FEMS Microbiology Ecology, 92, 2016, fiw038

doi: 10.1093/femsec/fiw038
Advance Access Publication Date: 21 February 2016
Research Article

RESEARCH ARTICLE

The effect of temperature change on the microbial diversity and community structure along the chronosequence of the sub-arctic glacier forefield of Styggedalsbreen (Norway)

Alejandro Mateos-Rivera[1,2,*], Jacob C. Yde[2], Bryan Wilson[1], Kai W. Finster[3,4], Laila J. Reigstad[5] and Lise Øvreås[1]

[1]Department of Biology, University of Bergen, NO-5020, Bergen, Norway, [2]Faculty of Engineering and Science, Sogn og Fjordane University College, NO-6851, Sogndal, Norway, [3]Department of Bioscience, Aarhus University, DK-8000, Aarhus, Denmark, [4]Stellar Astrophysics Centre, Department of Physics and Astronomy, Aarhus University, DK-8000, Aarhus, Denmark and [5]Department of Earth Science, University of Bergen, NO-5020, Bergen, Norway

*Corresponding author: Avdeling for ingeniør og naturfag, Høgskulen i Sogn og Fjordane, Røyrgata 6, NO - 6851, Sogndal, Norway. Tel: +47-951-681-49; E-mail: alejandro.mateos.rivera@hisf.no

One sentence summary: Samples collected along a chronosequence at the glacier forefield of Styggedalsbreen (Norway) reveal changes in the microbial diversity and community structure at different stages of soil development and temperature changes.

Editor: Rosa Margesin

ABSTRACT

Microbial communities in the glacier forefield of Styggedalsbreen, Norway, were investigated along a chronosequence from newly exposed soil to vegetated soils using next-generation sequencing of the 16S rRNA gene. In order to monitor the short-term effect of temperature on community successions along the soil gradient, the soil samples were incubated at three different temperatures (5°C, 10°C and 22°C). The microbial community composition along the chronosequence differed according to distance from the glacial terminus and incubation temperature. Samples close to the glacier terminus were dominated by *Proteobacteria* at 5°C and 10°C, while at 22°C members of *Chloroflexi*, *Acidobacteria* and *Verrucomicrobia* in addition to *Proteobacteria* accounted for most of the diversity, indicating that sites close to the glacier terminus are more closely related to former subglacial environments. Within the *Archaea* domain, members of the phylum *Euryarchaeota* dominated in samples closer to the glacier terminus with a shift to members of the phyla *Thaumarchaeota—Crenarchaeota* with increased soil age. Our data indicate that composition and diversity of the microbial communities along the glacier forefield depend not only on exposure time but are also to a large degree influenced by soil surface temperature and soil maturation.

Keywords: Styggedalsbreen; chronosequence; glacier forefield; microbial succession; next-generation sequencing; 16S rRNA gene

Received: 30 October 2015; **Accepted:** 18 February 2016

INTRODUCTION

The global temperature change has proven to be most pronounced in the Arctic region with an average winter temperature increase of 4°C in the last 50 years. Temperatures in the Arctic areas have increased approximately twice as fast as in temperate regions during the last century (Bernstein *et al.* 2007; Schütte *et al.* 2010; Mernild *et al.* 2014), and model calculations predict a rise in temperature of 6°C during the 21st century (ACIA 2004). This increase in temperature has dramatic consequences for Arctic ecosystems, such as the loss in area and volume of Arctic glaciers (Bárcena, Finster and Yde 2011; Zumsteg *et al.* 2013a; Bradley, Singarayer and Anesio 2014), a trend that will continue into the future (IPCC 2013).

As glaciers retreat, new bare soils appear, and as these gradually become exposed they represent ideal environments to study processes such as: (i) primary bacterial colonization of bare soils (Nicol *et al.* 2005; Zumsteg *et al.* 2012; Bajerski and Wagner 2013; Bradley, Singarayer and Anesio 2014); (ii) the effect of changes in environmental conditions along gradients such as the transition from subglacial anoxic to proglacial oxic conditions, the transition from restricted to increased nutrient availability, the effect of UV radiation, the contribution of atmospheric deposition of organic and inorganic compounds and changes in soil temperature (Schmidt *et al.* 2008; Schütte *et al.* 2010; Bárcena, Finster and Yde 2011); and (iii) the biotic–abiotic evolution of the exposed glacier forefield as a function of exposure time (Bernasconi *et al.* 2011). Therefore, glacier forefields provide excellent environments to study the effect of deglaciation on micro- and macrobiotal succession in polar and alpine regions.

There are approximately 200 000 land-based glaciers on Earth that all have distinct forefields (Pfeffer *et al.* 2014). This huge variety allows researchers to study the microbial communities along differing soil chronosequences and to determine similarities and differences between these forefields by identifying, for example, the patterns of microbial colonization and the drivers behind them (Rime *et al.* 2015). An understanding of the role of microbes in the succession and evolution of glacier forefields is necessary for several reasons; microorganisms are pioneer colonizers of proglacial areas (Hodkinson, Coulson and Webb 2003; Bradley, Singarayer and Anesio 2014), and are therefore important in primary succession processes in barren soils (Bardgett *et al.* 2007); in addition, microorganisms are the main drivers in pedogenesis and key players in biogeochemical cycling providing the foundation for plant growth (Lazzaro, Abegg and Zeyer 2009; Schütte *et al.* 2010). Most glacier forefield studies cover the period encompassing the last 250 years; this is when the Little Ice Age (LIA) ended and the glaciers were at their maximum expansion (Bernasconi *et al.* 2011; Rime *et al.* 2015). At present, these studies have focused primarily on glaciers in Greenland (Bárcena, Yde and Finster 2010; Bárcena, Finster and Yde 2011), Svalbard (Kastovska *et al.* 2005, 2007), Austria (Tscherko *et al.* 2003; Bardgett *et al.* 2007) and Switzerland (Bernasconi *et al.* 2011; Zumsteg *et al.* 2012; Frey *et al.* 2013; Guelland *et al.* 2013; Rime *et al.* 2015), with a particular emphasis on abiotic factors (Tscherko *et al.* 2003; Bardgett *et al.* 2007; Bárcena, Finster and Yde 2011; Bernasconi *et al.* 2011; Frey *et al.* 2013) and on the development of soils (Guelland *et al.* 2013). The features of the microbial communities along these chronosequences have been analyzed using a number of different molecular fingerprinting techniques such as DGGE (Denaturing Gradient Gel Electrophoresis) (Bárcena, Yde and Finster 2010), T-RFLP (Zumsteg *et al.* 2012) and 454-pyrosequencing (Rime *et al.* 2015). The predominant focus of these studies was the bacterial diversity, although in the case of Zumsteg *et al.* (2012) fungal, archaeal and bacterial diversity.

There are certain properties of glacial soils that have been analyzed. Studies have shown that the carbon and nitrogen content increase with soil age from 0.1 to 40 mg g^{-1}, and 0.1 to 2 mg g^{-1}, respectively (Tscherko *et al.* 2003; Bardgett *et al.* 2007; Lazzaro, Abegg and Zeyer 2009; Bradley, Singarayer and Anesio 2014) and soil pH has been found to become more acidic as soil age increases (Tscherko *et al.* 2003; Bajerski and Wagner 2013). A number of studies have analyzed the microbial communities present along chronosequences in glacier forefields. Zumsteg *et al.* (2012) found changes in fungal communities from *Ascomycota* to *Basidiomycota*, and from *Euryarchaeota* to *Crenarchaeota* in archaeal communities; the overall presence and variability in contribution to the total community of certain bacterial phyla including *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, *Cyanobacteria*, *Gemmatimonadetes*, *Proteobacteria* and *Verrucomicrobia* have also been observed (Nemergut *et al.* 2007; Bajerski and Wagner 2013; Zumsteg *et al.* 2013a).

Here we present the first study of microbial samples obtained from a forefield in front of the glacier Styggedalsbreen (central Norway), focusing on the putative short term effects of increasing temperature, using massive sequencing to detect changes in the whole community.

Samples were incubated at three different temperatures (5°C, 10°C and 22°C) for one week to detect short-term population changes in the whole community. Incubation temperatures were chosen according to seasonal variation at the field site. During winter, temperatures at Styggedalsbreen are below 0°C but in early and later summer, soil temperatures may be up to 10°C within a 100 m distance of the glacier snout (Haugland 2004). In this study, we also wanted to know how a dramatic increase in soil temperatures may affect the microbial community structure. Glacier forefields are ideal environments for psychrophilic and psychrotolerant microorganisms that can live at low temperatures. In selecting for 5°C, 10°C and 22°C, we hope to better distinguish the psychrophilic community (considered as microorganisms that grow optimally at temperatures below 15°C with a maximum growth temperature of around 20°C) from the psychrotolerant community (considered as microorganisms capable to grow at low temperatures that grow optimally at temperatures above 20°C) (Morita 1975), since we expect most of the psychrophilic fraction of the community to die off or reduce in number at 22°C.

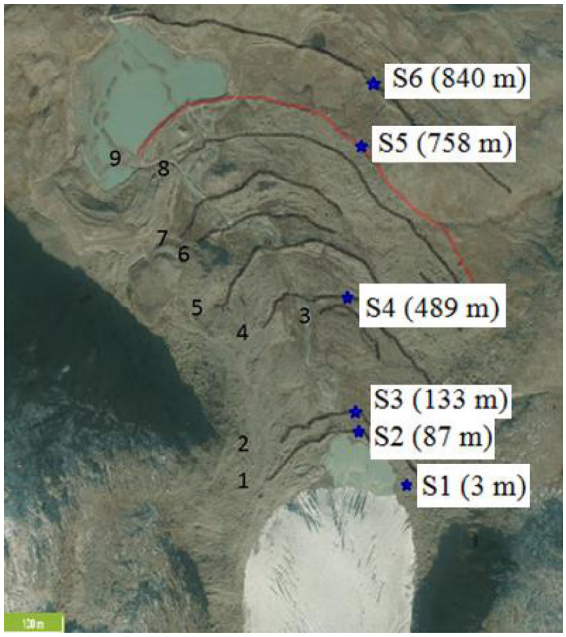
We used Illumina barcoded primers to amplify the 16S rRNA gene to assess the composition of microbial communities along different chronosequence stages of the glacier forefield. We addressed a number of relevant questions relating to the effects of increasing temperature on the performance of Arctic ecosystems under rapidly changing temperature regimes. (i) Is there a relation between microbial community diversity along a chronosequence in the Styggedalsbreen forefield area and the age and pedogenesis of the uncovered area and how does that relate to previous findings? (ii) Do the microbial communities respond to changes on short time scales (such as modifications in the soil temperature depending on the growth temperature) and what effects would these changes have on the ecosystem?

MATERIALS AND METHODS

Study area

The glacier Styggedalsbreen (61.5°N; 8.3°E) is located in the western part of the alpine landscape of Jotunheimen in central

(a)



(b)

Figure 1. (**a**) Location map of the Styggedalsbreen glacier zone. Picture: Google Maps; (**b**) aerial view of Styggedalsbreen; the different moraines are marked with black color and numbered. The sampling sites are marked with blue stars (S1–S6). LIA moraine is shown in red color (number 9).

Norway, ranging from 1295 to 1785 m a.s.l. (meters above sea level) (Fig. 1a). The glacier front has retreated around 800 m since the LIA, which ended circa 1750 CE (Common Era) with an approximate recession rate of 5 m year^{-1} in the 20th century. However during the last five years, the recession rate has been approximately 15 m year^{-1} (Norwegian Water Resources and Energy Directorate, NVE). The region has a typical northern hemispheric alpine climate, with a short growing season between June and September, strong winds with precipitation of snow during autumn and winter, and wet summers resulting in an average annual precipitation of 860 mm (Matthews 2005). The local mean air temperature is approximately –3.1°C, with a maximum of 5.7°C in July (Matthews 2005). The bedrock is composed of pyroxene granulite gneiss with inclusions of mylonite and rare peridotite intrusions (Matthews 2005). On the Styggedalsbreen forefield there are nine distinct moraines that formed at the termination of and after the LIA (Fig. 1b), in addition to numerous minor moraine ridges. In front of the LIA moraine, there are several older moraines of Holocene origin (Fig. 1b).

Samples S1–S6 (Fig. 1b) were collected from six locations: (i) 3 m in front of the ice margin (S1); (ii) the youngest moraines (S2 and S3); (iii) the highest post-LIA moraine (S4); (iv) the LIA moraine (S5); and (v) an older Holocene moraine (S6). Vegetation on the top of the moraines starts with moraine 4 (sample site S4) with a dominance of cyanobacterial mats, mosses and lichens. Sample sites S5 and S6 were fully covered by vegetation.

Sampling procedure

Samples were collected on the top of the moraines (S2–S6), with sampling site S1 being collected at the front of the glacial terminus (Fig. 1b). From each sampling point the top 15 cm were excavated with an ethanol-cleaned spade and the soil sample was transferred into sterile plastic bags (LDPE model, Norlip A/S, Denmark). Samples were homogenized to detect the total variability of each sampling point and stored at 5°C prior to transportation to the University of Bergen for further analyses. To study the effect of short term temperature changes on the microbial community composition, each soil sample (S1–S6) was divided into three different subsamples, of which one was incubated at 5°C, one at 10°C and the remaining subsample at 22°C for a time period of a week. Taking into account the original six soil samples and nine subsamples from each (three temperatures in triplicates), this gives a total of 54 samples (Fig. S1, Supporting Information). Finally samples were stored at –80°C prior to further analyses.

Lichenometric measurements

Lichenometry techniques have been used frequently to date moraines ridges younger than 500 years (Porter 1981). Different approaches to lichenometric dating can be used: (i) the single largest lichen-spot used to date other lichens (McCarroll 1994), (ii) the fixed-area largest lichen (Bull and Brandon 1998) or (iii) the lichen cover approach (Grab, Van Zyl and Mulder 2005). We have used the mean of the largest five lichens approach, which is a modification of the single largest lichen-spot method developed by Beschel (1950) from the *Rhizocarpon geographicum* species because of its ability to survive stressful climatic conditions, its longevity and its characteristic green–yellow color, which makes it easy to distinguish from other lichens in the field (Jomelli *et al.* 2007; Osborn *et al.* 2015). Based on the average diameter of the five largest *R. geographicum* spots, a calibration curve as described by Erikstad and Sollid (1986) and recently applied by Matthews (2005) was used to date the moraines. The relationship between lichen diameter and age at Jotunheimen is expressed by the equation: $\log (y + 150) = 5.0309 + 0.0074x$, where 'x' is the mean size (in mm) of the five largest lichens per moraine, and 'y' is the calculated age of the moraine in years (Matthews 2005). Two different approaches were used to calibrate the relationship: (i) the moraine or glacier position based on photographs, maps and historical measurements, and (ii) the age of the local termination of the LIA (1750 CE). Styggedalsbreen

Table 1. Oligonucleotide primers used in this study for PCR and real-time qPCR.

Primer	Target	Sequence (5′ – 3′)	Annealing t° (°C)	Reference
519F	16S rRNA gene	CAGCMGCCGCGGTAA	55	Øvreås *et al.* (1997)
806R	16S rRNA gene	GGACTACHVGGGTWTCTAAT	55	Caporaso *et al.* (2011)
Bac338F	Bacterial 16S rRNA gene	ACTCCTACGGGAGGCAGCAG	56	Sheik *et al.* (2012)
Eub518R	Bacterial 16S rRNA gene	ATTACCGCGGCTGCTGG	56	Sheik *et al.* (2012)
Arch349F	Archaeal 16S rRNA gene	GYGCASCAGKCGMGAAW	51	Swan *et al.* (2010)
Arch806R	Archaeal 16S rRNA gene	GGACTACVSGGGTATCTAAT	51	Swan *et al.* (2010)

has two moraines dated based on historical reports (moraines 3 and 4; Fig. 1b) with ± 1 year of accuracy and one moraine assumed to have originated during LIA (moraine 9; Fig. 1b) (Erikstad and Sollid 1986; Matthews 2005).

Soil characterization

Aliquots containing 1 g of homogenized soil were weighed and transferred into tin capsules and dried overnight at 80°C for quantification of total carbon (TC) and nitrogen (TN) contents using the CHN analyzer Flash 2000 EA (Thermo Scientific, USA). The current and potential acidity of the soil pH was determined from soil samples obtained from each sampling site (S1–S6). The pH was measured after suspension of 10 g soil in 10 mL of distilled water (current) and also in 10 mL of KCl (1 M) (potential), using a glass electrode connected to a MP220 pH-meter (Mettler Toledo, USA). The suspensions were stirred for 30 min and left to settle for 30 min, and this process was repeated four times (a total of 120 min) following the procedure of Bárcena, Yde and Finster (2010). All the assays were performed in triplicate.

DNA extraction, PCR amplification and Illumina sequencing

0.25 g from each of the 54 soil subsamples (Fig. S1, Supporting Information) were carefully weighed and used for DNA isolation using the UltraClean Soil DNA extraction kit (MoBio, Carlsbad, CA, USA), following the protocol provided by the manufacturer. The extracted DNA was quantified using a QuBit Fluorometer (Invitrogen, USA) and stored at –20°C for further analyses. 16S rRNA genes were PCR-amplified using the HotStar PCR Master Mix Kit (Qiagen, Hilden, Germany) with Illumina prokaryotic primer-pair: (FAdapter) 519F/(RAdapter) 806R (Table 1) targeting the variable V4 region. Amplification was performed using a dual indexing approach modified from Caporaso *et al.* (2012). Two PCR steps were performed to reduce the variability in the samples and increase its reproducibility (Berry *et al.* 2011), for the first PCR-amplification two replicates (per sample) were made, comprising 19 μL master mix and 1 μL extracted DNA, for the second PCR step, the protocol was as previously indicated, but using 18.5 μL of master mix, 2 μL of the specific primer pair (1 μM) and 2.5 μL of DNA (pooled from both replicates).

For the first PCR reaction, an initial denaturation step (95°C, 15 min) was followed by 25 cycles of denaturation (95°C for 20 s), annealing (55°C for 30 s) and extension (72°C for 30 s) and a final extension of 7 min at 72°C. In the second PCR only 10 cycles were run. Amplicons were purified using the GenElute PCR Clean-Up Kit (Sigma-Aldrich, USA), eluted in 25 μL DEPC-treated water and the DNA was quantified using the Qubit Fluorometer (Invitrogen). Finally the purified samples were mixed in equimolar amounts and sent for sequencing analyses using the Illumina 'MiSeq sequencing system' (Illumina, USA) at the Norwegian High-Throughput Sequencing Centre, Oslo (Norway).

Quantitative/real-time PCR

The extracted DNA was used to determine the abundance of bacterial and archaeal 16S rRNA genes in triplicate by quantitative real-time PCR (qPCR) on a C1000 Thermocycler (BioRad, USA). For bacterial 16S rRNA gene quantification, 2 μL of the primer-pair Bac338F/Eub518R (Table 1; Sheik *et al.* 2012), were added to 17 μL of the IQ SYBR Green Supermix (BioRad) and 1 μL of DNA. The PCR reaction conditions were as follows: initial activation for 5 min at 95°C, followed by 40 cycles of amplification (30 s denaturation at 95°C, annealing at 56°C for 30 s, extension at 72°C for 30 s) and final extension 10 min at 72°C (Sheik *et al.* 2012). For the archaeal 16S rRNA gene, the primer-pair Arch349F/Arch806R was used (Table 1; Swan *et al.* 2010) with the same reagent concentration as described above. During the qPCR program an initial activation at 95°C for 10 min was followed by 40 cycles of amplification (30 s at 95°C, annealing at 51°C for 30 s and 72°C for 30 s) and final extension 10 min at 72°C (Swan *et al.* 2010). The fluorescence was measured at the end of each cycle and a melting curve obtained from 65°C to 95°C with increments of 0.2°C. Dilution of plasmids containing bacterial or archaeal 16S rRNA gene were used to obtain the standard curves and the Ct values, and the assays were performed in triplicate. The archaeal and bacterial 16S rRNA genes used as controls were cloned into pCR 2.1 Topo TA (Invitrogen, USA), transformed into *Escherichia coli* strains and the recombinant clones selected for plasmid purification using the GenElute PCR Clean-Up Kit (Sigma-Aldrich, USA). Finally, quantification of plasmid DNA was performed using QuBit (Invitrogen, USA) in order to adjust the amount of plasmid DNA used for quantitative comparison with our microbial samples (Stoddard *et al.* 2015). The efficiency of the obtained qPCR was between 97% and 108% with R^2 values > 0.99 in all runs.

Statistical and multivariate analyses

16S rRNA gene sequences were processed using QIIME (Quantitative Insights into Microbial Ecology; Caporaso *et al.* 2010) at 97% pairwise identity of the OTUs (Operational Taxonomic Units) and taxonomy classified using the SILVA database (Quast *et al.* 2013) (see Supporting Information for details). Shannon diversity (H') was estimated for the prokaryotic community based on OTU abundances using the statistical software environment R (R Core Team 2013). The evenness index E_{var} (Smith and Wilson 1996) was used to quantify evenness of the microbial community at each sampling point along the chronosequence.

Relationships at phylum level between environmental parameters and microbial community structures for three different temperatures were assessed by non-metric

Table 2. Lichenometric dating of Styggedalsbreen moraines compared with the historical dates. ND indicates non-defined values.

Moraine ridge	Lichen size (mm)	Historical date (CE)	Estimated date (CE)
9	125 ± 4.3	1750	1758
8	101.2 ± 5.4	ND	1825
7	97.8 ± 7.6	ND	1834
6	85.3 ± 4.7	ND	1864
5	70.2 ± 6.9	ND	1896
4	72.2 ± 3.4	1906	1892
3	52.3 ± 5.8	1931	1930
2	8.2 ± 1.4	ND	1997
1	0 ± 0	ND	2007

multidimensional scaling (NMDS; Kruskal 1964) using the package *vegan* (Oksansen *et al.* 2013) with the Bray–Curtis coefficient used as a dissimilarity measure and 999 permutations. NMDS solutions differ depending on the dimensions (Rydgren 1993). For this study, the solution 2D was the one shown in graphs. The environmental variables (pH, Total Nitrogen (TN), Organic Carbon (OC) and soil age) were plotted using the function *envfit* with 999 permutations to determine how the variables affect the community composition. One-way ANOVA was used to test for differences in the environmental variables with distance from the glacier terminus being the categorical variable. For some physicochemical parameters such as acidity (KCl), OC, TN, archaeal copy numbers (qPCR) and bacterial copy numbers (qPCR) data were transformed into base 10 logarithms before analyses (Table S1, Supporting Information). Post-hoc analyses were performed to check for normal distribution and constant variance of the residuals. Permutational multivariate analyses of variance (PerMANOVA) were used to test differences in the microbial communities with temperature and distance from the glacier using the function *adonis* with a Monte-Carlo test and 9999 unrestricted permutations (Table S2, Supporting Information).

RESULTS AND DISCUSSION

Lichenometric dating of moraines

Despite the fact that Matthews (2005) dated the oldest Styggedalsbreen moraines in 1978, a new dating effort had to be conducted as new moraines have been formed since then. A total of nine moraines (including the LIA moraine at Styggedalsbreen forefield) were dated (Table 2). The means of the maximum diameter of the five largest lichens found on boulders on each moraine were combined with the historical dating of moraines 3, 4 and 9 (Fig. 1b) to perform the dating calibration. For example, the year of formation of moraine 9 was set to be 1750 CE based on historical observations at Nigardsbreen (located 40 km away; e.g. Matthews 2005), whereas the lichenometric methods estimated the year of formation to be 1758 CE. The new dating differ by up to 16 years from the previous datings by Matthews (2005). Beyond the LIA moraine there are some moraines that cannot be dated by lichenometry, as they are older than 500 years (Matthews 2005). The validity of lichenometry has been under discussion ever since the method was proposed mainly due to its lack of reproducibility and questions about thallus diameter as an age index, but despite the disputed accuracy of the method, lichenometry has been shown to yield reliable results in the Arctic and alpine environments (Armstrong 2004) and dating of lichens from rocks has been frequently used in glacial moraines. Although the use of lichens to date surfaces can be controversial in some situations, we have used the lichenometric analyses to date young surfaces (250 years old) where other dating methods such as radiocarbon analyses could not be trusted, and we have used a regional calibration curve that has been described and used before avoiding different microclimatic conditions. Despite the hypothetical low reproducibility of the method, we obtained similar results to the ones previously published (Matthews 2005). Error estimates of this method can vary depending on the publication, with certain authors assuming an error range of 30–45 years for a 150 year old surface, while others assume 10% accuracy on LIA or ± 20 years for older moraines (Osborn *et al.* 2015). Matthews (2005) estimated differences of –11.4 ± 17.1 years for the same Western Jotunheimen curve we have used in the present study, between the control points and the predicted ages. We underestimated the LIA moraine in 8 years, whereas we overestimated moraines 3 and 4 in 1 and 14 years, respectively, compared to the historical dates.

Soil characterization

The amounts of organic carbon (OC) and total nitrogen (TN) significantly increased ($P \leq 0.001$) with increasing soil age (Table 3). Measurements showed low concentrations of both OC and TN in bare soil, for OC we obtained 0.4 mg g^{-1} in sample S1, which gradually increased to 53.1 mg g^{-1} at S6. Quantification of the amount of total carbon (TC) showed that an average of more than 99% of the TC was present in the form of OC (data not shown). The amount of TN was below the detection limit of our method for samples S1–S3, and increased to approximately 3 mg g^{-1} in the oldest soils. The pH values also indicated an environmental change along the chronosequence; the pH significantly decreased ($P \leq 0.001$) from slightly alkaline (pH = 7.8) in the bare soil close to the glacier terminus, to acidic values (pH = 4.7) on

Table 3. pH, Organic Carbon (OC) and Total Nitrogen (TN) in soil samples from the different sampling sites at Styggedalsbreen forefield. b.d. indicates values below detection limit.

Sample name	OC (mg g^{-1})	TN (mg g^{-1})	Ratio OC/TN	pH	1 M KCl
S1	0.40 ± 0.02	b.d.	–	7.8 ± 0.6	7 ± 0.1
S2	0.45 ± 0.04	b.d.	–	7.6 ± 0.3	6.9 ± 0.5
S3	0.50 ± 0.05	b.d.	–	7.1 ± 0.6	5.6 ± 0.3
S4	1.29 ± 0.61	0.09 ± 0.04	14.79	7.0 ± 0.2	5.1 ± 0.6
S5	14.5 ± 1.9	0.72 ± 0.00	20.12	5.9 ± 0.3	4.2 ± 0.1
S6	53.1 ± 8.6	2.98 ± 0.84	17.84	4.7 ± 0.2	3.7 ± 0.1

Table 4. Total DNA content and qPCR 16S rRNA gene abundance data (archaeal and bacterial members) at the different sampling sites along the Styggedalsbreen chronosequence.

Sample name	Archaea 16S rRNA gene copies (g soil^{-1})	Bacteria 16S rRNA gene copies (g soil^{-1})	DNA content (μg g^{-1} soil)
S1	$3.6 \times 10^4 \pm 0.3 \times 10^4$	$13.4 \times 10^5 \pm 7.5 \times 10^5$	0.35 ± 0.08
S2	$1.5 \times 10^4 \pm 0.8 \times 10^4$	$3.6 \times 10^5 \pm 1.7 \times 10^5$	0.15 ± 0.02
S3	$26.5 \times 10^4 \pm 16.2 \times 10^4$	$103.3 \times 10^5 \pm 83.8 \times 10^5$	3.66 ± 1.71
S4	$25.4 \times 10^4 \pm 5.9 \times 10^4$	$95.7 \times 10^5 \pm 33.2 \times 10^5$	4.5 ± 0.66
S5	$93.2 \times 10^4 \pm 67.4 \times 10^4$	$411.3 \times 10^5 \pm 314.6 \times 10^5$	11.61 ± 2.20
S6	$378.7 \times 10^4 \pm 161 \times 10^4$	$1893.3 \times 10^5 \pm 193 \times 10^5$	23.33 ± 2.86

the vegetated moraines. The pH value in soil suspended in KCl (1 M) was 1 to 2 units lower than the pH of the same soil when it was suspended in distilled water.

The results obtained for OC and TN in our soil samples are consistent with results reported in previous studies at other alpine glaciers (Frey *et al.* 2013; Bradley, Singarayer and Anesio 2014). The obtained pH values in our soil samples showed equivalent value to those reported by Frey *et al.* (2013) from the Alps and by Bajerski and Wagner (2013) from Antarctica. In all cases, more acidic pH values were observed in samples furthest away from the glacier terminus.

Determination of gene copy numbers by qPCR

qPCR-based determination of bacterial and archaeal 16S rRNA gene abundances showed that bacterial gene copy numbers were 10–100 times higher than the archaeal copy numbers (10^6–10^8 g soil^{-1} for bacteria versus 10^4–10^6 g soil^{-1} for archaea) (Table 4). The total numbers of bacteria and archaea were lowest at the most recently exposed sampling site (S1) and increased significantly with age ($P \leq 0.001$) thus following the same trends as observed for soil OC and TN. The bacterial and archaeal copy numbers were not affected by increasing temperatures. The obtained results were similar at each sampling site (data not shown), and this was the case for the community structure (Fig. 2). These results indicate that the relative abundances of the community members changed as a response to increasing temperatures, while size of the community represented by community gene copy was unaffected by a rise in temperature (Table 4).

The trend seen in bacterial 16S rRNA gene copy numbers is consistent with the findings reported by Frey *et al.* (2013) on an alpine glacier forefield. To the best of our knowledge, trends in archaeal 16S rRNA gene copy numbers along proglacial chronosequences have not been reported so far. Our results indicate that members of the archaeal group follow the same pattern as bacteria with a 100-fold increase along the chronosequence. Similar results were found for the different incubation temperatures per sampling site, indicating that increased temperatures can change the relative abundances of different groups, modifying the dominance of distinct groups in the community without increasing the absolute value of copy number. The standard deviations of the gene copy numbers were high but in concordance with the results obtained from other glacier forefields (Philippot *et al.* 2010; Bajerski and Wagner 2013; Frey *et al.* 2013).

Diversity of the microbial communities

The microbial diversity present was addressed by next-generation Illumina sequencing. A mean of 130 000 reads per sample was obtained (Table S3, Supporting Information). The diversity bar charts revealed that the results for triplicate subsamples (Fig. S1, Supporting Information) were almost identical, and

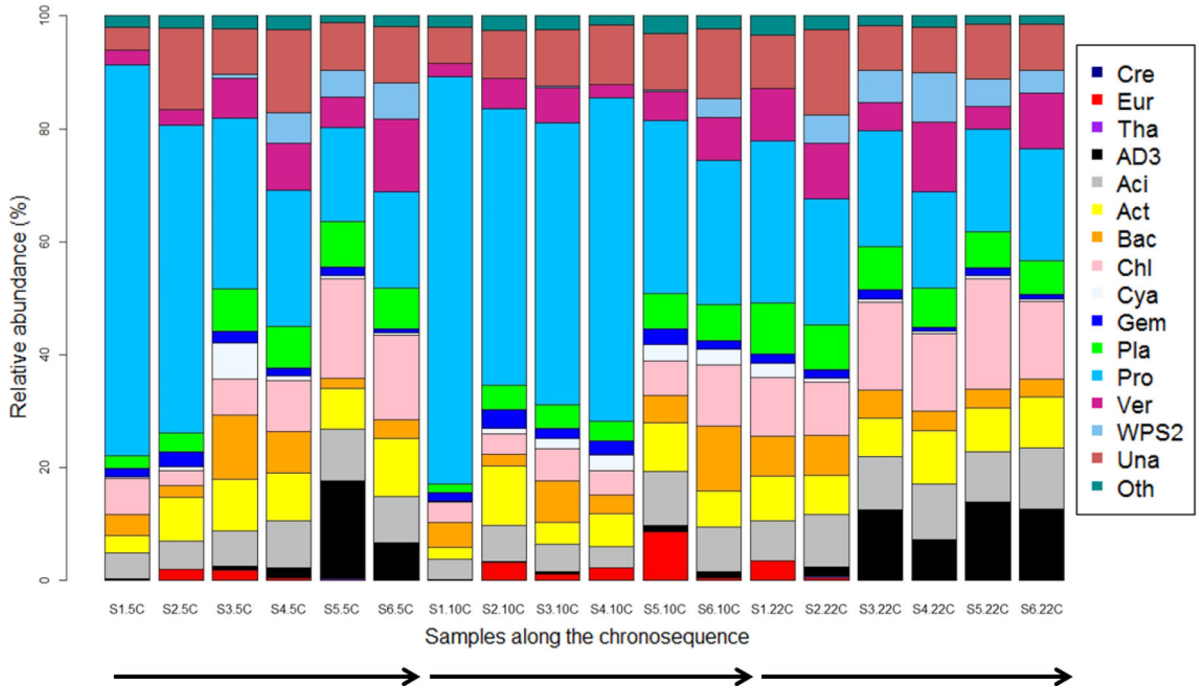


Figure 2. Taxonomic assignment of prokaryotic 16S rRNA gene at phylum level along the different stages of soil development at three different temperatures. Each colour represents the relative abundance in percentage (%) of a specific phylum. 'Cre' refers to *Crenarchaeota*, 'Eur' to *Euryarchaeota*, 'Tha' to *Thaumarchaeota*, 'AD3' to candidate division AD3, 'Aci' to *Acidobacteria*, 'Act' to *Actinobacteria*, 'Bac' to *Bacteroidetes*, 'Chl' to *Chloroflexi*, 'Cya' to *Cyanobacteria*, 'Gem' to *Gemmatimonadetes*, 'Pla' to *Planctomycetes*, 'Pro' to *Proteobacteria*, 'Ver' to *Verrucomicrobia*, 'WPS2' to candidate division WPS2, 'Una' to unassigned, which represents all the sequences that were not possible to assign, and 'Oth' to others, which represents sequences with lower abundances than 1%.

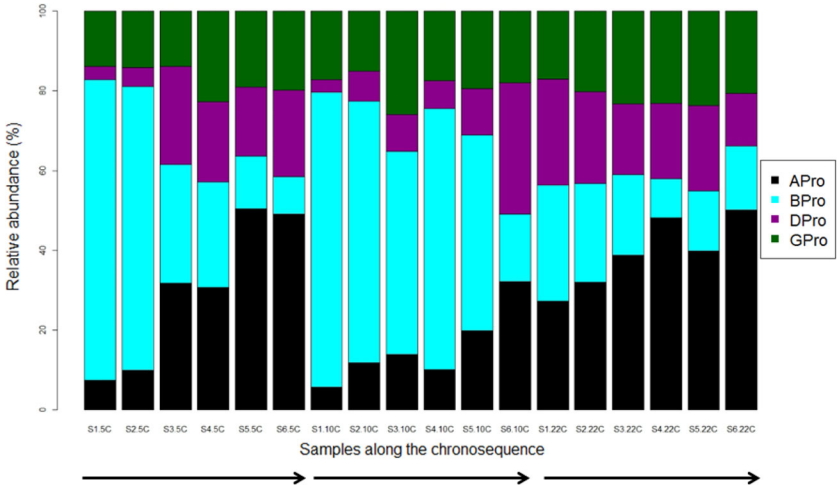


Figure 3. Taxonomic assignment of bacterial 16S rRNA gene within phylum *Proteobacteria* at class level at 5°C, 10°C and 22°C along the chronosequence. 'APro' refers to *Alphaproteobacteria*, 'BPro' refers to *Betaproteobacteria*, 'DPro' refers to *Deltaproteobacteria* and 'GPro' refers to *Gammaproteobacteria*.

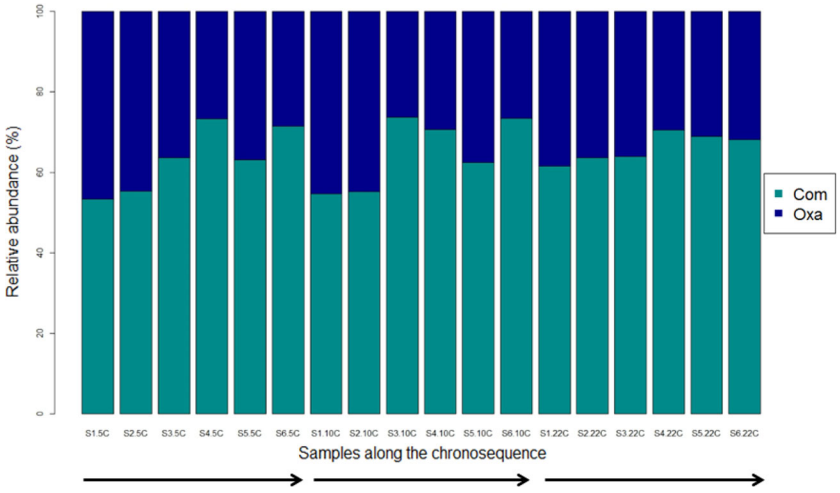


Figure 4. Taxonomic assignment of bacterial 16S rRNA gene of the two most abundant families within class *Betaproteobacteria* along the chronosequence. 'Com' refers to *Comamonadaceae* and 'Oxa' to *Oxalobacteraceae*.

thus we pooled the triplicates for the following analyses in 18 final bar charts (Fig. 2).

Diversity analyses for samples incubated at 5°C and 10°C showed a significant effect of distance from the glacier ($P = 0.037$ and $P = 0.014$ for 5°C and 10°C, respectively; Table S2, Supporting Information) with dominance of members from the bacterial phylum *Proteobacteria* for young soils (72% and 69%, for 5°C and 10°C, respectively, in sample S1) (Fig. 2). The relative contribution of sequences affiliated with this phylum decreased with soil age (17% and 25%, for 5°C and 10°C, respectively, in sample S6). Other abundant phyla were *Chloroflexi* (3%), *Verrucomicrobia* (3%), *Actinobacteria* (3%) and *Bacteroidetes* (2%). For these phyla we observed the opposite trend as their fraction increased with soil age (up to 18%, 12%, 10% and 11%, respectively, in sample S6). Candidate phylum AD3 contributes to the diversity of the community only in the mature soils, such as 17% at sample site S5 (LIA moraine). Among the *Proteobacteria*, members of the class *Betaproteobacteria* were the most abundant (50% in S1) (Fig. 3) with a decrease in dominance with soil age (4% in S6), thus reflecting the same trend as the phyla. The most abundant families of the *Betaproteobacteria* were *Comamonadaceae* and *Oxalobacteraceae*, accounting for 16% and 15%, respectively, of the diversity at sampling site S1 (Fig. 4). *Methylibium* and *Polaromonas* were the most abundant genera of *Comamonadaceae* (3.6% and 1.5%, respectively; Table S4, Supporting Information), whereas *Janthinobacterium*, was the most abundant genus within the *Oxalobacteraceae* family (2.8% in S1; Table S4, Supporting Information).

Incubations at 22°C had a strong impact on the microbial community structure, with a dramatic decrease in members of the *Proteobacteria* (Fig. 2), resulting in a more homogenous community composition with no significant differences along the chronosequence (Table S2, Supporting Information). Compared to results obtained at 5°C and 10°C significant differences were only found with the results at 10°C (Table S2, Supporting Information). Also after incubations at 22°C the phylum *Proteobacteria* was the most prominent (28% in S1) with a slight decrease in dominance along the chronosequence (20% at the oldest sampling site, S6). Members of *Chloroflexi* followed *Proteobacteria* in dominance, expressing a similar trend as in the samples that were incubated at lower temperatures (10% for S1 up to 20% for S5), followed by the *Verrucomicrobia* (up to 12% in S4) and

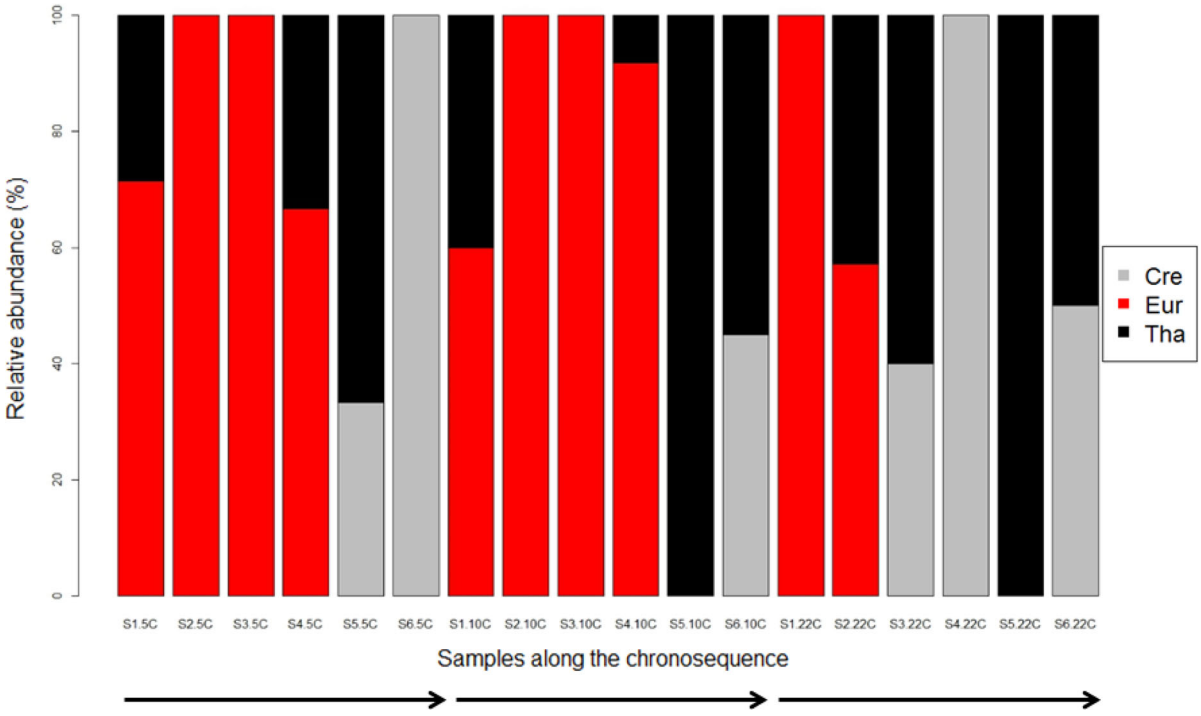


Figure 5. Taxonomic assignment of archaeal 16S rRNA gene at phylum level. Each site is represented (S1–S6) and each color shows the abundance within the domain *Archaea* of the phyla adjusted to 100%. 'Cre' refers to *Crenarchaeota*, 'Eur' refers to *Euryarchaeota* and 'Tha' refers to *Thaumarchaeota*.

the *Acidobacteria* (up to 11% in S6). Similarly to the low temperature incubations, candidate phylum AD3 increased in abundance along the chronosequence (up to 14% in S5). Within the *Proteobacteria*, we observed a dominance of members of the class *Alphaproteobacteria* (an average of 9% of the relative abundance), while members from the *Beta-*, *Gamma-* and *Deltaproteobacteria* accounted for equivalent fractions of approximately 4% of all sequences along the chronosequence (Fig. 2). The remaining phyla accounted for a mean of 2% of all sequences.

Members of the domain *Archaea* were found at all sites of the chronosequence, albeit they only represented around 0.7% of the total amount of the sequences. Our results showed a clear dominance of the *Euryarchaeota* phylum members (around 2% of the relative abundance) in the young soils (Fig. 5) and a co-dominance of the *Thaumarchaeota/Crenarchaeota* phyla members at the oldest sites of the chronosequence (S5–S6). The most abundant group was the order *Thermoplasmatales* (phylum *Euryarchaeota*), with increased abundance next to the glacial snout (up to 2.3% of the total sequences; Table S5, Supporting Information), followed by members of order *Nitrososphaerales* (*Thaumarchaeota*) that were found in most of the samples, with increased numbers with increasing soil age (up to 0.2% of all the sequences; Table S5, Supporting Information). Orders *Methanomicrobiales* and *Methanobacteriales* were also detected but with lower abundances. They accounted for less than 0.1% of the total prokaryotic sequences (Table S5, Supporting Information). A mean of 9% of the sequences were unassigned (Fig. 2).

Our results revealed different patterns in the microbial communities. At low and mid temperatures (where psychrophiles and psychrotolerants coexist), *Proteobacteria* dominated the community in samples from recently exposed soils. Schütte *et al.* (2010) reported similar results from glacier forefields on Svalbard (Norway). The phylum *Proteobacteria* encompasses metabolically diverse groups of microorganisms such as chemolithotrophs, heterotrophs and phototrophs. Many members of that phylum are well adapted to ecosystems with limited access to nutrients (Hodkinson, Coulson and Webb 2003; Zumsteg *et al.* 2012) suggesting that *Proteobacteria* are favored during the first phase after the glacial retreat and during the transition from anoxic to oxic conditions (Skidmore *et al.* 2005). Within the phylum *Proteobacteria*, representatives of the class *Betaproteobacteria* including the families *Comamonadaceae* and *Oxalobacteraceae* (of the order *Burkholderiales*) were prevalent. Abundance of *Betaproteobacteria* decreased along the glacial chronosequence. Our results are in agreement with the results of Nemergut *et al.* (2007), who studied bacterial abundance in a Peruvian glacier forefield. Skidmore *et al.* (2005) suggest that the decrease in *Proteobacteria* abundance is due to increased temperatures and distance from the glacier, as some of their members are adapted to live in low temperature habitats and subglacial areas with low organic matter content. Oligotrophic environments, such those that can be found underneath and in front of the glacier, favor members of the family *Comamonadaceae* that utilize H_2 or CO as energy sources (Willems *et al.* 1991). H_2 is produced in subglacial ecosystems by rock weathering and together with formate, acetate and oxalate is used as an energy and carbon source by subglacial bacteria under anaerobic conditions (Mader *et al.* 2006; Telling *et al.* 2015). Among members of the *Oxalobacteraceae*, there are nitrogen-fixing organisms that are favored in nitrogen-limited environments. At Styggedalsbreen, representatives of the chemoorganotrophic genus *Janthinobacterium* were the most numerous. Isolates belonging to this genus are adapted to growth at low temperatures and neutral pH (De Ley, Segers and Gillis 1978). These properties of *Janthinobacterium* may explain why members of this genus were more abundant in the youngest soils of the chronosequence (S1–S3) at 5°C and 10°C than in the older soils and at higher temperatures.

In contrast to the *Proteobacteria*, the proportion of members of the phyla *Acidobacteria*, *Actinobacteria* and *Verrucomicrobia* increased in abundance along the chronosequence. This may be caused by increasing acidity of the soil, as has previously been reported (Lauber *et al.* 2009; Philippot *et al.* 2010; Schütte *et al.* 2010). Members from the *Acidobacteria* are frequently involved in cellulose decomposition in oligotrophic soils (Zumsteg, Schmutz and Frey 2013b). Members from *Actinobacteria* catabolize organic carbon, which is present at higher concentrations in older soils (Heuer *et al.* 1997), whereas representatives of the *Verrucomicrobia* are often found in cold habitats, and their abundance can be associated with their ability to oxidize methane, which is provided by the atmosphere as well as by methanogenesis in the deeper anoxic soil layers (Wagner, Kobabe and Liebner 2009; Bergmann *et al.* 2011).

The phylum *Chloroflexi* shows the same trend as the phyla *Acidobacteria*, *Actinobacteria* and *Verrucomicrobia* along the chronosequence and the relative abundance of the *Chloroflexi* members increased with higher incubation temperatures, as it would be expected. Our results are in agreement with what has been reported previously from sites in Antarctica (Pearce *et al.* 2013) but contrasts with other studies that demonstrated the presence of *Chloroflexi* in proglacial environments but at low relative abundances (Shivaji *et al.* 2011; Srinivas *et al.* 2011). These results may be related to differences in soil nitrogen concentration and organic matter composition (Dini-Andreote *et al.* 2014).

The phylum *Bacteroidetes* also showed a similar trend to the phyla *Acidobacteria*, *Actinobacteria* and *Verrucomicrobia* with an increased abundance with increasing soil age and increasing pH. In the 5°C incubations, members of the *Bacteroidetes* increased in abundance in samples S1–S3, followed by a decrease in relative abundance in the remaining samples. However, in the 10°C incubations, a high *Bacteroidetes* abundance was only observed in the oldest soils of the chronosequence (Fig. 2). This result is not in agreement with previous findings that correlate this phylum with increasing pH and neighborhood to the glacier terminus (Lauber *et al.* 2009; Philippot *et al.* 2010; Bajerski and Wagner 2013), despite temperature being cited as the main factor affecting *Bacteroidetes* survival. This distribution was explained by the physiological properties of *Bacteroidetes* that thrive under anoxic conditions by performing fermentation on inorganic substrates resulting from weathering of subglacial ecosystems (Sheik *et al.* 2015). In the 22°C incubation, the relative abundance of *Bacteroidetes* decreased with increasing soil age (Fig. 2), as reported in previous studies.

Members of the phylum *Cyanobacteria* were generally present at low relative abundance along the chronosequence, although their presence increased in abundance in the intermediate stages of the chronosequence. *Cyanobacteria* are photoautotrophic organisms, which often have the ability to fix nitrogen. Therefore they produce organic carbon and biologically available nitrogen to the organisms along chronosequence and prepare the sites for later succession (Schmidt *et al.* 2008; Stibal *et al.* 2008). Our results are in accordance with what has been reported previously (Nemergut *et al.* 2007; Zumsteg *et al.* 2012), where the relative abundances of *Cyanobacteria* increased in the transitional stages and decreased with increasing soil age, as a result of reduced light availability due to increased vegetation and competition for nutrients with plants and other microorganisms (Frey *et al.* 2013).

The domain *Archaea* was represented by members of the phyla *Euryarchaeota*, *Thaumarchaeota* and *Crenarchaeota* in all samples. As was observed with the domain *Bacteria* (Lauber *et al.* 2008; Rousk, Brookes and Bååth 2009), changes in archaeal populations are correlated with pH and shifts in OC content (Dong *et al.* 2015). The only difference between low and mid-temperatures compared to high temperatures was the absence of *Euryarchaeota* in intermediate age soils (S3–S4). In young soil (S1–S4) *Euryarchaeota* showed the highest relative abundance of all archaeal phyla. *Euryarchaeota* include methanogens, such as members of the order *Methanomicrobiales* and *Methanobacteriales* that are dominant in subglacial ecosystems (Stibal *et al.* 2012; Dieser *et al.* 2014) but also typically found in environments with high water content (as found close to the glacier snout) and low access to oxygen (Angel, Claus and Conrad 2012), as is the case at the terminus of the Styggedalsbreen. In the oldest soils (S5–S6) the archaeal community showed higher relative abundances of *Thaumarchaeota—Crenarchaeota*. *Thaumarchaeota* constitute a recently described phylum (Brochier-Armanet *et al.* 2008) that comprise members with the ability to thrive on the oxidation of ammonia to nitrite and that often can also fix CO_2 (Schleper, Jurgens and Jonuscheit 2005; Spang *et al.* 2010) such as members of the order *Nitrososphaerales* that grow with NH_3 as energy and CO_2 as carbon sources (Pessi *et al.* 2015). By combining these properties they may play an important role in the cycling of both nitrogen and carbon (Nicol *et al.* 2008; Spang *et al.* 2010). The increase in relative abundance of the *Thaumarchaeota* community along the chronosequence is also strongly linked with soil pH. This is in concordance with observations reported by Hu *et al.* (2013) and Nicol *et al.* (2008).

Our results are also in agreement with results reported by Zumsteg *et al.* (2012) where population changes of *Euryarchaeota* or *Crenarchaeota* dominated communities in young soils or intermediate and old soils, respectively. In our study, members of the *Thaumarchaeota* were more frequently present than those of *Crenarchaeota* and we suggest that a major fraction of *Crenarchaeota* members (Zumsteg *et al.* 2012) actually belong to *Thaumarchaeota*, but have been wrongly classified in the taxonomic database due to the mixed nomenclature (Hu *et al.* 2013). Within the *Euryarchaeota*, Zumsteg *et al.* (2012) found that only 4% of all the archaeal sequences belong to *Methanomicrobiales* which supports our findings suggesting that other taxa (such as *Thermoplasmatales*) may be more abundant in glacier forefields, as described previously (Pessi *et al.* 2015).

Table 5. Coefficients for Shannon diversity index (H') and evenness (E_{var}) of the 16S rRNA genes at Styggedalsbreen.

Sample name	OTUs	Shannon (H')	Evenness (E_{var})
S1	597 ± 81.7	4.2 ± 0.4	0.57 ± 0.05
S2	470.6 ± 33.6	4.2 ± 0.3	0.62 ± 0.01
S3	579.6 ± 103.9	4.2 ± 0.2	0.63 ± 0.06
S4	522 ± 61.5	4.1 ± 0.4	0.62 ± 0.02
S5	416.3 ± 27.9	4.0 ± 0.4	0.63 ± 0.02
S6	495 ± 54.5	4.0 ± 0.3	0.57 ± 0.01

Shannon and evenness index of the microbial communities along the chronosequence

The number of OTUs in our samples did not follow a clear trend, as the largest number of OTU's was found in sample S1 while the lowest number was found in sample S5 (Table 5). The Shannon diversity (H') index is used to assess the species diversity in a community. In our case indices indicate a high diversity along the chronosequence (4.2–4), with the LIA moraine (S5) affiliating with the lowest H' index (4.01). E_{var} was calculated to determine

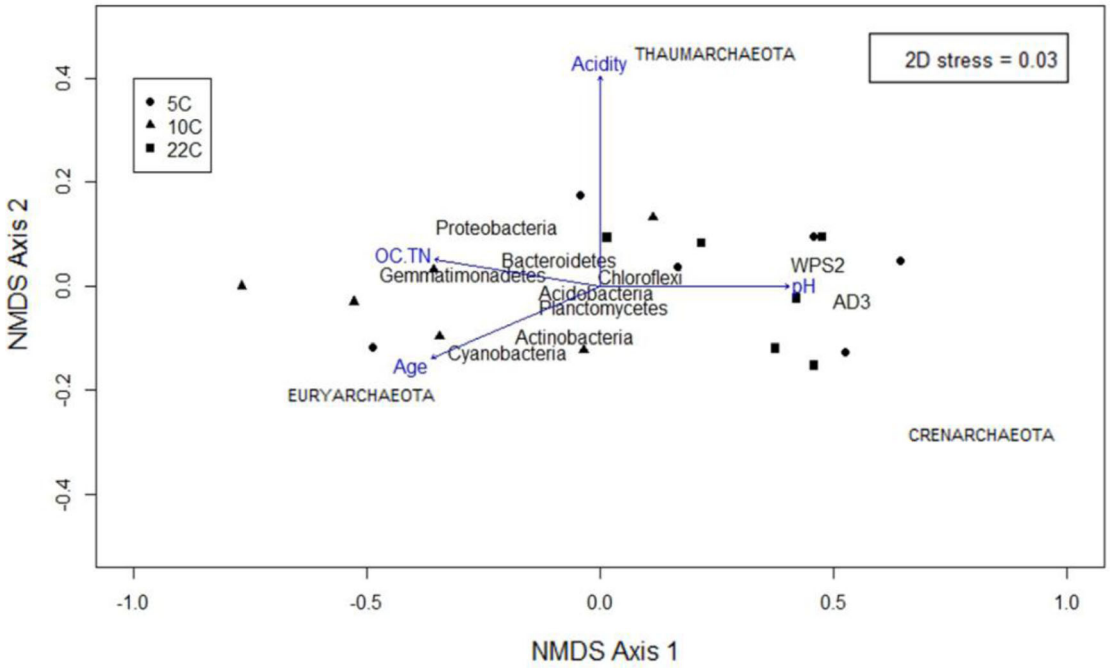


Figure 6. NMDS showing the relationship between community structures and sampling points at different temperatures. Prokaryotic phyla are represented with black color and environmental variables are shown with blue arrows. 'OC.TN' refers to the ratio C to N. Circles show the representation of the points at 5°C, triangles at 10°C and squares at 22°C. Archaeal phyla are shown with capital letters. Due to the amount of samples the x-axis explains the 100% of the variation.

whether diversity changes of the prokaryotic communities were higher in intermediate soils (0.62) compared with the youngest and oldest soils (0.57).

Our *H′* indices results showed a difference between samples at different stages of the chronosequence (4.2–4), as reported by Zumsteg *et al.* (2012). However, Nemergut *et al.* (2007) found an increased diversity in older soils. The high values of the Shannon index are in agreement with those from previous studies in other glacier forefields (Shivaji *et al.* 2011; Srinivas *et al.* 2011; Pessi *et al.* 2015), but conflict with other observations where Shannon indices were between 2.3 and 3.4 (Bernasconi *et al.* 2011; Zumsteg *et al.* 2012).

Non-metric multidimensional scaling

NMDS ordination visualized the relationship between the community structures and sampling site at three different temperatures (Fig. 6). The outcome points showed a strong positive correlation between soil exposure time and the presence of members of the phylum *Euryarchaeota* (sequences of *Euryarchaeota* were only found in young soils) and a negative relationship between soil exposure time and the presence of members of the phyla *Thaumarchaeota* and *Crenarchaeota*. There were also positive correlations between acidity (KCl) and members of phylum *Thaumarchaeota* and positive correlations between the candidate divisions WPS2 and AD3 and soil pH. *Proteobacteria* and *Gemmatimonadetes* showed a positive correlation with the C to N ratio and *Cyanobacteria* and *Acidobacteria* a positive relationship with soil age but this correlation was less strong than the relation of *Euryarchaeota*. In summary our results showed that a variety of environmental factors controls the microbial communities in the glacier forefields: (i) older soils are often found to be more acidic than newly formed soils; (ii) we found that the archaeal phyla are strongly correlated with soil age, with a shift from a *Euryarchaeota* dominated community in young soils to a *Crenarchaeota – Thaumarchaeota* dominated community in older soils; (iii) certain bacterial phyla such as *Proteobacteria* and *Gemmatimonadetes* showed a positive correlation with increased OC and TN.

Microorganisms are ubiquitous in cold environments, as we confirm in the present study. However the microbial diversity of the soil samples depends on various nutritional and/or environmental factors, such as the presence of carbon, nitrogen, oxygen, soil pH and temperature. As temperature is a very important factor for microbial community development, we analyzed how the microbial communities present in glacier moraines differ based on two factors: distance from the glacier forefront and the effects of incubation temperature. Glacier forefields have received increasing attention in recent years with focus on studying abiotic and biotic factors including microbial survival, colonization and the diversity of the microbial communities. However, the study of microbial population changes associated with soil samples obtained from glacier forefields based on increased incubation temperatures, has not been described yet. For this reason, we incubated our samples at three different temperatures (i) 5°C (the soil temperature during early and late summer), (ii) 10°C (the soil temperature in mid-summer) and (iii) 22°C, as a conservative estimate of a future increase in temperature, and how these temperature regimes would affect those microbial communities comprising psychrophilic and psychrotolerant microorganisms. Our results showed that the proglacial area of Styggedalsbreen represents a successional gradient establishing a diverse microbial community along the chronosequence. The community is dominated by *Proteobacteria* (mainly *Betaproteobacteria*) and *Euryarchaeota* in younger soil samples next to the glacier terminus, with a decrease in abundance with increasing soil age. Phyla such as *Chloroflexi*, *Acidobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Crenarchaeota* and *Thaumarchaeota* may be found along the chronosequence with increased abundance with increasing soil age. We suggest that the changes

in community composition observed are due to variation in soil composition along the chronosequence, as OC and TN content increases, whilst pH decreases. In addition, this analysis indicates that a strong increase in temperature (22°C) would modify the forefield microbial communities by altering the dominance of the different microbial groups along the chronosequence and we assume that their adaptation to higher temperature would allow them grow faster. Our results also indicate that despite the high temperature the community size was not affected as the higher temperature did not affect the amount of DNA that we could extract from the samples (Table 4); however, the higher temperature did affect the community composition. These data suggest that at low and mid temperatures (5°C and 10°C, respectively), the community is primarily constituted of psychrophilic and psychrotolerant microorganisms, while at higher temperatures (22°C), most of the psychrophiles would disappear while the psychrotolerant microorganisms would increase in number and thereby dominate the community. These considerations are also supported by the fact that the number of 16S rRNA gene copies did not change with incubation temperature, indicating that increasing temperatures favor certain microbial groups along the chronosequence. We conclude that the shifts in microbial diversity in proglacial environments to a large degree depend on environmental conditions and reveal that temperature changes will have a strong influence on the development of microbial communities of glacier forefields. Linking the response of the communities to the modeled future increase in temperatures is crucial to fully appreciate the consequences of climate change. Glacial forefields are dynamic environments that are highly influenced by increasing temperature in the Arctic and so detailed research in proglacial areas with contrasting climatic, geological and glaciological conditions is required to address this issue more thoroughly. In the next phase of the investigations, we will address whether the effect that follows change in community composition is buffered by the metabolic redundancy of the indigenous microbial community or whether central steps in element cycling are eliminated.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

ACKNOWLEDGEMENTS

We would like to express gratitude to Knut Rydgren for his help with statistical analyses, Antonio García Moyano and Steffen Leth Jørgensen with the qPCR assays, Elzbieta Petelenz-Kurdziel and Hilde Rief Armo for technical support and the rest of the Department of Biology at the University of Bergen (UiB) and Faculty of Engineering and Science at Høgskulen i Sogn og Fjordane (HiSF). We also thank the Norwegian Sequencing Centre (NSC) for the sequencing service. The work has been supported by Norwegian Research Council (NRC) grant number 227062—'Microorganisms in the Arctic: major drivers of biogeochemical cycles and climate change'.

Conflict of interest. None declared.

REFERENCES

ACIA. *Arctic Climate Impact and Assessment*. Cambridge, UK: Cambridge University Press. 2004.

Angel R, Claus P, Conrad R. Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. *ISME J* 2012;**6**:847–62.

Armstrong RA. Lichens, lichenometry and global warming. *Microbiologist* 2004;**5**:32–5.

Bajerski F, Wagner D. Bacterial succession in Antarctic soils of two glacier forefields on Larsemann Hills, East Antarctica. *FEMS Microbiol Ecol* 2013;**85**:128–42.

Bárcena TG, Finster KW, Yde JC. Spatial patterns of soil development, methane oxidation, and methanotrophic diversity along a receding glacier forefield, southeast Greenland. *Arct Antarct Alp Res* 2011;**43**:178–88.

Bárcena TG, Yde JC, Finster KW. Methane flux and high-affinity methanotrophic diversity along the chronosequence of a receding glacier in Greenland. *Ann Glaciol* 2010;**51**:23–31.

Bardgett RD, Richter A, Bol R *et al.* Heterotrophic microbial communities use ancient carbon following glacial retreat. *Biol Letters* 2007;**3**:487–90.

Bergmann G, Bates S, Eilers K *et al.* The under-recognized dominance of *Verrucomicrobia* in soil bacterial communities. *Soil Biol Biochem* 2011;**43**:1450–5.

Bernasconi SM, Bauder A, Bourdon B *et al.* Chemical and biological gradients along the Damma glacier soil chronosequence, Switzerland. *Vadose Zone J* 2011;**10**:867–83.

Bernstein L, Bosch P, Canziani O *et al.* Climate change 2007: synthesis report. http://www.ipcc.ch/ (29 February 2016, date last accessed), 2007.

Berry D, Mahfoudh KB, Wagner M *et al.* Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. *Appl Environ Microbiol* 2011;**77**:7846–9.

Beschel RE. Flechten als altermasstab Rezenter moraine. *Z Gletscherkunde Glazialgeologie* 1950;**1**:152–61.

Bradley JA, Singarayer JS, Anesio AM. Microbial community dynamics in the forefield of glaciers. *P Roy Soc Lond B Bio* 2014;**281**:2793–802.

Brochier-Armanet C, Boussau B, Gribaldo S *et al.* Mesophilic *Crenarchaeota*: proposal for a third archaeal phylum, the *Thaumarchaeota*. *Nat Rev Microbiol* 2008;**6**:245–52.

Bull WB, Brandon MT. Lichen dating of earthquake generated regional rockfall events, Southern Alps, New Zealand. *Geol Soc AM Bull* 1998;**110**:60–84.

Caporaso JG, Kuczynski J, Stombaugh J *et al.* QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010;**7**:335–6.

Caporaso JG, Lauber CL, Walters WA *et al.* Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *P Natl Acad Sci USA* 2011;**108**:4516–22.

Caporaso JG, Lauber CL, Walters WA *et al.* Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* 2012;**6**:1621–4.

De Ley J, Segers P, Gillis M. Intra and intergeneric similarities of *Chromobacterium* and *Janthinobacterium* ribosomal ribonucleic acid cistrons. *Int J Syst Bacteriol* 1978;**28**:154–68.

Dieser M, Broemsen ELJE, Cameron KA *et al.* Molecular and biogeochemical evidence for methane cycling beneath the western margin of the Greenland ice sheet. *ISME J* 2014;**8**:2305–16.

Dini-Andreote F, De Cássia Pereira e Silva M, Triado-Margarit X *et al.* Dynamics of bacterial community succession in a salt marsh chronosequence: evidences for temporal niche partitioning. *ISME J* 2014;**8**:1989–2001.

Dong K, Kim WS, Tripathi BM *et al.* Generalized soil *Thaumarchaeota* community in weathering rock and Saprolite. *Microb Ecol* 2015;**69**:356–60.

Erikstad L, Sollid JL. Neoglaciation in South Norway using lichenometric methods. *Norsk Geogr Tidsskr* 1986;**40**:85–105.
Frey B, Bühler L, Schmutz S *et al.* Molecular characterization of phototrophic microorganisms in the forefield of a receding glacier in the Swiss Alps. *Environ Res Lett* 2013;**8**:015033 (11pp).
Grab S, Van Zyl C, Mulder N. Control son basalt terrace formation in the Eastern Lesotho Highlands. *Geomorphology* 2005;**67**:473–85.
Guelland K, Hagedorn F, Smittenberg RH *et al.* Evolution of carbon fluxes during initial soil formation along the forefield of Damma Glacier, Switzerland. *Biogeochemistry* 2013;**113**:545–61.
Haugland JE. Formation of patterned ground and fine-scale soil development within two late Holocene glacial chronosequences: Jotunheimen, Norway. *Geomorphology* 2004;**61**:287–301.
Heuer H, Krsek M, Baker P *et al.* Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Appl Environ Microbiol* 1997;**63**:3233–41.
Hodkinson ID, Coulson SJ, Webb NR Community assembly along proglacial chronosequences in the high Arctic: vegetation and soil development in north-west Svalbard. *J Ecol* 2003;**91**:651–63.
Hu HW, Zhang LM, Dai Y *et al.* pH-dependent distribution of soil ammonia oxidizers across a large geographical scale as revealed by high-throughput pyrosequencing. *J Soils Sediments* 2013;**13**:1439–49.
IPCC. Summary for policymakers. In: Stocker TF, Qin D, Plattner G-K *et al.* (eds). *Climate Change 2013: The Physical Science Basis, Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge, UK: Cambridge University Press, 2013.
Jomelli V, Grancher D, Naveau P *et al.* Assessment study of lichenometric methods for dating surface. *Geomorphology* 2007;**86**:131–43.
Kastovska K, Elster J, Stibal M *et al.* Microbial assemblages in soil microbial succession after glacier retreat in Svalbard (high arctic). *Microb Ecol* 2005;**50**:396–407.
Kastovska K, Stibal M, Sabacka M *et al.* Microbial community structure and ecology of subglacial sediments in two polythermal Svalbard glaciers characterized by epifluorescence microscopy and PLFA. *Polar Biol* 2007;**30**:277–87.
Kruskal JB. Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika* 1964;**29**:115–29.
Lauber CL, Strickland MS, Bradford MA *et al.* The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Soil Biol Biochem* 2008;**40**:2407–15.
Lauber CL, Hamady M, Knight R *et al.* Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Appl Environ Microbiol* 2009;**75**:5111–20
Lazzaro A, Abegg C, Zeyer J. Bacterial community structure of glacier forefields on siliceous and calcareous bedrock. *Eur J Soil Sci* 2009;**60**:860–70.
McCarroll D. A new approach to lichenometric: dating single-age and diachronous surfaces. *The Holocene* 1994;**22**:383–96.
Mader HM, Pettitt ME, Wadham JL *et al.* Subsurface ice as a microbial habitat. *Geology* 2006;**34**:169–72.
Matthews JA. 'Little Ice Age' glacier variations in Jotunheimen, southern Norway: a study in regional lichenometric dating of recessional moraines with implications for climate and lichen growth rates. *The Holocene* 2005;**15**:1–19.
Mernild SH, Hanna E, Yde JC *et al.* Coastal Greenland air temperature extremes and trends 1890–2010: annual and monthly analysis. *Int J Climatol* 2014;**34**:1472–87.
Morita RY. Psychrophilic bacteria. *Bacteriol Rev* 1975;**39**:144–67.
Nemergut DR, Anderson SP, Cleveland CC *et al.* Microbial community succession in an unvegetated, recently deglaciated soil. *Microb Ecol* 2007;**53**:110–22.
Nicol GW, Tscherko D, Embley TM *et al.* Primary succession of soil *Crenarchaeota* across a receding glacier foreland. *Environ Microbiol* 2005;**7**:337–47.
Nicol GW, Leininger S, Schleper C *et al.* The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing Archaea and bacteria. *Environ Microbiol* 2008;**10**:2966–78.
Oksanen J, Blanchet FG, Kindt R *et al.* *Vegan: Community Ecology Package*. R package version 2.0–6, 2013.
Osborn G, McCarthy D, LaBrie A *et al.* Lichenometric dating: science or pseudoscience? *Quat Res* 2015;**83**:1–12.
Øvreås L, Forney L, Daae, FL *et al.* Distribution of bacterioplankton in meromictic Lake Saelenvannet, as determined by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. *Appl Environ Microb* 1997;**63**:3367–73.
Pessi IS, Osorio-Forero C, Galvez EJC *et al.* Distinct composition signatures of Archaeal and bacterial phylotypes in the Wanda Glacier forefield, Anctarctic Peninsula. *FEMS Microbiol Ecol* 2015;**91**:1–10.
Pfeffer WT, Arendt AA, Bliss A *et al.* The Randolph Glacier Inventory (2014): a globally complete inventory of glaciers. *J Glaciol* 2014;**60**:537–52.
Philippot L, Tscherko D, Bru D *et al.* Distribution of high bacterial taxa across the chronosequence of two alpine glacier forelands. *Microb Ecol* 2010;**61**:303–12.
Pearce DA, Hodgson D, Thorne M *et al.* Preliminary analysis of life within a former subglacial lake sediment in Antarctica diversity. *Diversity* 2013;**5**:680–702.
Porter SC. Lichenometric studies in the Cascade Range of Washington: establishments of *Rhizocarpon geographicus* growth curves at Mount Rainier. *Arct Alp Res* 1981;**13**:11–23.
Quast C, Pruesse E, Yilmaz P *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013;**41**:590–6.
R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/ (25 February 2016, date last accessed), 2013.
Rime T, Hartmann M, Brunner I *et al.* Vertical distribution of the soil microbiota along a successional gradient in a glacier forefield. *Mol Ecol* 2015;**24**:1091–108.
Rousk J, Brookes PC, Bååth E. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Appl Environ Microbiol* 2009;**75**:1589–96.
Rydgren K. Herb-rich spruce forests in W Nordland, N Norway: an ecological and methodological study. *Nord J Bot* 1993;**13**:667–90.
Schmidt SK, Reed SC, Nemergut DR *et al.* The earliest stages of ecosystem succession in high-elevation (5000 metres above sea level) recently deglaciated soils. *Proc R Soc Ser B* 2008;**275**:2793–802.
Schütte U, Abdo Z, Foster J *et al.* Bacterial diversity in a glacier foreland of the high Arctic. *Mol Ecol* 2010;**19**:54–66.

Schleper C, Jurgens G, Jonuscheit M. Genomic studies of uncultivated archaea. *Nat Rev Microbiol* 2005;**3**:479–88.

Sheik CS, Mitchell TW, Rizvi FZ *et al.* Exposure of soil microbial communities to chromium and arsenic alters their diversity and structure. *PLoS One* 2012;**7**:e40059.

Sheik CS, Stevenson EI, Den Uyl PA *et al.* Microbial communities of the Lemon Creek Glacier show subtle structural variation yet stable phylogenetic composition over space and time. *Front Microbiol* 2015;**6**:495.

Shivaji S, Pratibha MS, Sailaja B *et al.* Bacterial diversity of soil in the vicinity of Pindari glacier, Himalayan mountain ranges, India, using culturable bacteria and soil 16S rRNA gene clones. *Extremophiles* 2011;**15**:1–22.

Skidmore M, Anderson SP, Sharp M *et al.* Comparison of microbial community compositions of two subglacial environments reveals a possible role for microbes in chemical weathering processes. *Appl Environ Microbiol* 2005;**71**:6986–97.

Smith B, Wilson JB. A consumer's guide to evenness indices. *Oikos* 1996;**76**:70–82.

Spang A, Hatzenpichler R, Brochier-Armanet C *et al.* Distinct gene set in two different lineages of ammonia-oxidizing archaea supports the phylum *Thaumarchaeota*. *Trends Microbiol* 2010;**554**:331–40.

Srinivas TNR, Singh SM, Pradhan S *et al.* Comparison of bacterial diversity in proglacial soil from Kafni Glacier, Himalayan Mountain ranges, India, with the bacterial diversity of other glaciers in the world. *Extremophiles* 2011;**15**:673–90.

Stibal M, Tranter M, Benning LG *et al.* Microbial primary production on an Arctic glacier is insignificant in comparison with allochthonous organic carbon input. *Environ Microbiol* 2008;**10**:2172–8.

Stibal M, Wadham JL, Lis GP *et al.* Methanogenic potential of Arctic and Antarctic subglacial environments with contrasting organic carbon sources. *Global Change Biol* 2012;**18**:3332–45.

Stoddard SF, Smith BJ, Hein R *et al.* rrnDB: improved tools for interpreting rRNA gene abundance in bacteria and Archaea and a new foundation for future development. *Nucleic Acids Res* 2015;**43**:593–8

Swan BK, Ehrhardt CJ, Reifel KM *et al.* Archaeal and bacterial communities respond differently to environmental gradients in anoxic sediments of a California hypersaline lake, the Salton Sea. *Appl Environ Microbiol* 2010;**76**:757–68.

Telling J, Boyd ES, Bone N *et al.* Rock comminution as a source of hydrogen for subglacial ecosystems. *Nat Geosci* 2015;**8**:851–5.

Tscherko D, Rustemeier J, Richter A *et al.* Functional diversity of the soil microflora in primary succession across two glacier forelands in the Central Alps. *Eur J Soil Sci* 2003;**54**:685–96.

Wagner D, Kobabe S, Liebner S. Bacterial community structure and carbon turnover in permafrost-affected soils of the Lena Delta, northeastern Siberia. *Can J Microbiol* 2009;**55**:77–83.

Willems A, De Ley J, Gillis M *et al.* *Comamonadaceae*, a new family encompassing the Acidovorans rRNA complex, including *Variovorax paradoux* gen. nov., comb. nov., for *Alcaligenes paradoux* (Davis 1969). *Int J Syst Bacteriol* 1991;**41**:445–50.

Zumsteg A, Luster J, Göransson H *et al.* Bacterial, Archaeal and fungal succession in the forefield of a receding glacier. *Microb Ecol* 2012;**63**:552–64.

Zumsteg A, Bååth E, Stierli B *et al.* Bacterial and fungal community responses to reciprocal soil transfer along a temperature and soil moisture gradient in a glacier forefield. *Soil Biol Biochem* 2013a;**61**:121–32.

Zumsteg A, Schmutz S, Frey B. Identification of biomass utilizing bacteria in a carbon-depleted glacier forefield soil by the use of ^{13}C DNA stable isotope probing. *Environ Microbiol Rep* 2013b;**5**:424–37.