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

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Received 5 December 2012
Accepted for publication 20 February 2013
Published 12 March 2013
Online at stacks.iop.org/ERL/8/015033

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 Oscillatoriales. The diversity in sparsely vegetated soils was low, and sequences closely related to Nostoc sp. dominated. The majority of the algal phylotypes are related to members of the 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 et al 2007). The majority of glaciers in the European Alps have receded quickly at a mean rate of 8–11 m yr⁻¹ (estimates derived from data in 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 et al 2002, Nemergut et al 2007, Frey et al 2010, Lapanje et al 2011), fungi (Jumpponen 2003, Bruner et al 2011, Zumsteg et al 2012) and eukaryotic microalgae (Mataloni et al 2000,
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. 2009, 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'E08°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 N₀₅ and C₀₅. 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 rDNA gene sequences, 0.2 μM of each cyanobacteria-specific primer CYA359F (GAA TYT TCC GCT 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’ (5’-CTG CGG AAG GAT CAT TGA TTC-3’; Piercey-Normore and DePristo 2001) and a universal primer, ITS4-3’ (5’-TCC GCC TGT 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. Twenty-five ng of DNA was added as a template for all cyanobacterial and algal-specific PCR reactions. All PCR reaction steps were verified by gel electrophoresis on 1% agarose gels, stained with ethidium bromide, illuminated with trans-UV light.

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 HaeIII 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 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 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 TGG) 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, Quanti Tec 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
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 (cyanobacteria) 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 10⁻⁴ 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 forest edge 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 to 15.2 μg g⁻¹ dry soil in the developed soils. The total bacterial abundance increased from 6.8 × 10⁶ gene copies in barren soils to 1.1 × 10⁹ 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 × 10⁷ 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 LIBSUFF 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>
<thead>
<tr>
<th>Location</th>
<th>DNA content (μg g⁻¹ soil)</th>
<th>Cyanobacterial 16S rRNA gene copies (g⁻¹ soil)</th>
<th>Bacterial 16S rRNA gene copies (g⁻¹ soil)</th>
<th>Percentage cyanobacteria/total bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial soils</td>
<td>1.5 ± 0.6</td>
<td>4.7 × 10⁵ ± 2.4 × 10⁵</td>
<td>6.8 × 10⁶ ± 2.0 × 10⁶</td>
<td>6.9</td>
</tr>
<tr>
<td>Transient soils</td>
<td>8.4 ± 1.9</td>
<td>2.3 × 10⁷ ± 1.4 × 10⁷</td>
<td>2.8 × 10⁹ ± 6.1 × 10⁹</td>
<td>8.2</td>
</tr>
<tr>
<td>Developed soils</td>
<td>15.2 ± 2.3</td>
<td>1.5 × 10⁸ ± 8.3 × 10⁷</td>
<td>1.1 × 10⁹ ± 3.5 × 10⁹</td>
<td>1.4</td>
</tr>
</tbody>
</table>
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 CYAE8 clustered within the genus GpIV and clone CYBC8 within the genus GpI following the RDP classifier (Wang et al. 2007). Supports for key nodes are shown as posterior probabilities. The Clostridium perfringens (Y12669) sequence outgroup roots the tree. Scale bar represents substitutions per site.
phylotypes, clones CYAA1, CYAA3, CYAD3 and CYAD10, closely related to cyanobacterial sequences obtained from glaciers and glacial surface snow (Jungblut et al. 2010, Strunecky et al. 2010). Closest cultured relatives of these four cloned sequences were Arthronema gyaxiana UTCC 393 strain (AF218370) and Phormidium cf. murrayi CCALA 843. The closest related relative (99%) of clone CYAB4 was Microcoleus rushforthii UTCC 296 (AF218377). Clone CYAA5 was most similar (98%) to an Antarctic clone (AY151747) and a strain of Geitlerinema sp. (EU083410) from receding glaciers habitats (figure 1). Closest cultured relatives of Chamaesiphon subglobosus PCC9222 (AF317510). The closest relatives (99%) of the cloned sequence of CYAA12 were a cyanobacterial strain Chamaesiphon subglobosus PCC7430 (AY170472) from the order of the Chroococcales. Several cloned 16S rRNA gene sequences (e.g. CYAA4, CYAB7 and CYAC8) in the barren soils belong to uncharacterized Cyanobacteria or to the genus GpIV (clone CYAE8) following the RDP classifier (Wang et al. 2007) and was most closely related to an environmental clone (GQ397093) retrieved from a recently deglaciated soil (Sattin et al. 2009).

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 Cyanotheca aeruginosa strain NIVA-CYA 258/2 and clone CYBD1 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 Oscillatoriaceae 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 unclassified 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 class Chlorococcales, Trebouxiophyceae or Ulvophyceae. 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 Chlorophyceae most closely related to genus Oedogonium sp. (DQ413055) and to Ulvophyceae closely similar (clones ALAC8, ALAC11, ALB9, ALBC4) to the genera Planophila sp. (AJ416102) and Pseudendoclonium sp. (Z47996). Three phylotypes (clone ALAA1, ALBA9, ALBC8) were affiliated to Trebouxiophyceae (figure 2). In contrast, clone sequences retrieved from developed soils were predominantly found within the class Trebouxiophyceae, and were most closely related to the genera Asterochloris, Stichococcus and Trebouxia (figure 2). These algae have been

### Table 3. Results of sequence analysis of cyanobacteria from the barren (initial) soils, 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.)

<table>
<thead>
<tr>
<th>Location</th>
<th>Total clones</th>
<th>Clone OTUs</th>
<th>Number of clones sequenced</th>
<th>Total number of sequences</th>
<th>Sequence OTUs</th>
<th>Coverage (%)</th>
<th>H</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial soils</td>
<td>96</td>
<td>8</td>
<td>33</td>
<td>28</td>
<td>11</td>
<td>91</td>
<td>2.9</td>
<td>0.95</td>
</tr>
<tr>
<td>Transient soils</td>
<td>96</td>
<td>9</td>
<td>38</td>
<td>36</td>
<td>5</td>
<td>97</td>
<td>2.2</td>
<td>0.94</td>
</tr>
<tr>
<td>Developed soils</td>
<td>96</td>
<td>12</td>
<td>55</td>
<td>54</td>
<td>10</td>
<td>88</td>
<td>2.8</td>
<td>0.91</td>
</tr>
</tbody>
</table>

### Table 4. Results of sequence analysis of green algae from the barren (initial) soils, 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.)

<table>
<thead>
<tr>
<th>Location</th>
<th>Total clones</th>
<th>Clone OTUs</th>
<th>Number of clones sequenced</th>
<th>Total number of sequences</th>
<th>Sequence OTUs</th>
<th>Coverage (%)</th>
<th>H</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial soils</td>
<td>49</td>
<td>4</td>
<td>16</td>
<td>12</td>
<td>8</td>
<td>88</td>
<td>1.7</td>
<td>0.87</td>
</tr>
<tr>
<td>Transient soils</td>
<td>70</td>
<td>3</td>
<td>16</td>
<td>13</td>
<td>9</td>
<td>90</td>
<td>1.8</td>
<td>0.88</td>
</tr>
<tr>
<td>Developed soils</td>
<td>96</td>
<td>4</td>
<td>46</td>
<td>44</td>
<td>16</td>
<td>92</td>
<td>2.3</td>
<td>0.87</td>
</tr>
</tbody>
</table>
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 foreelds, 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 Oscillatoriales, Chroococcales and Nostocales, 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. scytonemins, 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). 13C-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 Oscillatoriales (filamentous cyanobacteria without heterocytes). 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, Belnap 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 (Mataloni et al. 2000, Belnap 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 Flora dolomite (Sigler et al. 2003). Interestingly, the oscillatory phylotypes Microcoleus sp. and Phormidium sp. seem to be absent or nearly absent if phototrophs 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 phylotypes 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 phylotypes (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 phylotypes 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 Pseudendoclonium 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 phototropic 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 phototrophic 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 phylotypes 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.

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