Molecular characterization of phototrophic microorganisms in the forefield of a receding glacier in the Swiss Alps

Beat Frey\textsuperscript{1,3}, Lukas Bühler\textsuperscript{1}, Stefan Schmutz\textsuperscript{1}, Anita Zumsteg\textsuperscript{1,2} and Gerhard Furrer\textsuperscript{2}

\textsuperscript{1} Rhizosphere Processes Group, WSL Swiss Federal Research Institute, 8903 Birmensdorf, Switzerland
\textsuperscript{2} Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, 8092 Zürich, Switzerland

E-mail: beat.frey@wsl.ch

Received 5 December 2012
Accepted for publication 20 February 2013
Published 12 March 2013

Abstract
Recently deglaciated areas are ideal environments to study soil formation and primary microbial succession where phototrophic microorganisms may play a role as primary producers. The aim of our study was to investigate the cyanobacterial and green algal community composition in three different successional stages of the Damma glacier forefield in the Swiss Alps using 16S rDNA and ITS rDNA clone libraries. Cyanobacterial target sequences varied along the glacier forefield, with the highest cyanobacterial 16S rRNA gene copies found in sparsely vegetated soils. Sequence analysis revealed that the phototrophic communities were distinct in each of the three soil environments. The majority of the cyanobacterial sequences retrieved from barren soils were related to the \textit{Oscillatoriales}. The diversity in sparsely vegetated soils was low, and sequences closely related to \textit{Nostoc} sp. dominated. The majority of the algal phylotypes are related to members of the \textit{Trebouxiophyceae} known to live as symbiotic partners in lichens. We conclude that the community composition appears to shift markedly along the chronosequence, indicating that each soil environment selects for its phototrophic community. When cyanobacteria occur together with eukaryotic microalgae, they form a rich source of organic matter and may be important contributors of carbon in nutrient-deficient deglaciated soils.

Keywords: glacier retreat, glacier forefield, soil chronosequence, primary production, organic matter, cyanobacteria, green algae, photoautotrophy

1. Introduction

Glaciers in alpine regions are highly sensitive to changes in climatic conditions (Oerlemans 2005), and increasing global atmospheric temperatures in recent decades have resulted in many glaciers receding (Häberli \textit{et al} 2007). The majority of glaciers in the European Alps have receded quickly at a mean rate of 8–11 m yr \textsuperscript{−1} (estimates derived from data in \url{http://glaciology.ethz.ch/messnetz/glaciers/}). Sites that have been recently deglaciated provide an ideal environment to study soil formation and primary microbial succession, since microorganisms like bacteria (Hodkinson \textit{et al} 2002, Nemergut \\textit{et al} 2007, Frey \textit{et al} 2010, Lapanje \textit{et al} 2011), fungi (Jumpponen 2003, Brunner \textit{et al} 2011, Zumsteg \textit{et al} 2012) and eukaryotic microalgae (Mataloni \textit{et al} 2000, \textit{Trebouxiophyceae} known to live as symbiotic partners in lichens. We conclude that the community composition appears to shift markedly along the chronosequence, indicating that each soil environment selects for its phototrophic community. When cyanobacteria occur together with eukaryotic microalgae, they form a rich source of organic matter and may be important contributors of carbon in nutrient-deficient deglaciated soils.

Abstract
Recently deglaciated areas are ideal environments to study soil formation and primary microbial succession where phototrophic microorganisms may play a role as primary producers. The aim of our study was to investigate the cyanobacterial and green algal community composition in three different successional stages of the Damma glacier forefield in the Swiss Alps using 16S rDNA and ITS rDNA clone libraries. Cyanobacterial target sequences varied along the glacier forefield, with the highest cyanobacterial 16S rRNA gene copies found in sparsely vegetated soils. Sequence analysis revealed that the phototrophic communities were distinct in each of the three soil environments. The majority of the cyanobacterial sequences retrieved from barren soils were related to the \textit{Oscillatoriales}. The diversity in sparsely vegetated soils was low, and sequences closely related to \textit{Nostoc} sp. dominated. The majority of the algal phylotypes are related to members of the \textit{Trebouxiophyceae} known to live as symbiotic partners in lichens. We conclude that the community composition appears to shift markedly along the chronosequence, indicating that each soil environment selects for its phototrophic community. When cyanobacteria occur together with eukaryotic microalgae, they form a rich source of organic matter and may be important contributors of carbon in nutrient-deficient deglaciated soils.

Keywords: glacier retreat, glacier forefield, soil chronosequence, primary production, organic matter, cyanobacteria, green algae, photoautotrophy

1. Introduction

Glaciers in alpine regions are highly sensitive to changes in climatic conditions (Oerlemans 2005), and increasing global atmospheric temperatures in recent decades have resulted in many glaciers receding (Häberli \textit{et al} 2007). The majority of glaciers in the European Alps have receded quickly at a mean rate of 8–11 m yr \textsuperscript{−1} (estimates derived from data in \url{http://glaciology.ethz.ch/messnetz/glaciers/}). Sites that have been recently deglaciated provide an ideal environment to study soil formation and primary microbial succession, since microorganisms like bacteria (Hodkinson \textit{et al} 2002, Nemergut \\textit{et al} 2007, Frey \textit{et al} 2010, Lapanje \textit{et al} 2011), fungi (Jumpponen 2003, Brunner \textit{et al} 2011, Zumsteg \textit{et al} 2012) and eukaryotic microalgae (Mataloni \textit{et al} 2000, \textit{Trebouxiophyceae} known to live as symbiotic partners in lichens. We conclude that the community composition appears to shift markedly along the chronosequence, indicating that each soil environment selects for its phototrophic community. When cyanobacteria occur together with eukaryotic microalgae, they form a rich source of organic matter and may be important contributors of carbon in nutrient-deficient deglaciated soils.

Keywords: glacier retreat, glacier forefield, soil chronosequence, primary production, organic matter, cyanobacteria, green algae, photoautotrophy
Freeman et al. 2009, Schmidt et al. 2011) are the first to colonize new substrates.

In the early stages of primary succession on glacier forefields, soil microbial activity in barren soils is limited by the low availability of both carbon and nitrogen (Yoshitake et al. 2007, Bernasconi et al. 2011). According to Göransson et al. (2011), bacterial growth rates in the temperate alpine glacier forefield they studied are carbon limited. Organic matter in barren soils is thought to come from Aeolian inputs (Bauer et al. 2002), from ancient carbon pools (Bardgett et al. 2007) or faecal deposition from higher animals (Mindl et al. 2007). Phototrophic microorganisms may also be a significant source of new organic carbon in glacial ecosystems (Stibal et al. 2008, Anesio et al. 2009, Freeman et al. 2009), as well as primary colonizers of recently deglaciated soil (Kastovska et al. 2005, Omelon et al. 2006, Stibal et al. 2006). These microorganisms have been well studied in arctic environments, but the occurrence and succession of cyanobacteria and, in particular, eukaryotic microalgae in temperate alpine glacier forefields have clearly been under-surveyed. 16S rRNA gene clone libraries revealed that heterotrophic bacteria, especially, Alpha- and Beta-proteobacteria, dominate the initial colonization of alpine glacier forefields (Nemergut et al. 2007, Sattin et al. 2009, Zumsteg et al. 2012). Cyanobacteria have also been identified but in lower numbers (Nemergut et al. 2007, Schmidt et al. 2008, Zumsteg et al. 2012). In contrast, no phylogenetic information on eukaryotic microalgae in temperate alpine glacier forefields has been collected.

The objectives of our study were, therefore, to investigate cyanobacterial and green algal diversity in a range of soil environments within a glacier forefield as well as the physicochemical parameters that may be essential in shaping the phototrophic communities. This study is part of the interdisciplinary research project, BigLink, which focuses on weathering, soil formation and ecosystem evolution along the temperate Damma glacier forefield in the Swiss Central Alps (Bernasconi et al. 2011). Heterotrophs are known to react strongly to soil parameters in this glacier forefield (Zumsteg et al. 2012). In contrast, photoautotrophs may play a role in generating pioneering organic carbon inputs in these carbon-limited soil environments and therefore, we hypothesized that the microbial primary producer communities changed with the development of plants, as plants increase the organic carbon input into the ecosystem. Cyanobacterial and green algal community composition were analysed using 16S rRNA and ITS rRNA gene sequences retrieved from three different successional stages of the microbial colonization, ranging from barren soil close to the glacier forefront (initial soils) to sparsely vegetated soils (transient soils), to soils with established plant cover (developed soils).

2. Material and methods

2.1. Location and sampling

The Damma glacier forefield is located in the Central Alps, within the Central Aar Granite, in Switzerland (N46°38′08″27″ at an altitude between 1950 and 2050 m above sea level (Bernasconi et al. 2011). The front of the Damma glacier has been monitored by the Swiss glacier monitoring network (http://glaciology.ethz.ch/messnetz/?locale=de) since 1921 when systematic measurements began and has been retreating at an average rate of approximately 10 m yr⁻¹. The recession of the Damma glacier since 1850 has not been continuous, but was reversed during 1920–28 and 1970–92, resulting in two small terminal moraines clearly visible in the field. Precipitation is around 2400 mm yr⁻¹, and the mean annual temperature ranges from 0 to 5 °C (Bernasconi et al. 2011).

2.2. Sampling

Since the retreat of the glacier has been monitored, the sites chosen for sampling can be classified as ice-free times for 0–10 yr (initial soils), 60–70 yr (transient soils) and 110–120 yr (developed soils). The initial soils are barren, with no vegetation, between the glacier terminus and the 1992 moraine (Bernasconi et al. 2011, Zumsteg et al. 2012). Transient soils are located between the 1992 and the 1928 moraines. The developed soils extend from the 1928 moraine onwards. Soil sampling was performed in September 2011. Surface soil samples (0–5 cm) were collected in five independent replicates from each site and treated separately. Each of the replicate samples consisted of five subsamples taken within an area of 4 m². The distance between the sampling areas for each replicate was approximately 20 m. Soils were maintained in sterile falcon tubes, immediately placed on ice, and kept in the dark at 4 °C for transport on the same day to the Research Institute WSL. Barren and transient soils are characterized by gravel with sandy–silty sediment between barren rocks. Larger stones were manually removed from these fine granitic sediments. Vegetated soil was passed through a 2 mm sieve and then larger stones and roots were handpicked from the sieve. The residues were used for analysis. Samples were then frozen at −80 °C until further processing.

2.3. Soil physicochemical parameters

Soil pH was determined in 0.01 M CaCl₂. Total carbon and nitrogen content in the soils were measured using dried and finely ground (disc mill) soil weighed into tin capsules and introduced into a Flash elemental analyzer (Thermo Fisher Scientific, Wohlen, Switzerland) operated with helium as a carrier gas (Zumsteg et al. 2012). Samples were combusted in the presence of O₂ in an oxidation column at 1030 °C, and the combustion gases were then passed through a reduction column (650 °C). The N₂ and CO₂ gases produced were separated chromatographically and the amount measured with a thermal conductivity detector (TCD). Contents were calibrated by bracketing with a standard soil with known Nₗot and Cₗot. The plant cover was determined using aerial photographs covering of approximately 25 m² of the sites, and then calculating the total green plant cover on this area with Adobe Photoshop CS3 (Adobe Systems Incorporated, San Jose, CA, USA). The correlation coefficients (R) with
their p-values were calculated according to Pearson with the statistical program SPSS statistics (IBM Corporation, Armonk NY, USA).

2.4. DNA extraction

Genomic DNA was extracted from thawed soil using the Smart Helix DNA extraction kit (Venturia, Ljubljana, Slovenia) according to the manufacturer’s instructions. Preliminary tests showed that this commercial kit efficiently extracted DNA from various types of soils (Zumsteg et al 2012). The extracted DNA was quantified with Pico Green (Invitrogen, Carlsbad CA, USA) as described by Frey et al (2008) and stored at −20°C until further use.

2.5. Cloning and sequencing of cyanobacterial 16S rRNA gene and algal ITS region

PCR amplifications to construct three clone libraries were performed on the five replicate DNA extracts of initial, transient and developed soils. For the PCR amplification of the cyanobacterial 16S rRNA gene sequences, 0.2 μM of each cyanobacteria-specific primer CYA359F (GAA TYT TCC GCA ATG GG) and CYA781R (GAC TAC T/AGG GGT ATC TAA TCC CA/TT T) was used according to the method of Nübel et al (1997), who found these primers provide a broad coverage of cyanobacterial taxa. PCR reactions were carried out in a total volume of 25 μl with 0.5 mM PCR buffer, 0.5 mM MgCl2, 400 μM dNTP, 0.6 mg ml−1 BSA and 0.05 U μl−1 Hot Star Taq Polymerase (Qiagen AG, Switzerland). The cycle conditions (35 cycles in total) were 15 min at 95°C for denaturation, 45 s at 95°C (denaturation), 1 min at 60°C (annealing) and 1 min at 72°C (elongation), with a final extension of 5 min at 72°C.

The internal transcribed spacer (ITS1-5.8-ITS2) of ribosomal DNA region was amplified using an algal-specific primer, nr-SSU-1780-5′-CTG CCG AAG CAT GAT TGA TTC-3′; Piercey-Normore and DePriest (2001) and a universal primer, ITS4-3′-TCC GCT TAT TGA TAT GC-3′; White et al (1990). PCR reactions were carried out in a total volume of 25 μl with 0.5 mM PCR buffer, 0.5 mM MgCl2, 0.2 μM of each primer, 400 μM dNTP, 0.6 mg ml−1 BSA and 0.05 U μl−1 Hot Star Taq Polymerase. The cycle conditions (35 cycles in total) were 15 min at 95°C for denaturation, 45 s at 95°C (denaturation), 1 min at 54°C (annealing) and 1.5 min at 72°C (elongation) with a final extension of 5 min at 72°C.

The PCR products from the five replicates were then pooled in one sample for each soil environment, ligated into the vector of the pGEM-T Easy Vector System and cloned into the competent cells JM109 (Promega Corporation, Madison, USA). A PCR reaction on the successfully transformed clones with the vector-specific primers M13f and M13r was performed as described in Frey et al (2011) to check if the length of the insert was correct. This insert was then restricted with MspI for cyanobacterial 16S rDNA and HaelIII for algal ITS rDNA in order to choose the clones to be sequenced with a unique RFLP pattern (clone OTUs). Representative clones from the most frequently occurring restriction patterns from each library were sequenced. The M13 amplicons were cleaned prior to sequencing with Montage SEQ96 sequencing reaction cleanup kit (Millipore Corporation, Billerica, MA) and were sequenced on both strands with Sp6 forward and T7 reverse primers, according to the procedure of Frey et al (2008). Cycle sequencing was carried out using the Big Dye-Terminator Cycle Sequencing Kit, version 1.1 (PE Applied Biosystems, Foster City, CA, USA). After the reaction, excess dye terminator was removed using a Montage SEQ96 sequencing reaction cleanup kit (Millipore). DNA sequencing was performed using an ABI 3730xl genetic analyser (Applied Biosystems).

The cyanobacterial 16S rDNA and the algal ITS rDNA sequences were subjected to a BLAST search. The sequences in the GenBank database with the greatest similarities were then imported into the BioEdit sequence alignment editor. All sequences were checked for chimeric characteristics. For phylogenetic placement, alignments against closest relatives and known taxonomic sequences were carried out using CLUSTALW for multiple sequence alignment, and the resulting alignments edited using BioEdit. Phylogenetic trees were inferred using MrBayes (version 3.2; Huelsenbeck and Ronquist 2001) for Bayesian analyses. Bayesian phylogenetic analyses were conducted on the aligned sequences using the GTR + gamma model of evolution with 2 million generations. Trees were viewed using FigTree (Rambaut 2008).

To estimate richness, evenness and Shannon index (H), sequences were collected into operational taxonomic units based on sequence identity at different fixed levels of identity from 95 to 100% in 1% increments. The open source computer program Mothur (Schloss et al 2009) was used to calculate operational taxonomic unit richness, clone library coverage based on Good’s coverage and known taxonomic sequences were carried out using Libshuff p-value to compare the phylogenetic diversity of the different clone libraries. The algal ITS gene sequences were deposited to GenBank under the following accession numbers JX435329–JX435396 and cyanobacterial 16S rRNA gene sequences under JX435397–JX435434.

2.6. Quantitative PCR of 16S rRNA gene sequences

Real-time PCR assays were performed in an ABI 7500 Fast real-time PCR system (Applied Biosystems) as described in by Frey et al (2011). Specific primers for cyanobacteria were CYA359F and CYA781R and for total bacteria the universal primers were 1369F (CGG TGA ATA CTC GC-3′) and 1492R (GGW TAC CTT GTT ACG GAC TT) (Frey et al 2008). Each 25 μl reaction contained 0.5 μM of each primer, 12.5 μl of SYBR Green PCR master mix, including HotStar Taq DNA polymerase, QuantTec SYBR Green PCR Buffer, dNTP mix, SYBR Green I, ROX and 5 mM MgCl2 (QuantiTect SYBR Green PCR Kit, Qiagen), 0.2 mg ml−1 BSA, 11 μl of diluted DNA corresponding to 2.5 ng of total DNA.
soil DNA, and RNase-free water to make up the 25 µl volume. PCR conditions are 15 min at 95 °C, followed by 40 cycles at 95 °C for 15 s, 60 °C (cyanobacterium) or 55 °C (bacteria) for 30 s (annealing), and 72 °C for 45 s (extension), followed by a final data acquisition step at 80 °C for 15 s. Each plate included the samples and the appropriate set of standards. After the DNA amplification cycles, melting curve analysis confirmed that the signals obtained were caused by the specific amplicon. All standard curves were constructed using plasmids from cloned 16S rRNA genes for cyanobacteria and bacteria, as described in Frey et al. (2008, 2011). The selected clones reflect the range of 16S rRNA gene sequences encountered. Tenfold serial dilutions of the plasmid, ranging from 10^{-1} to 10^{-9} copies, are used as templates to determine the calibration curve. The slope of each standard curve (regression lines of Ct versus log N, the log of initial DNA concentration in standard templates) was used to estimate the amplification efficiency in our qPCR assays. The slopes of the standard curves generated were −3.03 for cyanobacteria and −3.21 for bacteria. The correlation coefficients of all three standard curves were high (>0.99). The genes in each sample were quantified in triplicates. Data are presented as the average copy number of targets per gram of soil (dry weight).

3. Results

3.1. Physicochemical characteristics of the soil environments

The soil physicochemical parameters clearly differ in the three soil environments (table 1). With increasing soil age, the C and N content in the soil increases steadily from a C content of 0.07% in barren soils to 3.2% in developed soils (R = 0.81, P = 0.0001). Similarly, the N content rises (R = 0.77, P = 0.0001) from 0.01% to 0.15% along the chronosequence (table 1). The pH is highest in the bare soil (pH 4.7) and lowest in the developed soil (pH 3.7). In contrast, the plant cover increases along the forefield from no (barren soils) or very little vegetation (below 50% in transient soils) to 100% plant cover on developed soils (Zumsteg et al. 2012).

3.2. DNA content and qPCR of (cyano)bacterial 16S rRNA genes

The DNA contents in soils and bacterial 16S rRNA target sequences increased with distance from the glacier (table 2). The DNA content rose from 1.5 µg g⁻¹ dry soil close to the glacier terminus to 15.2 µg g⁻¹ dry soil in the developed soils. The total bacterial abundance increased from 6.8 × 10^6 gene copies in barren soils to 1.1 × 10^9 gene copies in developed soils (table 2). Cyanobacterial target sequences varied along the glacier forefield, with the highest percentage of cyanobacterial 16S rRNA gene copies in the transient soils where 2.3 × 10^7 gene copies represented 8.2% of the total bacterial 16S rRNA gene copy numbers. The lowest percentage of cyanobacterial 16S rRNA gene copies were recorded in the developed soils, namely only 1.4% of the total bacterial 16S rRNA gene copy numbers.

3.3. Phylogenetic analysis of cyanobacterial 16S rDNA sequences

Coverage of the sequences ranged between 88% and 97% of the sample sites (table 3). Shannon diversity (H) index showed a relatively high diversity (2.8–2.9) in the initial and developed soils, with the lowest H index (2.2) in the transient soils (table 3). Based on the LIBSHUFF analyses (data not shown), the clone libraries of the initial and developed soils differed significantly from that of transient soils (P < 0.001). The clone library of the initial soils was only weakly (P = 0.06), but not significantly, different from that of the developed soils.

The initial (barren) soils contain a diverse cyanobacterial community and more than half of the cloned sequences exist exclusively in this soil environment. Phylogenetic analysis identified phylotypes belonging to the orders Oscillatoriales and Chroococcales, and all cloned sequences were related to cyanobacterial sequences obtained from cold habitats. The majority of sequences (seven of the eleven OTUs) were closely related to phylotypes within the order of Oscillatoriales (figure 1). One cluster included four

Table 1. The physiochemical characteristics of the three soil environments.

| Soil development stage | Distance from the glacier front (m) | Age since deglaciation (a) | C_int (µg g⁻¹ soil) | N_int (µg g⁻¹ soil) | pH |
|------------------------|------------------------------------|---------------------------|---------------------|---------------------|-----|
| Initial soils          | 55                                 | 0–10                      | 0.07 ± 0.02         | 0.01 ± 0.01         | 4.7 ± 0.1 |
| Transient soils        | 240                                | 60–70                     | 0.33 ± 0.12         | 0.04 ± 0.02         | 4.3 ± 0.1 |
| Developed soils        | 710                                | 110–120                   | 3.23 ± 0.61         | 0.15 ± 0.04         | 3.7 ± 0.1 |

Table 2. Total DNA content, abundance of cyanobacterial and total bacterial target sequences (gene copy number g⁻¹ soil dry wt; mean ± SD; n = 5) analysed with quantitative real-time PCR targeting 16S rRNA gene fragments in the three soil environments. Note that the percentage cyanobacteria/total bacterial mass is calculated from the average values of the two strongly varying data sets.

| Location         | DNA content (µg g⁻¹ soil) | Cyanobacterial 16S rRNA gene copies (g⁻¹ soil) | Bacterial 16S rRNA gene copies (g⁻¹ soil) | Percentage cyanobacteria/total bacteria |
|------------------|--------------------------|-----------------------------------------------|------------------------------------------|----------------------------------------|
| Initial soils    | 1.5 ± 0.6                | 4.7 × 10^5 ± 2.4 × 10^5                        | 6.8 × 10^6 ± 2.0 × 10^6                   | 6.9                                    |
| Transient soils  | 8.4 ± 1.9                | 2.3 × 10^7 ± 1.4 × 10^7                        | 2.8 × 10^9 ± 6.1 × 10^7                   | 8.2                                    |
| Developed soils  | 15.2 ± 2.3               | 1.5 × 10^8 ± 8.3 × 10^8                        | 1.1 × 10^10 ± 3.5 × 10^9                  | 1.4                                    |
Figure 1. Bayesian phylogenetic tree showing cyanobacterial 16S RNA gene sequences from the three soil environments, their closest BLAST or RDP matches, and representatives of cultured strains. Several cloned sequences had no closest cultured relatives. Sequences in red are from initial (barren) soils, in green from transient soils and in blue from developed soils. Clone CYA8 clustered within the genus GpIV and clone CYBC8 within the genus Gp and clone CYBC8 within the genus Gp following the RDP classifier (Wang et al. 2007). Supports for key nodes are shown as a posterior probabilities. The *Clostridium perfringens* (Y12669) sequence outgroup roots the tree. Scale bar represents substitutions per site.
Table 3. Results of sequence analysis of cyanobacteria from the barren (initial), intermediate scarcely vegetated soils (transient) and the old soil with an established plant cover (developed), calculated with Mothur with a 97% identity for a unique genus. (Note: clone OTUs: unique OTU after RFLP with MspI, which occurs only once. Coverage: per cent library coverage based on Good’s estimate. H: Shannon–Weaver diversity index. E: evenness.)

| Location       | Total clones | Clone OTUs | Number of clones sequenced | Total number of sequences | Sequence OTUs | Coverage (%) | H   | E   |
|----------------|--------------|------------|---------------------------|--------------------------|---------------|--------------|-----|-----|
| Initial soils  | 96           | 8          | 33                        | 28                       | 11            | 91           | 2.9 | 0.95|
| Transient soils| 96           | 9          | 38                        | 36                       | 5             | 97           | 2.2 | 0.94|
| Developed soils| 96           | 12         | 55                        | 54                       | 10            | 88           | 2.8 | 0.91|

Table 4. Results of sequence analysis of green algae from the barren (initial), intermediate scarcely vegetated soils (transient) and the old soil with an established plant cover (developed), calculated with Mothur with a 97% identity for a unique genus. (Note: clone OTUs: unique OTU after RFLP with HaeIII, which occurs only once. Coverage: per cent library coverage based on Good’s estimate. H: Shannon–Weaver diversity index. E: evenness.)

| Location       | Total clones | Clone OTUs | Number of clones sequenced | Total number of sequences | Sequence OTUs | Coverage (%) | H   | E   |
|----------------|--------------|------------|---------------------------|--------------------------|---------------|--------------|-----|-----|
| Initial soils  | 49           | 4          | 16                        | 12                       | 8             | 88           | 1.7 | 0.87|
| Transient soils| 70           | 3          | 16                        | 13                       | 9             | 90           | 1.8 | 0.88|
| Developed soils| 96           | 4          | 46                        | 44                       | 16            | 92           | 2.3 | 0.87|

The diversity of the cyanobacterial sequences was very different in the sparsely vegetated transient soils, where the cyanobacteria were least diverse and were dominated by diazotrophic *Nostocales* phylotypes (figure 1). The majority of the sequences (clones CYBA4; CYBA9) in these soils were related (97–98% similarity) to *Nostoc* sp. strains or uncultured *Nostoc* sp. clones isolated from the cyanolichen *Peltigera* sp. (Kaasalainen et al. 2009). Within the *Chroococcales* clone CYBC8 was most similar (93%) to the *Cyanothecae aeruginosa* strain NIVA-CYA 258/2 and clone CYBDB1 was most similar (100%) to an environmental clone M-1999-12 (EU083410) from receding glaciers habitats (figure 1). One cloned sequence (CYBC8) clustered within the genus *GpI* following the RDP classifier (Wang et al. 2007) and most closely related to a cyanobacterium clone (FN811219) obtained from the Antarctic (Chong et al. 2012).

Samples from the developed soils contained a diverse suite of cyanobacterial phylotypes including sequences from soil, rhizosphere, alpine and endolithic habitats (figure 1). Here we only found a few cloned sequences, e.g. clones CYCB7 and CYCD3, which grouped within the order *Oscillatoriales* and were similar to sequences retrieved from barren soils (figure 1). In general, the cloned sequences we obtained from developed soils appeared to have few cultivated relatives and were most similar to uncultivated cyanobacteria.

3.4. Phylogenetic analysis of algal ITS gene sequences

Coverage of the sequences ranged between 88% and 92% in the different successional stages. Shannon diversity (H) index was highest (2.3) in the developed soils and lowest (1.7–1.8) in the younger soils (table 4). The sequences clustered into 29 OTUs, which were all affiliated with *Chlorophyta* from the classes *Chlorococcales*, *Trebouxioideae* or *Ulivoideae*. The algal sequences were related to various contrasting environments, including phylotypes retrieved from rocks, hot springs, river biofilm, freshwater and soil environments. The algal communities in the three successional stages differed and the younger sites, i.e. the initial and transient soils, were very different (P < 0.001) from the developed soils (figure 2). In the initial and transient soils, the majority of the cloned sequences (clones ALAB6, ALBD3, ALBD4, ALBC6) belonged to *Chlorococcales* most closely related to genus *Oedogonium* sp. (DQ413055) and to *Ulivoideae* closely similar (clones ALAC8, ALAC11, ALAB9, ALBC4) to the genera *Planophila* sp. (AJ161602) and *Pseudendoclonium* sp. (Z47996). Three phylotypes (clone ALAA1, ALBA9, ALBC8) were affiliated to *Trebouxioideae* (figure 2). In contrast, clone sequences retrieved from developed soils were predominantly found within the class *Trebouxioideae*, and were most closely related to the genera *Asterochloris*, *Stichococcus* and *Trebouxia* (figure 2). These algae have been

---

**Environ. Res. Lett. 8 (2013) 015033 B Frey et al.**
Figure 2. Bayesian phylogenetic tree showing ITS rDNA sequences from the three soil environments, their closest BLAST matches, and representatives of cultured strains. Sequences in red are from initial (barren) soils, in green from transient soils and in blue from developed soils. Supports for key nodes are shown as a posterior probabilities. The Raphidonema sempervirens (AJ431674) sequence outgroup roots the tree. Scale bar represents substitutions per site.

found in symbiotic lichen associations (Bákor et al. 2010, Peksa and Škaloud 2011).

4. Discussion

4.1. Cyanobacterial communities

Our study showed that new terrain can be successfully colonized by cyanobacteria after glacier retreat, as other researchers have also found (Nemergut et al. 2007, Schmidt et al. 2008, Zumsteg et al. 2012). The ground under the glacier (Mindl et al. 2007, Hodson et al. 2008) or Aeolian inputs (Bauer et al. 2002) could be the seeding propagules for the development of the phototrophic community after the glacier retreat. We found that cyanobacteria were highly abundant in barren and sparsely vegetated soils according to the qPCR of cyanobacterial 16S rRNA genes. The ratio of cyanobacterial 16S rRNA gene copies to the total bacterial 16S rRNA gene copy numbers decreased with the increase of organic matter along the chronosequence and the amount of heterotrophic organisms. Our copy numbers of cyanobacterial 16S rRNA genes could not be compared with other studies because no comparable data are available from glacier forefields, but our data clearly indicate that a large part (between 5 and 10%) of the total bacterial community consists of cyanobacteria. Kastovska et al. (2005), Stibal et al. (2006) and Rehakova et al. (2011) also found that cyanobacteria make up a significant proportion of the microbial communities in barren arctic...
environments and on arctic glacier surfaces. However, Sattin et al. (2009) showed that cyanobacteria were absent in the soils most recently exposed after glacier retreat in Alaska but were present in the 8 yr old soils.

The phylogenetic analysis of the cyanobacterial 16S rRNA gene sequences identified phylotypes belonging to the orders Oscillatoriiales, Chroococcales and Nostoccales, which is in accordance with other studies of glaciars (Xiang et al. 2009, Segawa and Takeuchi 2010), cold dry valleys in the Himalayas (Schmidt et al. 2011, Rehakova et al. 2011) and lithic environments in the Antarctic (Wood et al. 2008, Khan et al. 2011). The number of cyanobacterial OTUs on the Damma glacier forefield was, in general, similar to the number reported from other studies of glacier forefields. Nemergut et al. (2007) found only two cyanobacterial OTUs directly at the glacial forefront (approx. less than 1 yr after retreat), 14 cyanobacterial OTUs 100 m after the glacial forefront (approx. 4 yr) and 6 cyanobacterial OTUs 500 m after the glacial forefront (approx. 20 yr old soils). Zumsteg et al. (2012) found six cyanobacterial OTUs in barren soils (approx. 6 yr old soils), two cyanobacterial OTUs in transient soils (approx. 60 yr old) and no cyanobacterial OTUs in densely vegetated soils (approx. 110 yr old). Compared to our study, the last two worked with a different set of primers, namely universal bacterial 16S rRNA gene primers, to obtain cyanobacterial 16S rRNA gene sequences. Moreover, per cent library coverage of the two latter studies revealed that clone numbers were under-sampled so that these cyanobacterial OTUs are probably underestimated.

The factors influencing the occurrence of cyanobacteria include often in combination, soil pH, undeveloped and unstable soil substrate, moisture availability, light intensity and UV radiation (Castenholz and Garcia-Pichel 2000, Rehakova et al. 2011). These key environmental parameters may contribute to the differences in the species distribution and/or colonization we found along the soil chronosequence. Soil physicochemical differences may be the major driving force for the patterns of distribution of cyanobacterial phylotypes we observed in the three soil environments. Soil development involves the accumulation of organic matter and nitrogen and a decrease in pH with increasing time since deglaciation (Bernasconi et al. 2011, Zumsteg et al. 2012). Barren soils are poorly developed, with very low organic matter and nutrient content and are characterized by fine granitic sediments and rocks (Frey et al. 2010, Brunner et al. 2011). In the barren soils in high-altitude environments, in particular, cyanobacteria have to cope with large temperature fluctuations, repeated freeze-thaw cycles, low soil moisture and high UV radiation (Borin et al. 2010). Many cyanobacteria tolerate high levels of UV radiation (Castenholz and Garcia-Pichel 2000) and produce a wide range of UV protectants, e.g. scytomenin, carotenoids, or mycosporine-like amino acids. The tolerance of intense sunlight including UV radiation may have contributed to their success in colonizing high-altitude environments, such as the Damma glacier forefield. The cyanobacterial community in the barren soils of recently deglaciated soils in the Alps could, we suggest, be described as pioneers and in many cases organisms in this kind of environment are generalists (Frey et al. 2010, Lapanje et al. 2011).

However, not only the differences in soil physicochemical parameters may affect the microbial communities but also the photoautotrophic activity itself. The amounts of energy, carbon and nitrogen inputs into the system by photoautotrophic activity in early successional stages is obvious, therefore permitting the growth of a broader suite of microorganisms. In fact, we found that carbon availability is a key factor regulating microbial diversity in recently deglaciated bare soils (Zumsteg et al. 2013). $^{13}$C-labelled algal biomass was more efficiently utilized by the natural microbial community than fungal biomass indicating the important role of pioneering organic carbon inputs in these carbon-limited environments.

More than half of the cyanobacterial 16S rRNA gene sequences retrieved from barren soils exist exclusively in this soil environment. All cloned sequences obtained were related to cyanobacterial sequences obtained from cold habitats in the Arctic, Antarctic and Himalayas on river, lakes or rocks (Taton et al. 2003, Strunecky et al. 2010, Wong et al. 2010, Jungblut et al. 2010, Khan et al. 2011, Schmidt et al. 2011). It is often assumed that psychrophilic specialists occur only in cold environments and that they are outcompeted under other conditions (Casamatta et al. 2005, Jungblut et al. 2010). The barren soils were dominated by phylotypes affiliated within the Oscillatoriiales (filamentous cyanobacteria without heterocysts). These organisms do not require a stable substrate with a fine texture and high organic matter content (Kastovska et al. 2005, Khan et al. 2011). The majority of the oscillatorian sequences we retrieved from barren soils were most closely related to the Microcoleus sp. and Phormidium sp. Both organisms have evolved remarkable strategies to cope with water-related stress (Lange et al. 1994, Bellnap and Lange 2001) forming filaments surrounded by extracellular polymeric substances. These ‘sticky’ filaments may help to retain water during dry periods and provide an adhesive surface on rocks for bacteria attachment (Matalonii et al. 2000, Bellnap and Lange 2001). It is thus likely that these filamentous cyanobacteria are essential for the stabilization of the substrate in recently deglaciated areas, which then the substrate enables further to be colonized by more demanding organisms such as mosses and higher plants.

In contrast, cyanobacterial diversity was lowest in transient soils. These heterogeneous and sparsely vegetated soils were dominated by the Nostocales phylotypes, a group of filamentous cyanobacteria that form specialized cells (heterocysts) where nitrogen fixation is localized (Doods 1995). Hence, the presence of Nostoc phylotypes indicates diazotrophic potential in the community in addition to photoautotrophy. Interestingly, cyanobacterial 16S rRNA gene clone sequences that could be related to the genus Nostoc sp. exist exclusively in transient soils. Nostoc sp. are known to live in symbiotic communities in loose or tight association with plant roots (Gantar et al. 1991) or lichens (Kaasalainen et al. 2009), which make them less vulnerable to harsh environmental influences. Previous studies in the Swiss Alps indicate that cyanobacterial NifH sequences affiliated
to Nostoc sp. are well represented in the Damma glacier forefield (Duc et al. 2009), and have also been found in the Piora dolomite (Sigler et al. 2003). Interestingly, the oscillatory phototypes Microcoleus sp. and Phormidium sp. seem to be absent or nearly absent if photophors in the order Nostocales dominate. A similar pattern has also been observed by Rehakova et al. (2011) in Himalayan soils.

The change in cyanobacterial diversity from the initial to the developed soils could be explained by the spread of the vegetation cover and increase in organic matter. We found that the developed soils contain a diverse suite of cyanobacterial genes, including sequences from soil, rhizosphere, freshwater, Alpine and endolithic habitats (Heath et al. 2010, Jungblut et al. 2010, Wong et al. 2010, Schmidt et al. 2011). Densely vegetated soils had a finer texture, and proportionally more higher organic matter, water contents and nutrient concentrations compared with barren soils. However, a dense vegetation cover may result in less light being available for phototrophic microbes, and competition with vascular plants and root-associated microorganisms for nutrients. We suggest therefore that transient and developed soils appear to be quite heterogeneous in view to the ecological niches.

4.2. Green algal communities

The phylogenetic diversity of green algae (chlorophytes) was found to be relatively restricted along the chronosequence in the Damma glacier forefield. Eukaryotic microalgae in glacier forefields have been little investigated, which makes comparison with similar habitats difficult. Moreover, the ITS sequence is highly variable and much less information is available in publicly accessible databases about eukaryotic microalgae in general than about prokaryotes. The libraries were dominated by well-supported clades in the Chlorophyceae, Trebouxiophyceae and the Ulvophyceae. Chlorophytes are usually found in glacial ecosystems (Mataloni et al. 2000, Säwström et al. 2002, Kastovska et al. 2005, Stibal et al. 2006), cold dry valleys in the Antarctic (Schmidt et al. 2011) and in permafrost soil (Vishnivetskaya 2009), but they are also common in lithic niches (Wong et al. 2010, Khan et al. 2011).

The majority of the green algal phototypes we found, in particular in transient and developed soils, were related to the Trebouxiophyceae with closely related cultures to those of the genera Trebouxia sp. and Asterochloris sp., which are known to live as symbiotic partners in lichen. The most common photobiont genera, Trebouxia and Asterochloris, are present in approximately 20% of all lichen species (Bákor et al. 2010, Škaloud and Peksa 2010, Peksa and Škaloud 2011). Endolithic-lichenised communities typically comprise the chlorophyte Trebouxia sp. (Wong et al. 2010). As lichen symbionts, green algae can contribute substantially to carbon production and initial soil formation, as Freeman et al. (2009) also suggest. Trebouxia (including Asterochloris) photobionts may be free living (Ahmadjian 1987). These algae may belong to first settlers of newly developed habitats (Mukhtar et al. 1994). Stichococcus phototypes (also a member of the Trebouxiophyceae) have been mainly recovered from developed soils, but have also been found in an Alpine endolithon, whereas this green alga is most frequently found growing in soil and in freshwater habitats (Sigler et al. 2003). Stichococcus sp. shares a familial relationship with Trebouxia. They are predominantly free-living algal species, but it is possible that our Stichococcus-like phototypes may also be lichen photobionts. Green algae may have ecologically and physiologically adapted to withstand a harsh environment, such as barren soils. An unsuitable light or climatic regime may reduce the fitness of the photobiont, leading to its very low abundance or even absence in certain habitats. A number of Asterochloris clades were markedly tolerant to various climatic conditions and substrates (Peksa and Škaloud 2011).

One group of cloned sequences retrieved from the initial and transient soils we studied belong to the Ulvophyceae. Their closest culturable relatives are Pseudodolichonium basiliense and Planophila sp. Members of this rarely studied clade within the Ulvophyceae have also been detected in barren, rocky valleys in dry areas of the Himalayas (Schmidt et al. 2011), but their function in soil is completely unknown. These organisms seem to be capable of surviving in barren or sparsely vegetated soils. Another group of cloned sequences, obtained from the developed soils belong to the Chlorophyceae. Their closest culturable relatives are Mucidosphaerium sphagnale sp., also known as Dictyosphaerium sphagnale (Bock et al. 2011). Members of Dictyosphaerium are present worldwide mainly in freshwater habitats (Bock et al. 2011). Similarly, the green algae most closely related to Oedogonium sp. (DQ413055) are filamentous and grow in freshwater worldwide, usually attached to plants or algae (Mei et al. 2007). Possibly our sampling sites, in particular the transient and developed soils, are regularly temporarily flooded by meltwater streams in summer, which could explain the presence of these organisms.

5. Conclusions

We have shown that a diverse community of Cyanobacteria and eukaryotic members of the Chlorophyta are present in the Damma glacier forefield. The phototrophic community composition appeared to shift markedly along the chronosequence, indicating that each environment (soil age) selects different communities. The majority of the sequences retrieved from barren soils were most similar to sequences found in streams, lakes and rocks of cold habitats. In contrast, densely vegetated soils contained a diverse suite of photoautotrophs, including sequences from soil, rhizosphere, freshwater, Alpine and endolithic habitats. The change in phototropic diversity with distance from the glacier can be explained by the increase in organic matter and the development of vegetation cover, resulting in less light being available for phototrophic microbes. Cyanobacteria, together with eukaryotic microalgae, are significant drivers of organic matter input and could thus play an important role in the soil development as they contribute carbon to nutrient-deficient deglaciated barren soils. Furthermore, the occurrence of Nostoc phototypes also indicates that the community has diazotrophic potential in addition to photoautotrophy.
Acknowledgments

Financial support for this study was partly provided by the ‘Biosphere–geosphere interactions: Linking climate change, weathering, soil formation and ecosystem evolution (BigLink)’ project of the Competence Centre Environment and Sustainability (CCES) of the ETH Domain. This work was also partly financed by the Swiss National Science Foundation (project 31003A-138321). We thank the BigLink consortium, in particular Stefano Bernasconi (ETH), for scientific support, and Daniela Steiner (WSL) for valuable technical support in the laboratory. We also thank Ursula Graf and Alessandro Schlumpf of the WSL Central Laboratory for chemical measurements. We are grateful to the linguistic lecturer Silvia Dingwall for improving the English text.

References

Ahmadjian V 1987 The lichen alga Trebouxia: does it occur free-living? Prog. Syst. Evol. 158 243–7
Anesio A M, Hodson A J, Fritz A, Psenner R and Sattler B 2009 High microbial activity on glaciers: importance to the global carbon cycle Glob. Change Biol. 15 955–60
Bakor M, Peksa O, Skaloud P and Bacikova M 2010 Photobiont diversity in lichens from metal-rich substrata based on ITS rDNA sequences Ecotaxotol. Environ. Saf. 73 603–12
Bardgett R D et al 2007 Heterotrophic microbial communities use ancient carbon following glacial retreat Biol. Lett. 3 487–90
Bauer H, Kasper-Giebl A, Loflund M, Giebl H, Hitenberger R, et al
Bardgett R D
Bashay M, Peksa O, Skaloud P and Bacikova M 2010 Photobiont diversity in lichens from metal-rich substrata based on ITS rDNA sequences Ecotaxotol. Environ. Saf. 73 603–12
Belnap J and Lange O L 2001 Biological Soil Crusts: Structure, Function, and Management (Berlin: Springer)
Berntasconi S M et al 2011 Chemical and biological gradients along the Damma glacier soil chronosequence, Switzerland Vadose Zone J. 10 867–83
Bock C, Krienitz L and Pröschold K 2011 Taxonomic reassessment of the genus Chlorella (Trebouxioideae) using molecular signatures (barcodes), including description of seven new species Fottea 11 293–312
Borin S et al 2010 Rock weathering creates oasis of life in a high Arctic desert Environ. Microbiol. 12 293–303
Brummer I, Plotze M, Riedner S, Zumsteg A, Furrer G and Frey B 2009 Pioneering fungi from the Damma glacier forefield in the Swiss Alps can promote granite weathering Geobiology 9 266–79
Casamatta D A, Johansen J R, Vis M L and Broadwater S T 2005 Molecular and morphological characterization of ten polar and near-polar strains within the Oscillatoriales (Cyanobacteria) J. Phycol. 41 421–38
Castenholz R W and Garcia-Pichel F 2000 Cyanobacterial responses to UN-radiation Ecology of Cyanobacteria: Their Diversity in Time and Space ed B A Whitton and M Potts (Dordrecht: Kluwer) pp 591–611
Chong C W, Convey P, Pearce D A and Tan I K P 2012 Assessment of soil bacterial communities on Alexander Island (in the maritime and continental Antarctic transitional zone) Polar Biol. 35 387–99
Doods W 1995 The ecology of nostoc J. Phycol. 33 2–18
Duc L, Noll M, Meier B E, Bürgmann H and Zeyer J 2009 High diversity of diazotrophs in the forefield of a receding alpine glacier Microb. Ecol. 57 179–90
Freeman K R, Pescador M Y, Reed S C, Costello E K, Robeson M S and Schmidt S K 2009 Soil CO2 flux and phototrophic community composition in high elevation, ‘barren’ soils Environ. Microbiol. 11 674–86
Frey B, Niklaus P A, Kremer J, Lüscher P and Zimmermann S 2011 Heavy-machinery traffic impacts methane emissions as well as methanogen abundance and community structure in oxic forest soils Appl. Environ. Microbiol. 77 5060–8
Frey B, Pesaro M, Rüdiger A and Widmer F 2008 Dynamics of bacterial communities in bulk and poplar rhizosphere soil contaminated with heavy-metals Environ. Microbiol. 10 1433–49
Frey B, Rieder S, Brunner I, Plotze M, Koetzsch S, Lapanje A, Brandl H and Furrer G 2010 Weathering-associated bacteria from the Damma glacier forefield: physiological capabilities and impact on granite dissolution Appl. Environ. Microbiol. 76 4768–96
Gantar M, Kerby N W, Obereh Z and Rowell P 1991 Colonization of wheat (Triticum vulgare L.). I. A survey of soil cyanobacterial isolates forming associations with roots New Phytol. 118 477–83
Göransson H, Venterink H O and Baath E 2011 Soil bacterial growth and nutrient limitation along a chronosequence from a glacier forefield Soil Biol. Biochem. 43 1333–40
Häberli W, Hoelzle M, Paul F and Zemp M 2007 Integrated monitoring of mountain glaciers as key indicators of global climate change: the European Alps Ann. Glaciol. 46 150–60
Heath M W, Wood S A and Ryan K G 2010 Polyphasic assessment of fresh-water benthic mat-forming cyanobacteria isolated from New Zealand FEMS Microbiol. Ecol. 73 95–109
Hodkinson I D, Webb N R and Coulsen S J 2002 Primary community assembly on land—the missing stages: why are the heterotrophic organisms always there first? J. Ecol. 90 569–77
Hodson A et al 2008 Glacial ecosystems Ecol. Monogr. 78 41–67
Huelsenbeck J P and Ronquist F 2001 MrBayes: Bayesian inference of phylogeny Biometrics 17 754–5
Jumpponen A 2003 Soil fungal community assembly in a primary successional glacier forefield ecosystem as inferred from rDNA sequence analyses New Phytol. 158 569–78
Jungblut A D, Lovejoy C and Vincent W F 2010 Global distribution of cyanobacterial ecotypes in the cold biosphere ISME J. 4 191–202
Kaasalainen U, Jokela J, Fewer D P, Sivonen K and Rikkinen J 2009 Microcrystin production in the tripartite cyanobacterial Peltigera leucophlebia Mol. Plant-Microbe Interact. 22 695–702
Kastovsky K, Elster J, Stibal M and Santruckova H 2005 Microbial assemblages in soil microbial succession after glacial retreat in Svalbard (high Arctic) Microb. Ecol. 50 396–407
Khan N, Tuffin M, Stafford W, Cary C, Lacap D C, Pointing S B and Cowan D 2011 Hypolithic microbial communities of quartz rocks from Miers Valley, McMurdo Dry Valleys, Antarctica Polar Biol. 34 1657–68
Lange O L, Meyer A and Bubel B 1994 Net photosynthesis activation of a desiccated cyanobacterium without liquid water in high air humidity alone. Experiments with Microcoleus sociatus isolated from a desert soil crust Funct. Ecol. 8 52–7
Lapanje A, Wimmensberger C, Furrer G, Brunner I and Frey B 2011 Pattern of elemental release during the granite dissolution can be changed by aerobic heterotrophic bacterial strains isolated from Damma glacier (central Alps) deglaciated granite Microb. Ecol. 63 865–82
Mataloni G, Tell G and Wynn-Williams D D 2000 Structure and diversity of soil algal communities from Cierva Point (Antarctic Peninsula) Polar Biol. 23 205–11
Mindb B, Anesio A M, Meier K, Hodson A J, Laybourn-Parry J, Sommaruga R and Sattler B 2007 Factors influencing bacterial dynamics along a transect from supraglacial runoff to proglacial lakes of a high Arctic glacier FEMS Microbiol. Ecol. 59 307–17
Mukhtar A, Garty J and Galun M 1994 Does the lichen alga Trebouxia occur free-living in nature—further immunological evidence Symbiosis 17 247–53
Mei H, Luo W, Liu G X and Hu Z Y 2007 Phylogeny of Oedogoniaceae (Chlorophyceae, Chlorophyta) inferred from 18S rDNA sequences with emphasis on the relationships in the genus Oedogonium based on ITS-2 sequences Plant Syst. Evol. 265 179–91

Nemergut D R, Anderson S P, Cleveland C C, Martin A P, Miller A E, Seimon A and Schmidt S K 2007 Microbial community succession in an unvegetated, recently deglaciated soil Microb. Ecol. 53 110–22

Nübel U, Garcia-Pichel F and Muyzer G 1997 PCR primers to amplify 16S rRNA genes from cyanobacteria Appl. Environ. Microbiol. 63 3327–32

Oerlemans J 2005 Extracting a climate signal from 169 glacier records Science 308 675–7

Omelon C R, Pollard W H and Ferris F G 2006 Environmental controls on microbial colonization of high Arctic cryptoendolithic habitats Polar Biol. 30 19–29

Peksa O and Škaloud P 2011 Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga Asteroclorhis (Trebouxiophyceae) Mol. Ecol. 20 3936–48

Piercey-Normore M D and DePriest P T 2001 Algal switching among lichen symbioses Am. J. Bot. 88 1490–8

Rambaut A 2008 FigTree v1.1.2 (6 February 2008, http://tree.bio.ed.ac.uk/software/FigTree)

Rehakova K, Chlumska Z and Dolezal J 2011 Soil cyanobacterial and microalgal diversity in dry mountains of Ladakh, NW Himalaya, as related to site, altitude, and vegetation Microbiol. Ecol. 62 337–46

Sattin S R et al 2009 Functional shifts in unvegetated, perennial, recently-deglaciated soils do not correlate with shifts in soil bacterial community composition J. Microbiol. 47 675–81

Säwström C, Mumford P, Marshall W, Hodson A and Laybourn-Parry J 2002 The microbial communities and primary productivity of cymoconite holes in an Arctic glacier (Svalbard 79°N) Polar Biol. 25 591–6

Schloss P D et al 2009 Introducing Mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities Appl. Environ. Microbiol. 75 7537–41

Schmidt S K, Lynch R C, King A J, Karki D, Robeson M S, Nagy L, Williams M W, Mitter M S and Freeman K R 2011 Phylogeography of microbial phototrophs in the dry valleys of the high Himalayas and Antarctica Proc. R. Soc. B 278 702–8

Schmidt S K et al 2008 The earliest stages of ecosystem succession in high-elevation (5000 metres above sea level), recently deglaciated soils Proc. R. Soc. B 275 2793–802

Segawa T and Takeuchi N 2010 Cyanobacterial communities on Qiyi glacier, Qilian Shan China Ann. Glaciol. 51 153–62

Sigler W V, Bachofen R and Zeyer J 2003 Molecular characterization of endolithic cyanobacteria inhabiting exposed dolomite in central Switzerland Environ. Microbiol. 5 618–27

Skaloud P and Peksa O 2010 Evolutionary inferences based on ITS rDNA and actin sequences reveal extensive diversity of the common lichen alga Asteroclorhis (Trebouxiophyceae, Chlorophyta) Mol. Phylogenet. Evol. 54 36–46

Stibal M, Sabacka M and Kastovska K 2006 Microbial communities on glacier surfaces in Svalbard: impact of physical and chemical properties on abundance and structure of cyanobacteria and algae Microb. Ecol. 52 644–54

Stibal M, Trantar M, Benning L G and Rehak J 2008 Microbial primary production on an Arctic glacier is insignificant in comparison with allochthonous organic carbon input Environ. Microbiol. 10 2172–8

Streunecky O, Elster J and Komarek J 2010 Phylogenetic relationships between geographically separate Phormidium cyanobacteria: is there a link between north and south polar regions? Polar Biol. 33 1419–28

Taton A, Grubisic S, Brambilla E, de Wit R and Wilmotte A 2003 Cyanobacterial diversity in natural and artificial microbial mats of Lake Fryxell (McMurdo Dry Valleys, Antarctica): a morphological and molecular approach Appl. Environ. Microbiol. 69 5157–69

Vishnivetskaya T A 2009 Viable cyanobacteria and green algae from permafrost darkness Permafrost Soils ed R Margesin (Berlin: Springer) chapter 6, pp 73–84

Wang Q, Garrity G M, Tiedje J M and Cole J R 2007 Naive Bayesian classifier for rapid assessment of rRNA sequences into the new bacterial taxonomy Appl. Environ. Microbiol. 73 5261–7

White T J, Burns T, Lee S and Taylor J 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics PCR Protocols, A Guide to Methods and Applications ed D H Gelfand, J J Sninsky, T J White and A Innis (San Diego, CA: Academic) p 315

Wong F K Y, MacRae C, Lau M C Y, Aitchison J C, Wood S A, Rueckert A, Cowan D A and Pointing S B 2010 Hypolithic microbial community of quartz pavement in the high-altitude tundra of central Tibet Microb. Ecol. 60 730–9

Wood S A, Rueckert A, Cowan D A and Cary S C 2008 Sources of edaphic cyanobacterial diversity in the Dry Valleys of Eastern Antarctica ISME J. 2 308–20

Xiang S-R, Shang T-C, Chen Y, Jing Z-F and Yao T 2009 Dominant bacteria and biomass in the Kuytun 51 Glacier Appl. Environ. Microbiol. 75 7287–90

Yoshitake S, Uchida M, Koizumi H and Nakatsubo T 2007 Carbon and nitrogen limitation of soil microbial respiration in high arctic successional glacier foreland near Ny-Alesund, Svalbard Polar Res. 26 22–30

Zumsteg A, Luster J, Brunner I, Görransson H, Luster J, Smittenberg R, Zeyer J and Frey B 2012 Bacterial, archaeal and fungal succession in the forefield of a receding glacier Microb. Ecol. 63 552–64

Zumsteg A, Schmutz S and Frey B 2013 Identification of biomass utilizing bacteria in a carbon-depleted glacier forefield soils by the use of 13C DNA stable isotope probing Environ. Microbiol. Rep. at press (doi:10.1111/1758-2229.12027)