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ENVIRONMENTAL MICROBIOLOGY

Bacterial, Archaeal and Fungal Succession in the Forefield of a Receding Glacier

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Abstract Glacier forefield chronosequences, initially composed of barren substrate after glacier retreat, are ideal locations to study primary microbial colonization and succession in a natural environment. We characterized the structure and composition of bacterial, archaeal and fungal communities in exposed rock substrates along the Damma glacier forefield in central Switzerland. Soil samples were taken along the forefield from sites ranging from fine granite sand devoid of vegetation near the glacier terminus to well-developed soils covered with vegetation. The microbial communities were studied with genetic profiling (T-RFLP) and sequencing of clone libraries. According to the T-RFLP profiles, bacteria showed a high Shannon diversity index (*H*) (ranging from 2.3 to 3.4) with no trend along the forefield. The major bacterial lineages were *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Firmicutes* and *Cyanobacteria*. An interesting finding was that *Euryarchaeota* were predominantly colonizing young soils and *Crenarchaeota* mainly mature soils. Fungi shifted from an *Ascomycota*-dominated community in young soils to a more *Basidiomycota*-dominated community in old soils. Redundancy analysis indicated that base saturation, pH, soil C and N contents and plant coverage, all related to soil age, correlated with the microbial succession along the forefield.

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Introduction

In the Alps, glaciers have been retreating since the mid nineteenth century, with current rates of glacial retreat considerably higher than the long-term averages [52]. Due to this glacial retreat, new terrain has become exposed as a forefield chronosequence with varying physical, chemical and biological properties [60]. This is an ideal environment to study soil formation and primary microbial succession as microorganisms like bacteria [29] and fungi [24] are the first to colonize new substrates. Their ability to interact with minerals and organic compounds through physical and chemical processes makes them contributors to rock weathering [8, 9, 20]. These interactions mobilize otherwise inaccessible nutrients essential for higher organisms. This implies that fungal and bacterial colonizers could be key determinants of early ecosystem function and stability. The bacterial succession on glacier forefields has been investigated under differing climatic conditions and with changing soil parameters [48, 57, 59, 61]. These studies have been able to show a bacterial succession along the forefields but they are either restricted to a few sampling sites (<8) with relatively large intervals between the different soil ages or

to laboratory experiments. Fungi have also been studied in glacier forefields, but with focus on association with plants, and on relatively old soils [35, 36], without taking into account the complete chronosequence.

Much less information is available on the role of the archaea in glacier forefields. Nicol et al. [49, 50] found that the dominant archaeal communities in the alpine glacier forefield they studied were composed of the *Crenarchaeota*. However, it is not known to what extent the *Euryarchaeota* or other archaeal lineages are colonizing glacier forefields or if indeed they are present at all.

We therefore studied the bacterial, archaeal and fungal community structure and composition and their simultaneous successional distribution patterns along the temperate glacier Damma forefield. The study was part of the interdisciplinary research project BigLink [4, 5], which investigates weathering, soil formation and ecosystem evolution along the Damma glacier forefield, located in the Swiss Central Alps. We took samples along a transect at 22 sampling sites, ranging from bare soils close to the glacier terminus to densely vegetated soils up to 140 years old. In this chronosequence, increasing plant cover went along with a higher soil total organic carbon and nitrogen content, while the pH decreased [5]. The bacteria [20] and fungi [8] isolated from the bare soil near the glacier terminus were shown to be phylogenetically diverse and active in weathering.

We investigated whether all the three microbial groups, bacteria, archaea and fungi, undergo a community succession with increasing soil age, plant cover and nutrient content of the soil and whether the microbial diversity derived from SSU data changes along the chronosequence. For archaea, we also examined *Euryarchaeota* to see if they are colonizing the forefield. For fungi, we explored the community composition to find out whether it changes from a community dominated by free-living species to a more plant-associated fungal community. The small subunit rRNA genes from the different samples were analysed to determine the presence of diverse prokaryotes and eukaryotes. Soil chemical data were compared with microbial community data to identify the microbial inhabitants in the different soil ecosystems and to compare them. By simultaneously investigating the three phylogenetic groups, bacteria, archaea and fungi, along the chronosequence of a temperate glacier forefield, it should be possible to better understand the initial colonization and succession patterns of microorganisms in a changing environment.

Material and Methods

Location and Sampling

The Damma glacier forefield is located in the Central Alps, within the Central Aare Granite in Switzerland, at an altitude between 1,950 and 2,050 m above sea level. The front of the Damma glacier has been monitored by the Swiss glacier monitoring network (http://glaciology.ethz.ch/messnetz/?locale=de) and has retreated at an average rate of approximately 10 m per year since the beginning of systematic measurements in 1921, with two short expanding periods (1920–1928 and 1972–1992), which resulted in two small moraines. The precipitation is around 2,400 mm per year, and the mean annual temperature ranges from 0°C to 5°C [15].

Soil samples were taken at 22 sites along the forefield, where each site was defined by an area of 4 m^2 (Fig. 1). From each site, nine samples were taken randomly at 0–5-cm depth using an ethanol-cleaned shovel and then sieved through a 2-mm sieve to remove stones and larger plant material. They were frozen at −80°C until further processing. The texture of the sieved soil was loamy sand throughout the forefield. The youngest sites sampled for this study are 2 to 13 years old, enclosed by the glacier terminus and the 1992 moraine. The intermediate sites are located between the 1992 and the 1928 moraines and hence have soils aged between 58 and 78 years. The oldest sites are between 110 and 136 years old and extend from the 1928 moraine onwards.

Soil Physicochemical Parameters

Soil pH was determined in 0.01 M $CaCl_2$ [65]. For exchangeable metal cations, soil samples were extracted with 1 M NH_4Cl and measured using inductively coupled plasma optical emission spectrometry or, in case of exchangeable H^+ and exchangeable Al^+, through complexation and titration [64].

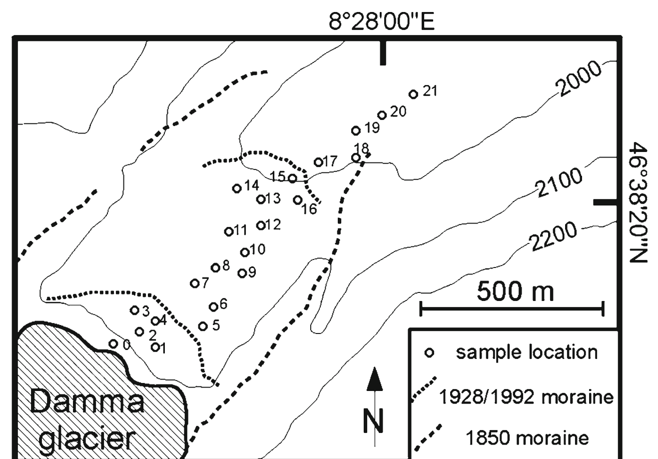


Figure 1 View of sampling sites 0 to 21 along the Damma glacier forefield showing also the two lateral moraines from 1850 and the 1928 and 1992 moraines. Sites 0 to 4 represent young, very scarcely vegetated soils (2 to 13 years), sites 5 to 16 intermediate soils (57 to 77 years) and sites 17 to 21 the oldest, well-vegetated soils (110 to 136 years). Soil age can also be approximated by the distance from the ice. The front of the glacier terminus can be noted on the *lower left* corner and the two end moraines are highlighted with *lines*, separating the age classes of the successional stages

The cation exchange capacity (CEC) was calculated as the sum of exchangeable H^+, Al^{3+}, Na^+, Mg^{2+}, K^+, Ca^{2+}, Mn^{2+}, Fe^{3+} and Zn^{2+} ($mmol_c\ kg^{-1}$). The base saturation (BS) was calculated as the sum of Na^+, Mg^{2+}, K^+ and Ca^{2+} divided by CEC (%).

Total carbon (C) and nitrogen (N) content in soils were measured using 10–50 μg dried finely ground (disk mill) soil weighed into tin capsules introduced into a Flash elemental analyzer (Thermo Fisher Scientific, Wohlen, Switzerland) operated with He as a carrier gas. The samples were combusted in the presence of O_2 in an oxidation column at 1030°C and the combustion gases passed through a reduction column (650°C). The N_2 and CO_2 gases produced were separated chromatographically and the amount measured with a thermal conductivity detector. The contents were calibrated by bracketing with a standard soil with known C and N.

The plant cover was determined using aerial photographs covering approximately 25 m^2 of the sites and calculating the total green plant cover of this area with Adobe Photoshop CS3 (Adobe Systems Incorporated, San Jose, CA, USA).

DNA Extraction and PCR Amplification

Genomic DNA was isolated in three replicates from the pooled soil samples using the Smart Helix DNA extraction kit (Venturia, Ljubljana, Slovenia) according to the manufacturer's instructions. The extracted DNA was quantified with Pico Green (Invitrogen, Carlsbad, CA, USA) and stored at −20°C until further use. Primers for the specific PCR amplification of the bacterial and archaeal 16S rRNA gene and the fungal 18S rRNA gene are documented in Table 1. Primers were obtained from Microsynth GmbH, Balgach, Switzerland. A total of 20 to 80 ng of DNA was added as template for the PCR reactions, performed in three replicates for each site. For the bacterial 16S rRNA gene, the PCR conditions were as described previously [21]. Archaeal-specific PCR reactions were carried out according to the protocol of Chin et al. [11]. Fungal-specific PCR reactions were carried out according to the protocol of Bornemann and Hartin [7].

T-RFLP Analysis

The three replicate PCR products were digested with 0.1 U of either the restriction enzyme *MspI* for bacteria, *HhaI* for archaea or *AluI* for fungi according to the manufacturer's recommendations (Catalys AG, Wallisellen, Switzerland). T-RFLP analyses were performed on ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and T-RFLP profiles were analysed using Genotyper v.3.7 NT (Applied Biosystems) according to Frey et al. [21]. The threshold for analysis was set to 100 fluorescence units. The profiles were analysed after transforming the raw peak heights in percentage values over the sum of the total peak heights detected in the samples and taking the mean of the three replicates [19].

Clone Libraries

Three clone libraries were constructed, one for site 0 (2-year-old site), one for site 6 (62-year-old site) and one for site 17 (110-year-old site). These sites were chosen because they contained the representative terminal restriction fragments (T-RFs) for the soil ages "young", "intermediate" and "old", respectively. PCR amplifications for the clone libraries were performed on the three replicate DNA extracts of each site, which were then pooled for cloning. Reactions were performed with the same primers as described above but unlabeled. The PCR products were then ligated into the vector of the pGEM-T Easy Vector System and cloned into the competent cells JM109 (Promega Corporation, Fitchburg, WI, USA), according to the manufacturer's instructions. A PCR reaction on the successfully transformed clones with the vector-specific primers M13f and M13r was performed, as described earlier in Widmer et al. [68], to check whether the length of the insert was correct. Inserts were restricted with *MspI* for bacteria, *HhaI* for archaea and *AluI* for fungi in order to select clones to be sequenced according to unique RFLP patterns (clone operational taxonomic units, OTUs). Bacteria were sequenced with the 27F primer and the two internal primers 341f and 901rev, archaea with the primers A109f and A934r and fungi with the M13f and M13r primers (Table 1). The products were sequenced with ABI 3730xl sequencer (Applied Biosystems), and the sequences were then sorted and aligned using BioEdit software (www.mbio.ncsu.edu/BioEdit/bioedit.html). A chimera check was performed on the Bellerophon server [32] and manually counter-checked with NCBI BLAST [1]. Chimeras were excluded from further analysis. The clones were also subjected to T-RFLP analysis, using the same primers as for the T-RFLP analysis of the environmental samples. The T-RFs of the clones were then compared to the in silico T-RFs of the cloned sequences. Only the T-RFs abundant in young or in old soils T-RFLP profiles were compared to the T-RFs of the clones.

Phylogeny

The sequences of bacteria, archaea and fungi were analysed by BLAST search on NCBI. Phylogenetic trees were generated by analysing the ClustalW sequence alignment performed in BioEdit using Bayesian inference with the computer program MrBayes 3.2 [33] and the LG+I+G model. A Markov chain Monte Carlo was run for 4,000,000

Table 1 List of PCR primers used in this study for T-RFLP and sequencing

Gene region	Sequence	Application in this study	References
Bacteria 16S rRNA gene			
27f – FAM	AGAGTTTGATCMTGGCTCAG-5′	T-RFLP, sequencing	[27]
1378r	CGGTGTGTACAAGGCCCGGGAACG-3′	T-RFLP, sequencing	[27]
341f	CCTACGGGAGGCAGCAG-5′	sequencing	Lopez-Gutierrez et al. (2004)
901rev	CCGTCAATTCCTTTRAGTTT-3′	sequencing	Lane (1991)
Archaea 16S rRNA gene			
A109f	ACKGCTCAGTAACACGT-5′	T-RFLP, sequencing	[11]
A934r - FAM	CTCCCCGCCAATTCCTT TA-3′	T-RFLP, sequencing	[11]
Fungi 18S rRNA gene			
nu-SSU0817-FAM	TTAGCATGGAATAATRRAATAGGA -5′	T-RFLP, sequencing	[7]
nu-SSU1536	ATTGCAATGCYCTATCCCCA -3′	T-RFLP, sequencing	[7]
M13 vector			
M13f	TGTAAAACGACGGCCAGT-5′	Vector primer, sequencing	Promega
M13r	CAGGAAACAGCTATGACC-3′	Vector primer, sequencing	Promega

generations, sampling every 100th generation, until the standard deviation of the split frequency was below 0.01. The tree was then visualized with the softwares Mesquite and FigTree. For clarity, we removed similar sequences in the trees. As outgroups, we chose the spirochete *Borrelia burgdorferi* (L36160) in the bacterial tree, a *Korarchaeota* (AF255604) for the archaeal tree and a member of the *Chytridiomycota*, *Cladochytrium* sp. (AB586077), in the fungal tree. The posterior probabilities of a node are only shown when below 75%.

Statistical Analysis

The correlation coefficients (R) with their p-values were calculated according to Pearson with the statistical program IBM SPSS statistics (IBM Corporation, Armonk NY, USA).

From the T-RFLP profiles, the Shannon diversity index (H) of the individual T-RFs was calculated according to Sigler and Zeyer [61]. The mean of the three replicate DNA extracts was always taken. Redundancy analysis (RDA) using the CANOCO program for Windows [63] was chosen to analyse the T-RFLP data combined with the environmental parameters pH, BS, C,N, PC and soil age. Initial analysis by detrended correspondence analysis revealed that the data exhibited a linear, rather than unimodal, response to the environmental variables. Furthermore, a Monte Carlo test was performed in CANOCO within the RDA to assess the significance of the correlation of T-RFLP profiles with the environmental parameters.

The number of sequence OTUs and the evenness were calculated from the sequence data using the open-source computer program Mothur [56]. The identity for a unique OTU was set to 97%. The coverage (C) was calculated according to $C=1-$(number of sequence OTUs/number of total sequences) [2].

Accession Numbers

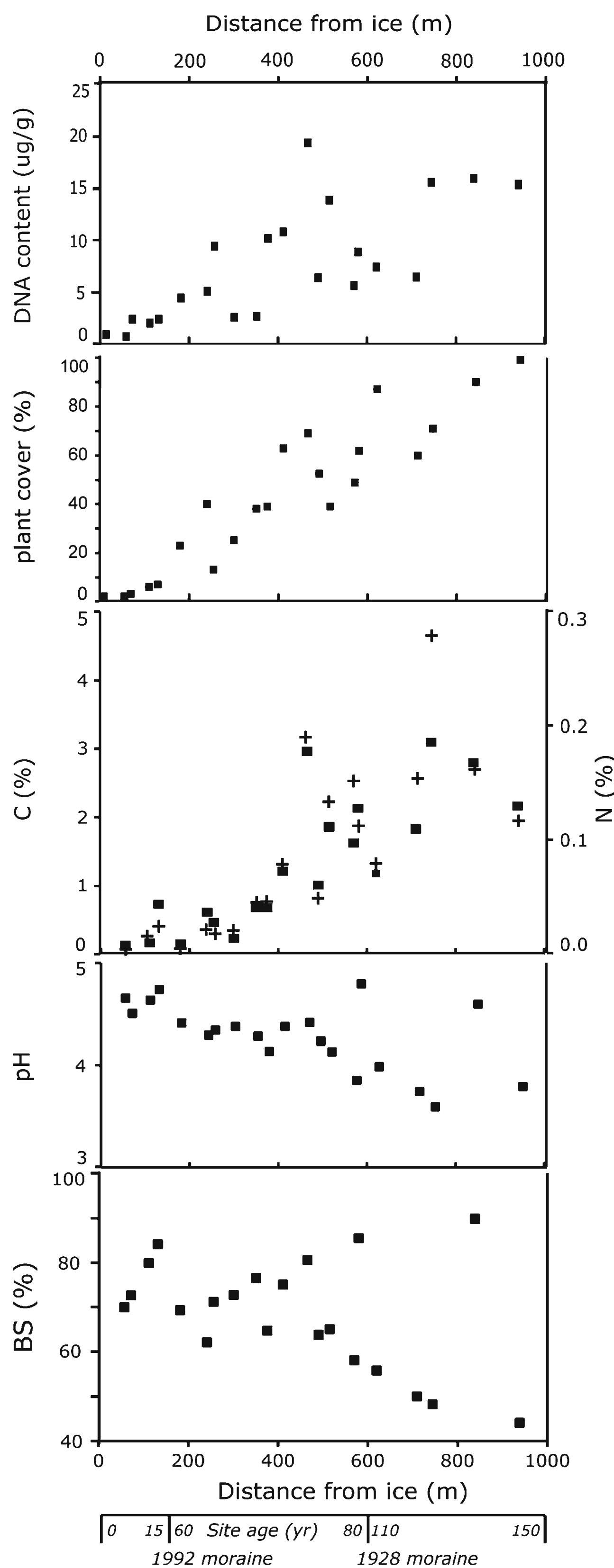
All sequences from this study were deposited in GenBank. The sequences from the bacterial libraries were assigned to accession numbers HM065582–HM065763; sequences from the archaeal libraries were assigned to accession numbers HM065764–HM065903 and the sequences from the fungal libraries to accession numbers HM065904–HM066004.

Results

Physicochemical Characteristics of the Glacier Forefield

With increasing soil age and thus increasing distance from ice (Fig. 1), the carbon and nitrogen content in the soil increased steadily from a very low C content of 0.07% in young soils to over 2% in the oldest soils ($R=0.72$, $p=0.0001$), while the N content rose from 0.01% to 0.13% ($R=0.76$, $p=0.0001$) (Fig. 2). Within the chronosequence, pH ranged from 3.8 to 4.8, with the higher values generally found at the young soil sites and the lower values at the old soil sites ($R=-0.69$, $p=0.0001$) (Fig. 2). The base saturation exhibited the same trend, between 70% and 80% at the young sites and around 50% at the old sites ($R=-0.56$, $p=0.007$) (Fig. 2). In contrast, the plant cover increased along the forefield from no or scarce vegetation at the young sites (below 50%) to 100% plant cover at the old sites ($R=0.92$, $p=0.0001$) (Fig. 2). The DNA content rose from 0.9 to

Figure 2 Environmental parameters measured along the chronosequence. DNA content, plant cover, carbon (*cross*), nitrogen (*square*), pH and base saturation (*BS*) are shown. The values are shown in percentages for all the values except for pH and DNA content (µg g^{-1} dry soil). The distance from the ice is marked on the *X*-axis together with a scale of the three age classes



15.4 µg g^{-1} dry soil along the forefield (R=0.78, p=0.0001) (Fig. 2).

Bacterial, Archaeal and Fungal Community Structures Derived from T-RFLP Profiling

The numbers of bacterial T-RFs (41 to 93) were higher than the numbers of archaeal and fungal T-RFs (Fig. 3). The Shannon diversity index (*H*) based on the T-RFLP profiles was in the range of 2.3 to 3.4, with no clear trend along the forefield (Fig. 3a). The numbers of archaeal T-RFs (0 to 41) decreased with soil age. Similarly, *H* (0.1 to 2) indicates a lower T-RF diversity of archaea in old soils compared to young soils (Fig. 3b). The numbers of fungal T-RFs [2–12] were low throughout the chronosequence. The Shannon index based on the T-RFLP profiles ranged from 0.3 to 1.2 without any clear trend (Fig. 3c).

The RDA axes 1 and 2 were found to explain 18.7% and 8% of the overall variance for bacteria (Fig. 4a), 14.7% and 8.8% for archaea (Fig. 4b) and 29% and 2.8% for fungi (Fig. 4c), within the T-RFLP data correlated to the environmental data. The microbial communities of the young, sparsely vegetated soils (2 to 13 years) were separated from the old, densely vegetated soils (110 to 136 years) for all the phylogenetic groups. Species environment correlations were 0.89 for bacteria, 0.71 for archaea and 0.68 for fungi, indicating that the data were strongly correlated with environmental parameters (Table 2). Monte Carlo significance tests revealed that the axes explain a significant amount of the variation in the bacteria data (0.001) and thus indicate a clear shift in bacterial communities. Archaeal and fungal variations, on the other hand, were not significantly explained (0.185 and 0.087) and thus only indicated a trend towards a community change along the chronosequence.

Bacterial, Archaeal and Fungal Community Compositions Derived from SSU rRNA Gene Clone Libraries

Coverage for the clone libraries was generally low (Table S1 in "Electronic supplementary material"). Only fungal sampling led to coverage of over 50% in the older soils. The cloned sequences revealed that the dominant bacterial group in this forefield were *Proteobacteria*, with 49% of the total number of sequences belonging to this group in 100% which referred to the total number of sequences of all three soil ages (Fig. 5a). *Alpha-proteobacteria* were more abundant (21%) than *Beta-proteobacteria* (16%). Both classes decreased in

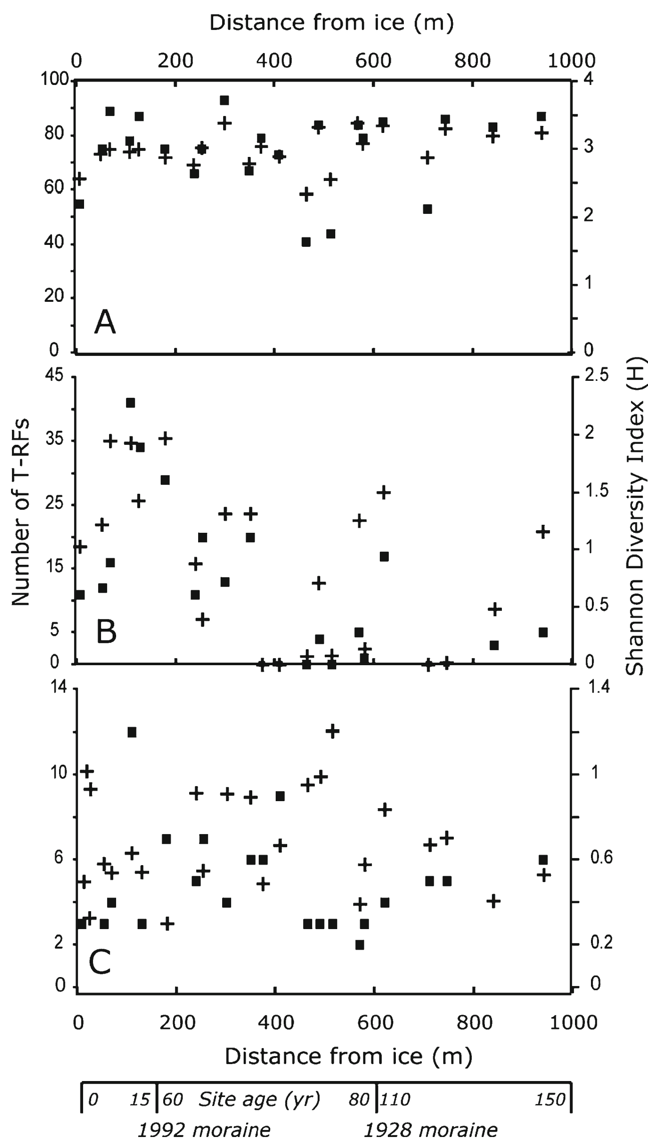


Figure 3 Number of terminal restriction fragments (*cross*) and the Shannon diversity index (*square*) are shown for bacteria (**a**), for archaea (**b**) and for fungi (**c**). The distance from the ice is marked on the *X*-axis together with a scale of the three age classes

percentage with increasing soil age. *Gamma-proteobacteria* (7%) were abundant in the young and old soils, but not in the intermediate soil. *Delta-proteobacteria* (5%) were only present in the intermediate and old soils (Fig. 5a). Comparison of the in silico T-RFs of the sequences and the T-RFLP analysis

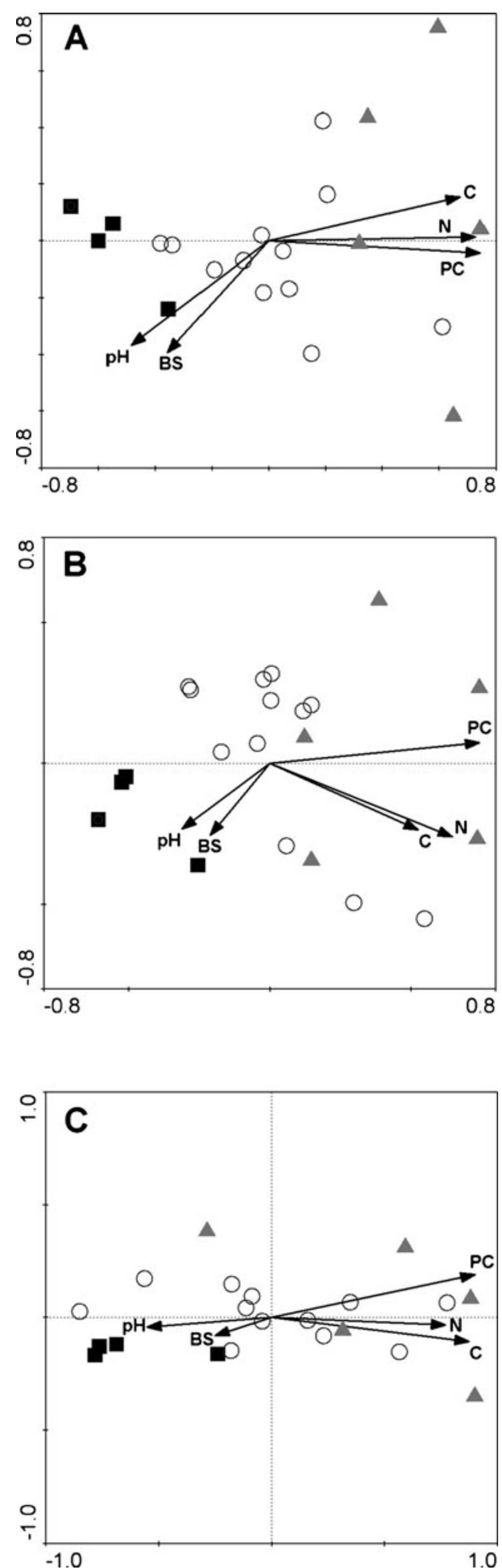


Figure 4 Ordination diagrams of RDA of T-RFLP profiling are shown for bacteria (**a**), for archaea (**b**) and for fungi (**c**) from the three successional stages of the Damma glacier forefield: young soils deglaciated for 2 to 13 years (*black squares*), intermediate soils on sites deglaciated for 57 to 77 years (*white circles*) and oldest soils on sites deglaciated for 110 to 136 years (*dark triangles*). *Arrows* indicate the environmental variables (*C* = TC (%), *N* = TN (%), *PC* = plant cover (%), *BS* = base saturation (%)). Bacterial axes explain a variance of 18.7% and 8%. For archaea, the axes explain 14.7% and 8.8%. Fungal axes explain 29% and 2.8%

Table 2 Results of RDA from T-RFLP profiling and corresponding environmental parameters. Values are shown for the first axes. The second axes explain less than 10% of the variance and thus do not account for a valuable part of the variance. The Eigenvalues are shown in the diagram of Fig. 4

	Bacteria	Archaea	Fungi
Cumulative percentage variance			
Of species data	19	15	29
Of species–environment relations	51	52	79
Species–environment correlations	0.89	0.71	0.68
Monte Carlo significance test			
F-ratio	3.7	2.8	6.1
P-value	0.001	0.185	0.087
Correlations (100×r)			
pH	−55	−28	−30
Base saturation	−40	−19	−13
Plant cover	84	67	48
C content	76	47	47
N content	82	58	41

of the environmental samples also revealed a decrease in *Alpha-proteobacteria* (T-RFs 70 bp; 125 and 129 bp) with increasing soil age, whereas here the T-RF specific for *Beta-proteobacteria* (135 bp) increased (Table 3). Other abundant classes were *Actinobacteria* (12%), *Acidobacteria* (9%) and *Cyanobacteria* (5%). The percentage of *Actinobacteria* and *Cyanobacteria* decreased drastically with soil age, whereas *Acidobacteria* appeared only from the intermediate site onwards. The T-RF assigned to *Cyanobacteria* (61 bp) could not be found in all the samples of the young soil and is thus not listed in Table 3. The T-RFs for *Actinobacteria* (138 bp and 145 bp), on the other hand, were represented in both the young and old soils, but T-RF 138 bp was more dominant in the young soils. The T-RFs specific for *Acidobacteria* (93 and 265 bp) were dominant in the young and old soils (Table 3). *Bacteroidetes* (6%) were most abundant in the intermediate soil, *Verrucomicrobia* (5%) in the young soil, *Firmicutes* (5%) in the old soil and TM7 (5%) in the young and intermediate soils. A comparison of the T-RFLP analysis of the clones and environmental samples confirmed the results for *Bacteroidetes* (206 bp) and *Firmicutes* (140 bp; 154 and 290 bp) (Table 3). *Gemmatimonadetes* (2%) and Chloroflexii (1%) were minor classes, each representing less than 2% of the total clone numbers (Fig. 5a). In the bacterial phylogenetic tree, the distribution of our clones in the classes mentioned above can

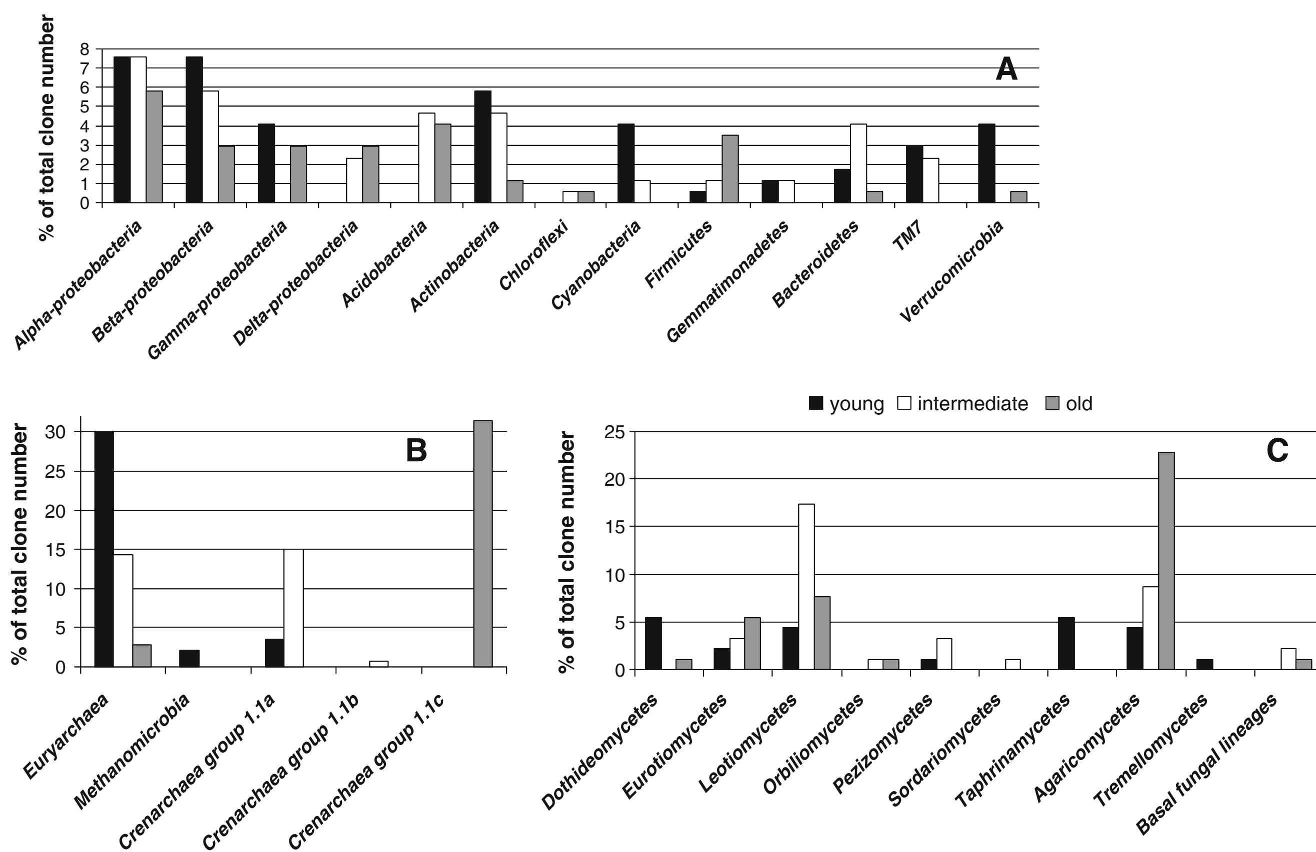


Figure 5 Comparison of phylogenetic classes over the three successional stages: 2-year-old site (young soil, *black*), 62-year-old site (intermediate soil, *white*) and 110-year-old site (old soil, *dark*). Sequences were obtained from 16S rRNA gene clone libraries of bacteria (**a**), archaea (**b**) and 18S rRNA gene clone library of fungi (**c**). Phylogenetic classes are shown in percentages of the total clone number

Table 3 Comparison of the T-RFs of the cloned sequences (in silico T-RF) with the T-RFLP profiles of the environmental samples (predicted T-RF). Only T-RFs from the environmental T-RFLP profiles present in either all the young (sites 0–4) or all the old soils (sites 17–21) are shown

Predicted T-RF	Young soils (site 0–4)	Old soils (site 17–21)	T-RF in silico from sequence	Clone name	Closest relative clone	Accession number (NCBI)	Corresponding phylogenetic class
Bacteria							
68	++	++	63	1-b44	*ncd390d05c1*	HM321946	*Gemmatimonadetes*
70	++	+	69	1-b49	*Bas-7-52*	GQ495410	*Alpha-proteobacteria*
74	++	+	60	18-b135	*WT15*	HQ738646	*Gamma-proteobacteria*
93	+	++	96	18-b203	*Acidobacteria Amb_16S_1563*	EF019024	*Acidobacteria*
125	++	+	128	18-b70	*Elev_16S_841*	EF019683	*Alpha-proteobacteria*
129	++	+	135	1-b69	*hfmB137*	AB600435	*Alpha-proteobacteria*
135	+	++	141	18-b192	*Eubacterium WD2102*	AJ292626	*Beta-proteobacteria*
138	++	+	132	1-b14	*Pseudonocardiaceae Elev_16S_454*	EF019272	*Actinobacteria*
141	+	++	147	18-b199	*ncd1274h02c1*	JF086927	*Firmicutes*
146	++	++	149	18-b73, 18-b88	*ncd1276f09c1*, *Frankineae MWM1-27*	JF107313, HQ674868	*Firmicutes*, *Actinobacteria*
153	++	+	152	18-b186	*Gamma-proteobacterium AKYG891*	AY922074	*Gamma-proteobacteria*
206	++	++	212	1-b25	*FFCH12595*	EU133712	*Bacteroidetes*
265	++	+	269	18-b103	*Ellin5095*	AY234512	*Acidobacteria*
291	++	+	297	18-b38	*RABS_A82*	HQ660787	*Firmicutes*
Archaea							
73	++	+	72	18-a37	*LAa02.18*	EU782009	*Euryarchaeota*
120	+	++	114	18-a111	*B51*	HQ233425	*Crenarchaeota 1.1c*
135	++	−	135	1-a48	*CaR3b.h03*	EU244277	*Euryarchaeota*
157	++	+	156, 154	1-a56, 18-a118	*SDG-195*, *mrR1.11*	EU365243, FJ746506	*Euryarchaeota*
162	+	++	168	18-a17	*Crenarchaeote PsRTC_28*	GU815318	*Crenarchaeota 1.1c*
Fungi							
100	++	++	101	18-f27	*Soil.06.17*	AY099411	*Basal fungal lineages*
271	++	+	276	18-f102, 18-f123	*S_Canopy_450_01_07*, *fungus 26_16*	AY382458	*Agaricomycetes*
448	+	++	452, 451, 455	1-f177, 1-f185, 18-f155	*Cladophialophora minutissima voucher UAMH 10710*	EF016375	*Eurotiomycetes*
450	++	++	456, 452, 455, 455	1-f138, 18-f26, 18-f189, 18-f133	*M228*, *Ceraceomyces borealis CFMR:L-8014*, *soil fungus 849*, *6034RhFu*	EU940063, GU187624, GU568164, GU201458	*Leotiomycetes*, *Agaricomycetes*, *Agaricomycetes*, *Eurotiomycetes*

++ T-RF present at all sites, + T-RF present at a few sites, − T-RF not present

be seen. It also indicates that our cloned bacterial sequences were highly similar to cloned sequences from other cold habitats (Fig. S1 in "Electronic supplementary material").

The fraction of *Crenarchaeota* increased with soil age (51%) (Fig. 5b). They belonged mainly to the group 1.1c *Crenarchaeota* (62%), which was only present in the old site. The group 1.1a (37%) appeared in both the young and intermediate soils, whereas the group 1.1b (1%) was only in the intermediate soil (Fig. 5b). Most cloned sequences retrieved from the young and intermediate soils were *Euryarchaeota* (49%), of which only 4% could be assigned to a taxon, *Methanomicrobia*. The remaining 96% could be assigned to *Euryarchaeota*, but not to specific taxa (Fig. S2 in "Electronic supplementary material"). This clearly indicates a community shift from *Euryarchaeota* in bare soils to *Crenarchaeota* in densely vegetated soils. A comparison of the in silico T-RFs of the sequences and the T-RFLP analysis of the environmental samples confirmed this shift as the abundance of T-RFs assigned to *Euryarchaeota* (73 bp; 135 and 157 bp) was higher in the young soils and the abundance of T-RFs assigned to *Crenarchaeota* (120 and 162 bp) was higher in the old soils (Table 3).

The fungal community was mainly composed of the phyla *Ascomycota* (60%) and *Basidiomycota* (37%). The other 3% are from the basal fungal lineages (Fig. 5c). Cloned sequences retrieved from the young soil belonged mostly to *Ascomycota* and the sequences retrieved from the old soil to *Basidiomycota* (Fig. S3 in "Electronic supplementary material"). *Leotiomycetes* (49%) and *Eurotiomycetes* (18%) were the most abundant *Ascomycota* classes (Fig. 5c). They were found in the clone libraries of all three soil ages. The proportion of cloned sequences belonging to *Eurotiomycetes* increased with soil age, whereas *Leotiomycetes* were the most abundant genera in the intermediate soil. The classes *Dothideomycetes* (11%) and *Taphrinomycetes* (9%) were only present near the glacier terminus. *Pezizomycetes* (7%), *Sordariomycetes* (2%) and *Orbiliomycetes* (4%) were the other fungal classes found (Fig. 5c). On the other hand, *Basidiomycota* were dominated by *Agaricomycetes* (97%), which rose in numbers of cloned sequences with soil age. In the fungal T-RFLP analysis of the clones, one T-RF represented many phylogenetic classes (450 bp). The T-RF for *Eurotiomycetes* (448 bp) confirmed the increase in the class along the gradient, whereas the T-RF for *Agaricomycetes* (271 bp) did not. Phylogenetic affiliations of the fungal-specific T-RFs from the environmental samples proved to be difficult to assign as the T-RFs were all of a similar size without great variation (Table 3).

Discussion

This is the first simultaneous investigation of bacteria, archaea and fungi along the chronosequence of a temperate glacier forefield. The Shannon diversity indices from T-RFLP profiling (Fig. 3) for bacteria were remaining constant along the chronosequence. This finding is in accordance with that of Schutte et al. [58], but in contradiction to Nemergut et al. [48] who detected an increase in diversity with soil age in an alpine forefield. These different results suggest that the factors influencing bacterial diversity are not solely related to soil age but also to other factors such as climatic conditions, bedrock composition, soil texture and pH [40, 44].

The RDA and clone library analyses indicated a change in the community structure from bare to densely vegetated soils for bacteria, archaea and fungi and thus a microbial succession along this forefield (Figs. 4 and 5; Table 3). Besides physical factors, in the initial phase of colonizing bare soils, the bacterial community composition is influenced by the chemical composition of the rock. Thus, different minerals are colonized by different bacteria [23]. It has also been shown that plant root exudates have a strong influence on cell counts even 10 cm away from the roots [46], which implies that plant colonization is important for microbial communities [15] and that the bacterial community structures therefore change with altering soil parameters and plant colonization. Limited C and N sources on bare soil select for a specific bacterial community. Mainly C and N fixers [12, 62] and weathering associated microorganisms can survive here [20]. Some microorganisms that utilize C and N from atmospheric deposition and from plants and mosses can also be expected to be present. The N-fixing diazotrophic community was shown to be abundant and diverse in the Damma glacier forefield and is a potentially important contributor to the N input in this environment [14]. In the older, vegetated soils, decomposers of plant material and nitrate reducers were thriving and outcompeted those bacteria that are successful in bare soils [13, 58]. This is also indicated in the RDA as the change in bacterial community structure is clearly correlated to the soil parameters (Fig. 4a).

The most frequently clone sequences and T-RFs found in environmental samples belonged to *Proteobacteria* (Fig. 5a; Fig. S1 in "Electronic supplementary material"; Table 3). This is consistent with the findings of Kersters et al. [39], who demonstrated that this phylum is phenotypically very versatile. *Proteobacteria* consist of numerous phototrophs, photoheterotrophs and chemolithotrophs, which are advantageous traits in this initial ecosystem with limited nutrient resources [30]. Within *Actinobacteria*, *Actinomycetales* were the dominant order (56.3%) (data not shown). *Actinomycetales* are active in the decomposition of organic materials in soil, including lignin and other recalcitrant polymers [27]. As they were mainly present near the glacier terminus, they may decompose materials from dead microbes, arthropods, fungal spores and pollen [25, 34] and other organics deposited by air on the forefield [53].

Cyanobacteria are photoautotrophic organisms that survive on bare rocks as the first colonizers [16], and in lichens they have a symbiotic relationship with fungi. Initial colonization of barren soils by *Cyanobacteria* is known to raise the nutrient status as their photosynthetic activities provide a significant input of C and N into the upper soil layers, which is important for the further succession of heterotrophs [18, 57].

Cyanobacteria were also shown to form the dominant ground cover for 60 years on an Arctic glacier chronosequence before they declined [29]. This was also the case in our clone libraries. However, the T-RF specific for *Cyanobacteria* was only present in a few of the young soils. In general, however, the T-RF for *Cyanobacteria* was less abundant in the old soils than in the young soils (data not shown).

Acidobacteria are ubiquitous in soil samples around the world in various habitats [42], including endolithically in granite rock [31], which implies that they should be capable of surviving on this forefield. Interestingly though, they were not found in the clone library of the bare soil, but the T-RFs assigned to *Acidobacteria* were present in the T-RFLP analysis from the young soils. The low coverage of the clone libraries could account for these contrasting results. Thus, autotrophs and chemolithotrophs as *Cyanobacteria* and *Proteobacteria* declined with rising soil age.

Archaea are known to be able to live under extreme conditions, but recently archaea that only adapted to moderate environmental conditions have also been found [6, 10]. Unlike bacteria and fungi, the Shannon diversity index of archaeal T-RFLP profiles declined along the chronosequence (Fig. 3b), which is contradictory to the observations made by Nicol et al. [50] who reported an increase in diversity along a successional gradient. The environmental conditions occurring in bare soils are extreme compared to those in vegetated soils where there are fewer moisture and temperature fluctuations and more organic matter. Our findings suggest that bare soils provide a large range of microenvironments where different archaeal species can survive, especially favouring those adapted to more extreme conditions. Indeed the RDA demonstrated that the archaeal community structure in the young soils differed from that in the old soils (Fig. 4b). Sequence analysis of archaeal clone libraries and comparison of the in silico T-RFs of the sequences with the predicted T-RFs of the environmental samples confirmed this finding, showing a shift from a *Euryarchaeota*-dominated archaeal community in the young soil to a *Crenarchaeota*-dominated archaeal community in the old soil (Fig. 5; Table 3; Fig. S2 in "Electronic supplementary material"). To the best of our knowledge, this is the first time the dominance of *Euryarchaeota* sequences on bare soil close to a glacier terminus has been shown. Contrary to our findings, Nicol et al. [50] reported a dominance of *Crenarchaeota* over the whole forefield. Their finding might be explained by the fact that they used different primers. Archaea, in general, are known to be influenced mainly by soil properties and not plant cover [51]. *Crenarchaeota* are often found in plant rhizospheres [66] but can also particularly adapt to soils with a low nutrient status [3]. They have also been found to be resistant to freeze–thaw cycles [54]. *Crenarchaeota* are also influenced by soil organic matter [69], which is barely present in the young soils on this forefield; thus, the *Euryarchaeota* found here are better adapted to the harsh environment with such low nutrient conditions. We suggest that, in the Damma glacier forefield, the rhizosphere-inhabiting *Crenarchaeota* species predominantly colonize the later successional stages of the forefield, whilst *Euryarchaeota* species predominantly colonize the bare soils. Alternatively, the *Euryarchaea* might be outcompeted by *Crenarchaeota*, and/or other microorganisms, in rhizosphere soils rich in organic matter.

We studied fungi using primers detecting the 18S rRNA gene [36]. As with bacteria and archaea, we also found distinctly different fungal communities in the bare and the vegetated soils. This may be related to the establishment of vegetation along the chronosequence as fungi are closely associated with plants [51]. Here the fungi found were either saprophytic or mycorrhizal, with some exceptions like *Taphrinamycetes* which are either plant parasites or pathogens. Thus, a change in the community structure can be expected as the nutrient supply and plant cover change along the gradient. The clone libraries show that the fungal community changed from *Ascomycota* which are able to live on rocks [22] or as dark septate endophytes like some *Dothidiomycetes* to the *Basidiomycota*, which colonized the vegetated soils (Fig. 5c; Fig. S3 in "Electronic supplementary material"). These findings corroborate those of Jumpponen [36] on the Lyman glacier forefield. The RDA also supports this interpretation as the fungal community structure in bare soils is negatively correlated to the C and N content in the soil and to plant cover, whereas the fungal community structures in old soils correlated positively with these parameters (Fig. 4c). We are aware that the plant community also strongly influences the soil microbial communities [15, 46], at least in the older soils [67], but we did not specifically investigate plant–microbial interactions. Rather, we consider the changing plant community to be part of the environmental conditions, and thus an external factor for the microbial community in the same sense as C and N.

Of the *Ascomycota* detected, most species belonged to the subphylum *Pezizomycotina*. This includes *Lecanoromycetes,* to which most of the lichen-forming fungal species belong [45]. Lichens are known to colonize rocks and stones and are important in bioweathering [26, 41]. Dark septate endophytes, as some *Dothidiomycetes*, can enhance plant growth by improving nutrient and water acquisition, especially in unfavourable environments, which could explain why *Dothidiomycetes* were found mainly in the young soil [37]. The dominance of *Basidiomycota* in the older soils can

be explained by the mycorrhizal associations of the *Agaricomycetes* with *Pinaceae* and angiosperms, including first-colonizing plants on glacier forefields [28, 47], and the plant litter decomposing activities of other *Basidiomycota* classes [43].

It has been suggested that fungi are more influenced by plant growth than prokaryotes, which are more influenced by soil properties [51]. This would explain the successional trend in community structure and composition of archaea and fungi as both plant colonization and soil properties change along the forefield. Soil pH has also been suggested as one of the main factors influencing microbial communities in soil [17]. Bacteria are the most competitive regarding the availability of simple organic substrates and this is probably reflected in the pH [69]. Bacteria may be able to outcompete the other groups for resources, thus maintaining a constant SSU diversity and having the community structure that changed the least along the forefield.

Very few fungal T-RFs present in the environmental T-RFLP profiles could be found in the in silico analysis of the clones. The restriction site seems to be located in close proximity in all phylogenetic classes found here. Thus, many T-RFs included multiple phylogenetic groups. Most likely, this is due to high sequence conservation of the fungal 18S rRNA gene [2]. With this close proximity of the fungal *AluI* restriction site, we may have underestimated the fungal T-RF diversity. In all analyses, it should be noted that differences can occur between the size of theoretical digest (in silico T-RF) and the actual size from the capillary sequencer (predicted T-RF) (Table 3) [38, 55]. Despite such differences, we were still able to confirm the increase or decrease in certain bacterial, archaeal or fungal phylogenetic classes in the clone library by comparing in silico T-RFs of the clones to the environmental T-RFLP profiles and assigning them to specific classes.

Overall, we were able to successfully link the two approaches, T-RFLP analyses and clone libraries and to show that bacteria, archaea and fungi all exhibit successional patterns along a glacier forefield chronosequence that range from bare soils to >100-year-old vegetated soils with high organic matter content. The autotrophic *Cyanobacteria* and the versatile *Proteobacteria* appeared to decline along the gradient, but a higher number of sequences from heterotrophs were found at the older sites. The archaea underwent a distinct community shift from a dominance of *Euryarchaeota* in the bare soils to a dominance of *Crenarchaeota* in vegetated soils. The fungi showed a community change from an *Ascomycota*- to a *Basidiomycota*-dominated community along the chronosequence. RDA analysis showed that the environmental factors pH, base saturation, carbon and nitrogen content and plant cover can explain 25–30% of the community changes. Further research is needed to determine other potential drivers of the community shifts, such as microclimatic conditions, and other nutrients. However, an inherent variability in the nature of glacier forefields may lie behind the majority of the observed differences.

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