no statistically significant changes in any of the measured characteristics of the soil/sediment samples after one year of the transplant experiment. The soil used for transplant experiment in 2004 and soil from control treatment collected in 2005 were compared. The subglacial sediments had a pH of 6.9 - 9.3 and a relatively low water content (5.5 -16 % w). The organic matter (0.19 - 2.0 % dw) and nutrient contents (0.08 - 0.58% C, < 0.03% N) were also low. The proglacial sediments were similar to the subglacial ones in most characteristics, except for the significantly lower pH and nitrogen content in 2005 (Table 1). The vegetated soil samples were the most distinct of the sediment types investigated in having higher water (8.8 - 31 % w), organic matter (1.1 - 8.4 % dw), carbon (0.9 - 3.3 % dw) and nitrogen (0.07 - 0.09 % dw) contents (Table 1). No significant differences in the texture of the sediments were found.

Table 2 shows the abundances and biovolume of the photoautotrophic microorganisms in the soils/sediments at the beginning of transplant experiments in August 2004. The total numbers of all photoautotrophic cells were between 1 - 75 cells per mg of sediment in subglacial sediments, 15 - 190 in proglacial, and 49 - 53 in vegetated soils. The mean abundance proportion of cyanobacteria within the phototrophic community was between 0.93 in vegetated and 0.95 in subglacial sediments. The biovolume proportions of cyanobacteria within the phototrophic community were shifted in the favour of algae due to their greater cell volume in proglacial and vegetated soil, and were between 0.41 in proglacial and 0.97 in subglacial sediments (Table 2). The values were very variable and no significant differences between sediment types were found.

Table 3 summarises the autochthonous and allochthonous recovery rates of the transplanted photoautotrophic microbial communities. The recovery rates by import of cells of the microbial communities in subglacial sediments were between 27 - 430%,

which means that the time needed for allochthonous recovery of the original size of the community was very variable, between ~3 months and 4 years. The recovery rates were lower for the proglacial barren soil communities (4 - 50%), and the potential recovery times thus much longer (~2 - 27 years). Only two localities could be used for vegetated soils, due to the limited availability of sites with developed vegetation in the catchment area. The recovery of the photoautotrophic community in both of them was relatively quick, with the recovery time being less than 1 year.

Negative or zero change in abundance, and thus no net growth, of the microbial photoautotrophic community was documented in all the investigated subglacial sites. Active growth was observed in two of the studied barren proglacial soil sites, Upper Kvisla and Tonedalen, whereas the other two localities showed no growth. In vegetated soil, there was active growth at one locality in Tonedalen (460%), while at Kvartsittsletta no growth was documented (Table 3). Therefore, the time needed for the recovery of the initial community by autochthonous growth was very variable among the soil types. In barren proglacial soil the community could potentially be restored in ~1.4 years, whereas in subglacial sediments, where the growth of the original community was very small or zero, the photoautotrophic community cannot be renewed by growth only.

The transplant experiments showed the colonisation potential of soil/sediment photoautotrophic communities within the catchment (Table 3). The numbers of cells in subglacial sediments transferred to proglacial barren soils increased up to ~11 times after 1 year of transplantation, while they did not have net growth when transferred to older vegetated soil. There was a negative change in the microbial abundance in samples from barren proglacial soil transplanted to both subglacial soils (0.2 - 22%) and to vegetated soils (0 - 62%). The communities from vegetated soil transferred to subglacial environments of Upper and Lower Kvisla increased up to ~3 times, whereas those transplanted to Tonedalen and Sněženka declined. Great variability in the transplant success was also found in vegetated samples transferred to proglacial soil (between 90 and 1980% of the original cell numbers).

Discussion

Terrestrial environments in the catchment of Werenskioldbreen are linked via wind and water pathways and viable cells of cyanobacteria and microalgae are transported along with soil/sediment particles between these environments. This is supported by the documented import of allochthonous cells into all the studied environments (Table 3). There are differences between the environment types and also between individual sites in terms of cell import and their subsequent survival, which may have arisen as a result of various factors, including the state of the original community of photoautotrophs, the physico-chemical properties of the soil/sediment and the transport pathways.

Soils on Svalbard and in other glaciated regions are poorly developed and mostly consist of coarse mineral substrate with a low content and a patchy distribution of organic matter (Kaštovská et al. 2005; 2007; Bargett et al. 2007). The physical and chemical properties of the investigated soils/sediments (Table 1) were consistent with previous studies of similar environments on Svalbard (Hodkinson et al. 2003; Kaštovská et al. 2005; Nakatsubo et al. 2008) and other recently deglaciated areas (Foght et al. 2004; Nemergut et al. 2007). Vegetated soils had a finer texture, higher organic matter and water contents and nutrient concentrations compared with subglacial and barren proglacial soils, however, a dense vegetation cover meant less available light for photoautotrophic microbes. The principal difference between otherwise very similar subglacial sediments and barren proglacial soils is obviously the lack of incident solar radiation in the former. All the sediment/soil environments appeared relatively stable, since no significant changes in their physical and chemical characteristics were recorded between the two years of the study (Table 1).

The abundance and biovolume of photoautotrophic microbes in the soils/sediments were very low and variable (Table 2), and consistent with previous studies from Werenskioldbreen and other polar glacial environments (Davey and Rothery 1993; Kaštovská et al. 2005; 2007). The heterogeneous distribution of photoautotrophs in the samples is likely to be a result of the patchiness of organic matter and fine clay

particles in the soil/sediment, which has also been suggested for microbial communities in cryogenic soils in Antarctica (Davey and Rothery 1993). Another reason for the observed variability in the abundance of photoautotrophs might be the intrinsic variability in the cell input to the soil/sediment environment, dependent on various, mostly unknown, environmental factors.

A simple conceptual model of the circulation of photoautotrophs within the glacier catchment is presented in Figure 3. It consists of three main elements: the actual pool of cyanobacterial and microalgal cells, based on the determined abundance, the degree of autochthony/allochthony, i.e. the ratio between growth and import of cells, and the potential sources for the cells, both of the latter based on the transplant experiments.

The barren proglacial soil environment is the most important site of photoautotrophic activity within the catchment. It harbours the most abundant community of photoautotrophs (Table 2), which results in the longest time needed for the recovery of the original size of the community (Table 3). The active growth of the local photoautotrophs is documented by the relatively short autochthonous recovery (Table 3). This is probably the result of the available light and the relative scarcity of competition of higher plants (Belnap and Lange 2001). Although the photoautotrophic community is not dependent only on allochthonous cell input here due to active growth, cells deposited in barren proglacial soils have the highest survival rate (Table 3). This is of great importance for the colonisation process, either of fresh exposed till after glacier retreat or after physical disturbance, e.g. by glacial water or solifluction, and indicates that new photoautotrophic communities may be readily started by imported cells from elsewhere. However, the primary production rate of photoautotrophs on very young sites is probably too low to fully sustain a heterotrophic community, since it has been shown that heterotrophic microbes mainly respire ancient organic carbon in recently deglaciated sites (Bardgett et al. 2007). It is likely that, despite the relative favourableness in comparison with the other

environments, the low temperature and the nutrient stress exert a strong control upon the activity of photoautotrophs in barren proglacial soils.

There was a great difference between the two studied vegetated soil sites, which makes a general assessment of the role of vegetated soil sites in the overall cell circulation very difficult. However, it is evident that there are factors which render older vegetated soils less favourable for photoautotrophic growth than young barren soils, most likely low light levels, caused by vegetation cover, and competition for nutrients from mosses and other higher plants. The result may be a very slow autochthonous growth, as was the case at the coastal plain site (Table 3), and the consequent lower abundance of photoautotrophs in vegetated soil compared with barren proglacial soil (Table 2). A similar observation was made by Davey and Rothery (1993) showing a negative correlation between the total biovolume of photoautotrophs and the occurrence of mosses in Antarctic soils. On the other hand, high growth may be achieved at sites with lower vegetation cover, such as Tonedalen (Table 3). Vegetated soils can thus be a source of photoautotrophic cells for other environments, especially in autumn when the ground becomes frozen and small soil particles together with the attached microorganims can be easily lifted by wind and transported to other environments (Marshall and Chalmer 1997). This is supported by the relatively high survival rate of photoautotrophs transferred from vegetated soil to both barren proglacial and subglacial environments (Table 3).

Subglacial environments are not expected to play an active role in the circulation of photoautotrophs within the catchment due to the absence of essential solar radiation, as supported by the virtual absence of autochthonous growth (Table 3). However, it is very important to quantify the input of photoautotrophic cells into this environment as well as their survival potential. This is because cyanobacteria and algae may be a significant source of organic carbon for indigenous subglacial microbes (Skidmore et al. 2000; 2005; Foght et al. 2004), and if preserved in a viable state in the sediment, they may give rise to a new community after deglaciation (Kaštovská et al. 2005; 2007). Significant abundances of photoautotrophs were found in the subglacial sediments of

Werenskioldbreen (Table 2), and more importantly, the allochthonous input of cells is relatively high, as the original cell abundance can be renewed in less than a year (Table 3). The main source of these new cells is probably the glacier surface, which is an efficient „trap“ for microbial cells, and provides a favourable environment for active growth (Stibal et al. 2006). During the melt season approximately 75% of supraglacial melt water from Werenskioldbreen is routed to subglacial environments (Řehák et al. 2007) and transports a significant proportion of the deposited microbial cells to the glacier bed (Stibal et al. 2008). This is in agreement with a study from John Evans Glacier in Arctic Canada, where seasonal inoculation of subglacial systems by bacteria from supraglacial waters was found (Bhatia et al. 2006). The relatively high survival rate of photoautotrophs transplanted to subglacial sediments from vegetated sites (Table 3) support the assertion that photoautotrophic cells may remain viable for some time under the glacier, and have a role in the recolonisation process of newly deglaciated soils (Kaštovská et al. 2005; 2007).

Circulation of photoautotrophic microbes in a glacierised catchment is a very important process, particularly with regard to colonisation of new substrata. Given the changes of climate and the ongoing glacial retreat, the proglacial areas will be expanding and the interaction between melt waters and sediments/soils in the glacial forefield will become more significant (Hodson et al. 2008). We show here that new proglacial substrata can be successfully colonised by photoautotrophic microbes. With progressing deglaciation, the pool of microbial cells available for circulation will increase, and the coupling between all the glacial environments is likely to be enhanced. Transport of cells may in some cases exceed *in situ* microbial growth and thus may be important in environments with low productivity. While the subglacial environment is rather a conduit for photoautotrophic communities than a place of growth and production, the supply of viable photautotrophs is relatively high and can serve as a significant resource of nutrients for subglacial microbial communities.

Acknowledgements

This work was supported by the Program Support of Targeted Research in the Academy of Sciences of the Czech Republic (AV 0Z60050516) and by the grants GA ASCR KJB6005409. University of Wrocław kindly enabled us to stay at Baranowski station. J. Elster stimulated this research and helped with the sample collection. Field assistance of S. Řehák is also greatly acknowledged.

References

Bardgett RD, Richter A, Bol R, Garnett MH, Bäumler R, Xu XL, Lopez-Capel E, Manning DAC, Hobbs PJ, Hartley IR, Wanek W (2007) Heterotrophic microbial communities use ancient carbon following glacial retreat. Biol Lett 3: 487–490. doi 10.1098/rsbl.2007.0242

Belnap J, Lange OL (2001) Structure and function of biological soil crusts: synthesis. In: Belnap J, Lange OL (eds) Biological soil crusts: Structure, function, and management. Springer, Berlin, pp 471–480

Bhatia M, Sharp M, Foght J (2006) Distinct bacterial communities exist beneath a High Arctic polythermal glacier. Appl Environ Microbiol 72:5838-5845. doi 10.1128/AEM.00595-06

Breen K, Lévesque E (2008) The influence of biological soil crusts on soil characteristics along a High Arctic glacier foreland, Nunavut, Canada. Arct Antarct Alp Res 40:287–297. doi 10.1657/1523-0430(06-098)[BREEN]2.0.CO;2

Davey MC, Rothery P (1993) Primary colonization by microalgae in relation to spatial variation in edaphic factors on Antarctic fellfield soils. J Ecol 81:335-343

Duc L, Noll M, Meier BE, Bürgmann H, Zeyer J (2009) High diversity of diazotrophs in the forefield of a receding Alpine glacier. Microb Ecol 57: 179-190. doi:10.1007/s00248-008-9408-5

Elster J (2002) Ecological classification of terrestrial algal communities in polar environments. In: Beyer L, Bölter M (eds) Geoecology of Antarctic ice-free coastal landscapes. Springer, Berlin, pp 303–326

Foght J, Aislabie J, Turner S, Brown CE, Ryburn J, Saul DJ, Lawson W (2004) Culturable bacteria in subglacial sediments and ice from two Southern Hemisphere glaciers. Microb Ecol 47(4): 329-340. doi 10.1007/s00248-003-1036-5

Hillebrand H, Dürselen C-D, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35:403-424

Hodkinson ID, Coulson SJ, Webb NR (2003) Community assembly along proglacial chronosequences in the high Arctic: vegetation and soil development in north-west Svalbard. J Ecol 91:651-663

Hodson A, Anesio AM, Tranter M, Fountain AG, Osborn M, Priscu J, Laybourn-Parry J, Sattler B (2008) Glacial ecosystems. Ecol Monogr 78:41-67

Kaštovská K, Elster J, Stibal M, Šantrůčková H (2005) Microbial assemblages in soil microbial succession after glacial retreat in Svalbard (High Arctic). Microb Ecol 50:396-407. doi 10.1007/s00248-005-0246-4

Kaštovská K, Stibal M, Šabacká M, Černá B, Šantrůčková H, Elster J (2007) Microbial community structure and ecology of subglacial sediments in two polythermal Svalbard glaciers characterized by the epifluorescence microscopy and PLFA. Polar Biol 30: 277-287. doi 10.1007/s00300-006-0181-y

Liengen T, Olsen RA (1997) Nitrogen fixation by free-living cyanobacteria from different coastal sites in a high arctic tundra, Spitsbergen. Arct Alp Res 29:470–477

Marshall WA, Chalmers MO (1997) Airborne dispersal of Antarctic algae and cyanobacteria. Ecography 20:585–594

Mueller DR, Pollard WH (2004) Gradient analysis of cryoconite ecosystems from two polar glaciers. Polar Biol 27:66–74. doi 10.1007/s00300-003-0580-2

Nakatsubo T, Yoshitake S, Uchida M, Uchida M, Shibata Y, Koizumi H (2008) Organic carbon and microbial biomass in a raised beach deposit under terrestrial vegetation in the High Arctic, Ny-Ålesund, Svalbard. Polar Res 27:23-27. doi 10.1111/j.1751-8369.2008.00037.x

Nemergut DR, Anderson SP, Cleveland CC, Martin AP, Miller AE, Seimon A, Schmidt SK (2007) Microbial community succession in an unvegetated, recently deglaciated soil. Microb Ecol 53:110-122. doi 10.1007/s00248-006-9144-7

Ohtonen R, Fritze H, Pennanen T, Jumpponen A, Trappe J (1999) Ecosystem properties and microbial communities changes in primary succession on a glacier forefront. Oecologia 119:239–246

Pearce DA, Bridge PD, Hughes KA, Sattler B, Psenner R, Russell NJ (2009) Microorganisms in the atmosphere over Antarctica. FEMS Microbiol Ecol 143-157. doi 10.1111/j.1574-6941.2009.00706.x

Řehák J, Řehák S, Stibal M, Řeháková K, Šabacká M, Kostka S (2007) Glacier caves and drainage systems of the northern part of Hornsund area, southwest Spitsbergen, Svalbard. In: Abstracts of the 8th GLACKIPR Symposium, Sosnowiec, Poland, p 111

Säwström C, Mumford P, Marshall W, Hodson A, Laybourn-Parry J (2002) The microbial communities and primary productivity of cryoconite holes in Arctic glacier (Svalbard 79°N). Polar Biol 25:591-596 doi 10.1007/s00300-002-0388-5

Sigler WV, Crivii S, Zeyer J (2002) Bacterial success in glacial forefield soils characterized by community structure, activity and opportunistic growth dynamics. Microb Ecol 44:306–316

Skidmore ML, Foght JM, Sharp MJ (2000) Microbial life beneath a high Arctic glacier. Appl Environ Microbiol 66:3214–3220

Skidmore M, Anderson SP, Sharp M, Foght J, Lanoil BD (2005) Comparison of microbial community compositions of two subglacial environments reveals a possible role for microbes in chemical weathering processes. Appl Environ Microbiol 71:6986–6997 doi 10.1128/AEM.71.11.6986-6997.2005

Stibal M, Šabacká M, Kaštovská K (2006) Microbial communities on glacier surfaces in Svalbard: the impact of physical and chemical properties on abundance and structure of cyanobacteria and algae. Microb Ecol 52:644-654. doi 10.1007/s00248-006-9083-3

Stibal M, Tranter M (2007) Laboratory investigation of inorganic carbon uptake by cryoconite debris from Werenskioldbreen, Svalbard. J Geophys Res-Biogeosciences 112: G04S33. doi:10.1029/2007JG000429

Stibal M, Tranter M, Benning LG, Řehák J (2008) Microbial primary production on an Arctic glacier is insignificant in comparison with allochthonous organic carbon input. Environ Microbiol 10:2172-2178. doi 10.1111/j.1462-2920.2008.01620.x

Tscherko D, Rustemeier J, Richter A, Wanek W, Kandeler E (2003) Functional diversity of the soil microflora in primary succession across two glacier forelands in the Central Alps. Eur J Soil Sci 54:685-696. doi 10.1046/j.1365-2389.2003.00570.x

Vincent WF (2000) Cyanobacterial dominance in the polar regions. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria. Kluwer, Dordrecht, pp 321-340

Wharton RA Jr, McKay CP, Simmons GM Jr, Parker BC (1985) Cryoconite holes on glaciers. BioScience 35:499-503

T**able 1.** Physico-chemical characteristics of soils/sediments at the beginning in summer 2004 and at the end of transplant experiments in summer 2005. Values with the same letter are not significantly different at p=0.05 from other soil/sediment types (one-way ANOVA with Tukey's honest significant difference test). No significant differences were found in the same samples after 1 year of incubation.

	subglacial		proglacial		vegetated	
	2004	2005	2004	2005	2004	2005
pH	7.4±0.39 a	8.2±0.48 a	7.3±0.47 a	7.1±.1.1 b	6.6±0.78 a	7.2±0.14 b
water content (%w)	7.3±1.6 a	13±2.8 a	7.3±3.7 a	14±9.3 ab	16±0.1 b	22±8.4 b
organic matter (%dw)	0.99±0.65 a	1.2±0.61 a	0.87±0.30 a	1.4±1.2 a	2.5±0.26 b	3.7±8.3 a
texture (% >0.5mm)	79±14 a	80±16 a	83±22 a	76±35 a	62±4.3 a	76±19 a
carbon (%dw)	0.50±0.08 a	0.10±0.03 a	0.63±0.18 a	0.37±0.34 a	1.1±0.23 b	2.2±1.0 b
nitrogen (%dw)	0.011±0.02 a	0.021±0.01 a	0.013±0.01 a	0.0063±0.01 b	0.015±0.04 a	0.076±0.01 c

Table 2. Abundance (A_{2004}) and biovolume of phototrophic microorganisms in soils/sediments at the beginning of transplant experiments in summer 2004. No statistical differences were found between soil/sediment types.

	subglacial	proglacial	vegetated
total abundance (cells mg^{-1})	22±36	120±76	51±2.4
total biovolume ($\mu m^3 mg^{-1}$)	1800±3600	7500±6900	3400±3500
cyanobacterial abundance (cells mg^{-1})	21±36	110±74	48±5.2
cyanobacterial biovolume ($\mu m^3 mg^{-1}$)	1800±3400	3100±2100	2000±1600
abundance proportion of cyanobacteria	0.95	0.94	0.93
biovolume proportion of cyanobacteria	0.97	0.41	0.56

Table 3. Recovery of local/transplanted photoautotrophs in different glacial soils/sediments after 1 year of incubation *in situ*. Numbers are means of 4 replicate samples. No significant differences between soil/sediment types were found (one-way ANOVA + Tukey HSD test).

type of soil/sediment	locality	R_{allo} (%)	T_{allo} (yrs)	R_{auto} (%)	T_{auto} (yrs)	$TS_{subglac}$ (%)	TS_{barren} (%)	TS_{veget} (%)
subglacial	Upper Kvisla	430	0.24	0	-		1164	0
	Lower Kvisla	120	0.81	0	-		0	0
	Tonedalen	27	3.8	42	2.4	-	60	0
	Sněženka	n.d.	n.d.	0	-		4	0
	mean±sd	192±211	1.6±1.9	11±21	n.d.		307±572	0±0
barren proglacial	Upper Kvisla	14	7.0	213	0.47	22		26
	Lower Kvisla	9.8	10	31	3.2	13		0
	Tonedalen	50	2.0	123	0.81	7	-	62
	Sněženka	3.7	27	81	1.2	0.2		0
	mean±sd	19±21	12±11	112±77	1.4±1.2	11±9.3		22±29
vegetated proglacial	Kvartsittsletta/Upper Kvisla	205	0.49	0	-	139	161	
	Kvartsittsletta/Lower Kvisla					333	1976	
	Kvartsittsletta/Sněženka					48	112	-
	Tonedalen	140	0.73	460	0.22	46	90	
	mean±sd	172±46	0.61±0.17	230±325	n.d.	142±135	585±928	

R_{allo} – recovery of phototrophic microbial population by import of cells
T_{allo} – years needed to recover the original population by import of cells
R_{auto} – recovery by growth
T_{autoh} – years needed to recover the original population by growth
TS_x – transplantation success of phototrophic community in *x*
n.d. – not determined
- can not be calculated, because R_{auto} is 0

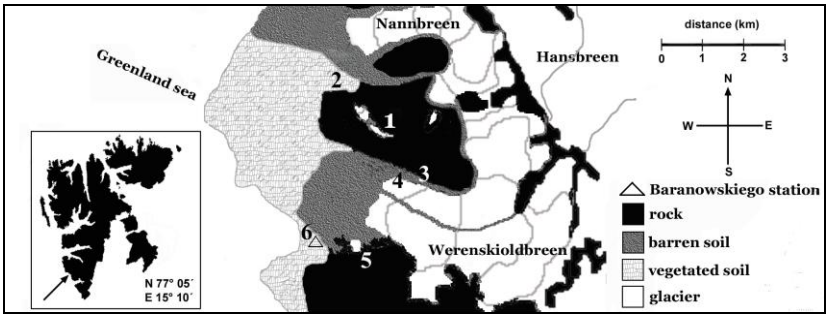

Figure 1. Location of the study area on Svalbard. 1. Tonedalen - subglacial and barren soil. 2. Tonedalen - vegetated soil. 3. Upper Kvisla - subglacial and barren soil. 4. Lower Kvisla - subglacial and barren soil. 5. Sněženka - subglacial and barren soil. 6. Kvartsittsletta coastal plain - vegetated soil.

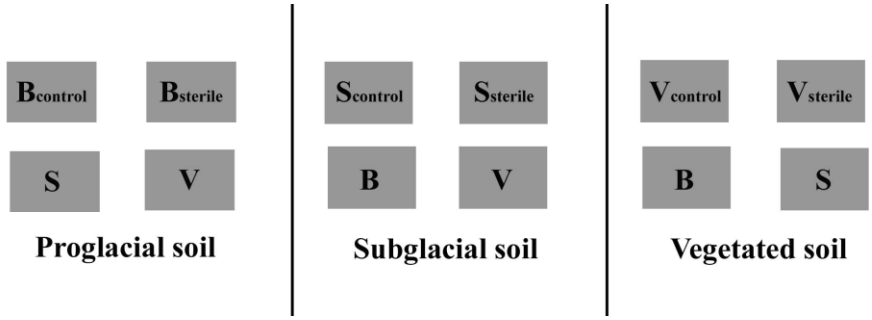

Figure 2. Schematic of the transplant procedure. Each grey square represents a quadriplicate sample incubated in environment X that was 1) transplanted from subglacial (S), barren proglacial (B) or vegetated (V) soil/sediment, 2) sterilised and left in place ($X_{sterile}$) or 3) untreated and left in place as control ($X_{control}$).

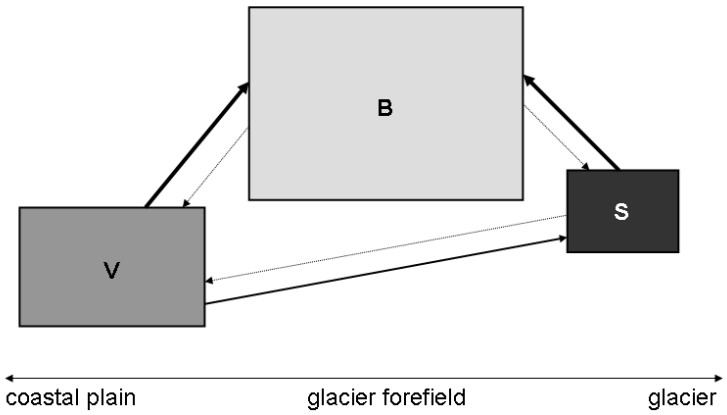

Figure 3. Conceptual model of the circulation of photoautotrophic microbes in Werenskioldbreen catchment. The size of the boxes represents the abundance of photoautotrophs in the given soil/sediment environment. The darkness of the box area represents the degree of allochthony of the environment, i.e. the darker the more allochthonous (dependent on input of cells rather than on growth) is the environment. The thickness of the arrows represents the survival rate of cells transferred to the given environment. S=subglacial sediments, B=barren proglacial soil, V=vegetated soil.