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Moss and underlying soil bacterial community structures are linked to moss functional traits

Ingeborg J. Klarenberg[1,2,3] | **Christoph Keuschnig**[4,5] | **Alejandro Salazar**[6] | **Liane G. Benning**[5,7] | **Oddur Vilhelmsson**[1,8]

[1]Natural Resource Sciences, University of Akureyri, Akureyri, Iceland

[2]Faculty of Life and Environmental Sciences, University of Iceland, Reykjavík, Iceland

[3]Department of Ecological Science, Vrije Universiteit Amsterdam, Amsterdam, Netherlands

[4]Environmental Microbial Genomics, Laboratoire Ampère, CNRS, École Centrale de Lyon, Écully, France

[5]German Research Centre for Geosciences (GFZ), Interface Geochemistry, Potsdam, Germany

[6]Faculty of Environmental and Forest Sciences, Agricultural University of Iceland, Reykjavík, Iceland

[7]Department of Earth Sciences, Free University of Berlin, Berlin, Germany

[8]BioMedical Center, University of Iceland, Reykjavík, Iceland

Correspondence
Ingeborg J. Klarenberg
Email: ingeborg.klarenberg@gmail.com

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Abstract

Mosses are among the first colonizing organisms after glacier retreat and can develop into thick moss mats during later successional stages. They are key players in N_2 fixation through their microbiome, which is an important process for nutrient buildup during primary succession. How these moss–microbe interactions develop during succession is not well studied and is relevant in the light of climate change and increased glacier retreat. We examined how the bacterial communities associated with two moss species of the genus *Racomitrium* and the underlying soil, as well as moss traits and nitrogen fixation, develop along a successional gradient in the glacier forefield of Fláajökull in southeast Iceland. In addition, we tested whether moss functional traits, such as total carbon (TC) and total nitrogen (TN) contents, moss moisture content, and moss shoot length are drivers of moss and underlying soil bacterial communities. Although time since deglaciation did not affect TN and moss moisture contents, TC and shoot length increased with time since deglaciation. Moss and underlying soil bacterial communities were distinct. While the soil bacterial community structure was driven by moss C/N ratios, the moss bacterial community structure was linked to time since deglaciation, moss C/N ratio, and moss moisture content. Moss N_2-fixation rates were linked to bacterial community composition and *nifH* gene abundance rather than moss TN or time since deglaciation. This was accompanied by a shift from autotrophic to heterotrophic diazotrophs. Overall, our results suggest that there is little lateral transfer between moss and soil bacterial communities and that moss traits affect moss and soil bacterial community structure. Only moss bacterial community changed with time since deglaciation. In addition, moss N_2-fixation rates are determined by bacterial community structure, rather than moss traits or time since deglaciation. This study on the interplay between succession, mosses, soils, and their bacterial communities will inform future work on the fate of newly exposed areas as a result of glacier retreat.

KEYWORDS
carbon, glacier retreat, moss, moss bacterial communities, moss traits, nitrogen, nitrogen fixation, plant–soil–microbe interactions, primary succession, soil, soil bacterial communities

INTRODUCTION

Formerly ice-covered terrains are becoming increasingly exposed as glaciers retreat due to climate change (Roe et al., 2017). Such glacier forefields are subject to rapid ecosystem development, with microbial communities as the first colonizers. These early colonizing microbial communities are responsible for the first stages of soil development, which often involves the formation of biological soil crusts capable of stabilizing the soil and fixing carbon (C) and nitrogen (N) (Bradley et al., 2014; Breen & Lévesque, 2008). The subsequent increase in C and N availability facilitates the colonization of other organisms, such as mosses (Vilmundardóttir et al., 2015b). Mosses can develop into thick moss mats during succession, especially in regions with high precipitation and little competition from higher plants (Tallis, 1958). Moss establishment further enhances soil development in newly exposed terrain, by contributing to N (Arroniz-Crespo et al., 2014; Bowden, 1991; Vilmundardóttir et al., 2015b), retaining moisture, and contributing to organic matter buildup (Vilmundardóttir et al., 2015b; Wietrzyk-Pełka et al., 2020), which additionally promotes soil microbial growth (Bardgett & Walker, 2004). Thus, while microbial communities can facilitate moss establishment, mosses influence underlying soil microbial communities via leachate, similar to how different litter types influence soil microbial communities (Fanin et al., 2014; Young et al., 2022). Despite an increasing number of studies linking the development of soil microbial communities to establishment of plants in glacier forefields (Arroniz-Crespo et al., 2014; de Mesquita et al., 2017; Knelman et al., 2012, 2018), we have a very limited understanding of the dynamics of moss-associated bacterial communities during ecosystem development in these environments. The interplay between mosses, their bacterial communities, and the soil they colonize could be important processes for future carbon accumulation in newly deglaciated areas.

Due to their diazotrophic microbiome (Ininbergs et al., 2011), mosses are the most important source for new N in Arctic ecosystems (Rousk et al., 2017). As glacier forefields are typically nutrient-limited, moss microbiomes may be key players in biogeochemical N cycling during primary succession (Arroniz-Crespo et al., 2014). N_2-fixation rates are variable and can be influenced by moss species (Jean et al., 2020; Stuart et al., 2020), N availability (Arroniz-Crespo et al., 2014), moisture (Rousk et al., 2018), temperature (Rousk, 2017), diazotroph composition (Ininbergs et al., 2011), diazotroph abundance (Arroniz-Crespo et al., 2014), and diazotroph activity (Warshan et al., 2016) throughout succession.

Moss-associated bacterial community composition may be driven by host identity (Holland-Moritz et al., 2018). Moss traits such as C and N contents, may also affect moss-associated bacterial community composition, similar to how phyllosphere microbial taxa are linked to leaf traits (Laforest-Lapointe et al., 2017; Li et al., 2018). These moss traits can change during succession. For instance, *Sphagnum* and bryophyte C/N ratio increased with peatland succession (Laine et al., 2021) and time since deglaciation in Chilean glacier forefields (Arroniz-Crespo et al., 2014). These changes might subsequently affect the composition of the moss microbiome.

The development of a moss microbiome during succession may furthermore depend on where the microbes are inherited from (Poosakkannu et al., 2017). Plant-associated microbial communities are thought to be mainly inherited from the surrounding soil, which is also referred to as horizontal transfer (Compant et al., 2019). While mosses might not have a large rhizosphere, some have rhizoids and are thus connected to the soil. In higher plants, microorganisms can also be transferred vertically, via the seed (Hardoim et al., 2012). For mosses, microbial organisms might indeed be transferred between the sporophyte and the gametophyte (Bragina et al., 2012) or via vegetative regeneration (Tallis, 1959). Depending on which source is more important for the composition of moss-associated bacterial communities, successional changes in soil microbial communities can be reflected in changes in the moss microbiome, or alternatively the moss microbiome may stay relatively stable throughout the development of a succession.

Here we examine the bacterial communities of two moss species of the genus *Racomitrium* and the underlying soil along a successional gradient in the glacier forefield of Fláajökull, in southeast Iceland. Mosses of the genus *Racomitrium* are important colonizers in Icelandic glacier forefields (Glausen & Tanner, 2019; Vilmundardóttir et al., 2015a).

We hypothesized that: (1) moss total N (TN) and moss total C (TC) increase with time since deglaciation;

(2) changes in moss functional traits (C/N ratio, TN, and moss moisture content) and time since deglaciation lead to shifts in moss-associated bacterial communities and the underlying soil bacterial community. We also hypothesized that moss-associated N_2-fixation rates and *nifH* gene abundance (3) will decrease with time since deglaciation as TN increases since high levels of N availability could reduce physiological needs to fix N, (4) increase with moss moisture content, and (5) depend on bacterial community composition.

MATERIALS AND METHODS

The chronosequence we studied lies in the proglacial area of Fláajökull glacier (64.328124°; −15.527791°), which is an outlet glacier on the southeastern side of the Vatnajökull icecap (Figure 1). The Fláajökull glacier forefield is characterized by a number of moraine ridges and other landforms such as drumlins and eskers (Evans et al., 2016; Jónsson et al., 2016). The oldest moraine dates from the glacier's furthest advance toward the end of the Little Ice Age in 1894 (Hannesdóttir et al., 2015). The extent of the glacier in the last 120 years has been estimated using multiple dating methods, including glaciological methods, lichenometry, and historical records (Dabski, 2002; Evans et al., 2016; Icelandic Glaciological Society, 2018). Our furthest sampling point in 2018 lays more than 3000 m from the front of the glacier. The length of the transect was approximately 1.70 km.

The two closest weather stations are located in Fagurhólsmýri (72 km) and Höfn (18 km), which have a mean annual temperature of 4.8 and 4.6°C, respectively, and a mean annual precipitation of 1814 and 1381 mm, respectively. The climate can be described as subpolar oceanic (Einarsson, 1984).

The substrate in the glacier forefield is characterized by gravel, silt, and sand (Jónsson et al., 2016) and the soils are classified as cambic vitrisols and further from the glacier as andosols (Arnalds & Óskarsson, 2009).

The area closest to the glacier is mostly unvegetated, with some scattered mosses and lichens. Moss cover (mainly *Racomitrium* sp.) increases with distance from the glacier with 25%–50% cover on the oldest moraine (Wojcik et al., 2020).

Sampling

We collected samples of moss and underlying soil in May 2018. Samples were taken in triplicate along one transect on moraine ridges, at the same locations where Wojcik et al. (2020) collected soil samples (Figure 1).

Moss samples were collected aseptically with a tweezer. Soil samples of 10-cm depth were taken just below the moss cover with a sterilized (with ethanol) hand corer. We collected a total of 27 moss and 27 soil samples. After collection, samples were transported on ice packs for one day and stored at −20°C until further analyses.

The moss samples were split into three parts, one for moss species determination and acetylene reduction assays (ARAs), one for biogeochemical analysis, and one for DNA extraction.

Additional soil samples were taken in late April 2021, to measure pH and soil moisture. These soil samples were collected at the same coordinates as the samples taken in 2018 and were taken of soil under moss cover and additionally of bare soil. Methods and results of these measurements are given in Appendix S1.

Moss shoot length, moss moisture content, and chemical analysis

Moss shoot length was measured for five shoots of each sample. Moss samples were dried at 70°C for 24 h and analyzed for field moisture content. Samples were subsequently milled to a fine powder, and the TN and TC contents and the carbon isotopic composition ($\delta^{13}C$) were analyzed. The analysis was carried out at GFZ Potsdam using a mass spectrometer (DELTAplusXL, ThermoFisher) coupled via a ConFlowIII interface with an elemental analyzer (Carlo-Erba NC2500). The analytical precision for $\delta^{13}C$ was 0.2% and for TC and TN it was 0.01% and replicate determinations showed a standard deviation <0.02%.

Moss N_2-fixation rates

Moss N_2-fixation rates were assessed using the ARA method (Hardy et al., 1968). The upper 5 cm of five shoots of each moss sample were weighed and wetted until saturated and then acclimated for 24 h at 15°C in 22-mL vials. Then, we replaced 10% of the headspace (2.2 mL) with acetylene and incubated the samples at 15°C, under 60 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) for 24 h in a growth chamber (Termaks series 8000, Bergen, Norway). Ethylene and acetylene were quantified by gas chromatography. Acetylene reduction rates were expressed as ethylene per gram dry mass (field weight) of the moss per day (as in Hardy et al., 1968).

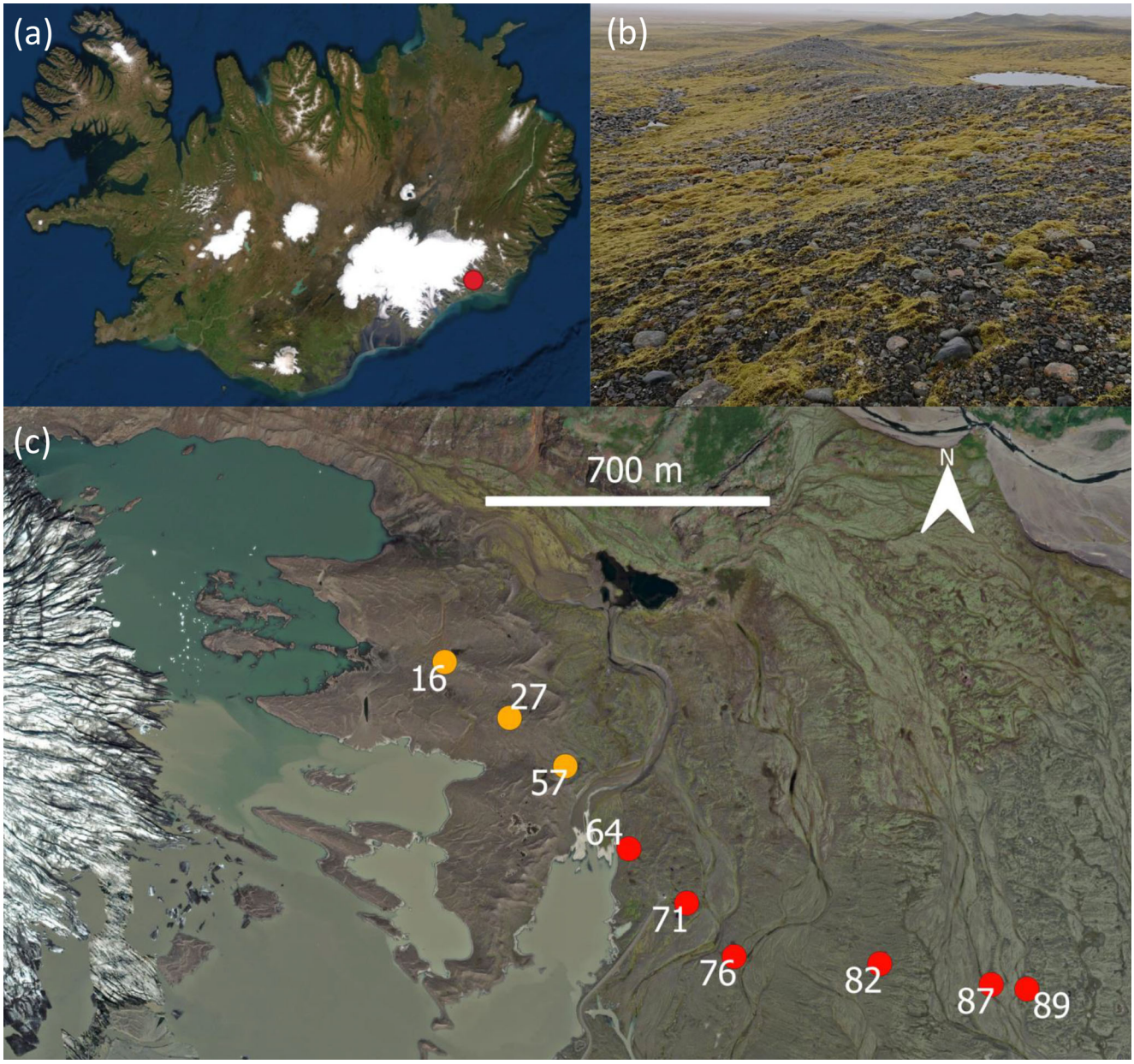

FIGURE 1 Overview of (a) the location of the Fláajökull glacier forefield, (b) *Racomitrium* spp. at the sampling site, and (c) the sampling locations along the chronosequence. The sites with *R. ericoides* are depicted in yellow and the sites with *R. lanuginosum* are in red. At each sampling location the time since deglaciation in 2018 and year of deglaciation are indicated. Time since deglaciation was determined from the study by Evans et al. (2016) and the Icelandic Glaciological Society (2018). The aerial and satellite images that form the basis for panels (a) and (c) are in the public domain; the photo in panel (b) was taken by Ingeborg Klarenberg in the present study.

DNA extraction

DNA was extracted for quantification of *nifH* and 16S rRNA gene sequencing. Before nucleic acid extraction, moss samples were ground in liquid N. DNA from the soil and the moss samples was extracted using the DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. DNA concentrations were assessed with a NanoDrop (NanoDrop Technologies, Wilmington, DE).

Quantitative real-time PCR of *nifH* genes

Quantification of *nifH* genes was performed by quantitative PCR (Corbett Rotor-Gene) using the primer set PolF/PolR (Poly et al., 2001). We confirmed the specificity of the *nifH* primers for our samples by Sanger sequencing of 10 clone fragments. Standards for *nifH* reactions were obtained by amplifying one cloned *nifH* sequence with flanking regions of the plasmid vector

using the M13 primer sites on the plasmid (TOPO TA cloning Kit, Invitrogen). Standard curves were obtained by serial dilutions (10^6 to 10^1 copies per reaction; $E = 0.9–1.1$, $R^2 = >0.99$ for all reactions). Each reaction had a volume of 20 μL, containing 10 μL of 2× QuantiFast SYBR Green PCR Master Mix (QIAGEN), 0.2 μL of each primer (10 μM), 0.8 μL of Bovine Serum Albumin (5 μg/μL), 6.8 μL of RNase free water, and 2 μL of template. The cycling program was 5 min at 95°C, 30 cycles of 10 s at 95°C and 30 s at 60°C. Samples with less than 10 *nifH* gene copies per microliter reaction were considered negative.

Sequencing and bioinformatics

Library preparation and paired-end (2 × 300 nt) sequencing of the V3–V4 region of the 16S rRNA gene on an Illumina HiSeq 2500 platform was performed by the Beijing Genomics Institute, using 338F/806R primer pair (Klindworth et al., 2013) and the standard Illumina protocol. We processed the raw sequences using the DADA2 pipeline (Callahan et al., 2017, 2016) (DADA2 version 1.24.0 in R version 4.2.1), which does not cluster sequences into operational taxonomic units (OTUs) but uses exact sequences or amplicon sequence variants (ASVs). We used the DADA2 function filterAndTrim to truncate forward and reverse reads and to filter out reads with ambiguous nucleotides, reads with a quality score less than or equal to 11, and reads with more than 2 expected errors. Forward reads were truncated at 250 bp and reverse reads at 220 bp. Errors introduced by PCR amplification and sequencing were modeled using the function learnErrors and this information was then used to infer sequences with the function dada. To infer ASVs, we used the function mergePairs. To identify and remove chimeras, we used the function removeBimeraDenovo. Assembled ASVs were assigned taxonomy to the SILVA (version 132) database (Quast et al., 2013) using the Ribosomal Database Project (RDP) naïve Bayesian classifier (Wang et al., 2007) in DADA2. We retained 1,959,094 reads (over 21,276 ASVs) from the DADA2 pipeline, after which we removed ASVs assigned to chloroplasts and mitochondria, and singletons, after which a total of 1,020,635 reads (over 13,108 ASVs) remained. Then we used prevalence filtering to keep ASVs present in at least 5% of the samples. In total, for 47 samples, a total of 721,269 reads remained (with on average 14,142 reads per sample) over 2972 ASVs. To account for uneven sequencing depths, the data were normalized using cumulative sum scaling (CSS) (Paulson et al., 2013).

Statistics

We used linear models (lm from the R package "stats" version 4.2.1) to investigate the responses of TC, TN, C/N ratio, moss tissue $\delta^{13}C$, N_2-fixation rates, *nifH* gene abundance, as well as richness and diversity of the soil- and moss-associated bacterial communities to moss species and time since deglaciation. We used a post hoc Tukey test to mean differences in richness and diversity of bacterial communities between moss species and underlying soil.

We used principal coordinates analysis (PCoA) based on weighted UniFrac distances to display the beta diversity between soil and moss bacterial communities. To explore the effect of time since deglaciation and moss traits on the soil and moss bacterial communities, we used constrained analysis of principal coordinates (CAP) based on weighted UniFrac distances with the "phyloseq" function ordinate (McMurdie & Holmes, 2013). To test the effect of time since deglaciation and moss traits on the bacterial community composition of the mosses and the underlying soil, we used permutational multivariate analysis of variances (PERMANOVAs) on weighted UniFrac distance matrices (adonis2 from the R package "vegan," version 2.6.2; Oksanen et al., 2022). We used moss species as strata in the PERMANOVAs to check whether time since deglaciation and moss traits could explain variation in the bacterial communities in the whole dataset, but we also ran PERMANOVAs on the two moss species separately. To avoid multicollinearity in the linear regression, we only included explanatory factors in the PERMANOVAs with correlation coefficients lower than 0.7 (Appendix S1: Table S1).

To identify soil- and moss-associated bacterial taxa whose $\log_2$-fold change increases or decreases with time since deglaciation, we used the R package "DESeq2" (Love et al., 2014). We used the non-normalized data and an adjusted p value cutoff of 0.1. For the moss-associated taxa, we included moss species and time since deglaciation in the model (species + time since deglaciation), to correct for moss species, similar to the linear models.

To explore the direct and indirect relationships between time since deglaciation, moss moisture content, TN, moss-associated bacterial community structure, and N_2 fixation, we constructed a structural equation model (SEM) (using the R package "lavaan"; Rosseel, 2012). For moss-associated bacterial community structure, we used the position on the first PCoA axis, based on a PCoA based on weighted UniFrac distances of only the *R. lanuginosum* samples. The reason for this choice was that controlling for moss species in the SEM was not possible, and thus we only used the data from *R. lanuginosum* for the SEM.

RESULTS

Moss functional traits and N_2 fixation in the glacier forefield

Moss shoot length increased with time since deglaciation, from 16.5 to 46.9 mm ($p = 0.02$) (Figure 2a, Appendix S1: Tables S2 and S3). Moss moisture content, TC, TN, C/N ratio, and $\delta^{13}C$ did not change significantly (Figure 2b–e, Appendix S1: Tables S2, S4–S8), Nevertheless, a nonsignificant increasing trend with time since deglaciation was found for TC and C/N ratio (Figure 2d,e, Appendix S1: Tables S2, S6, and S7). *nifH* gene abundance decreased ($p = 0.01$) with time since deglaciation (Figure 2g, Appendix S1: Tables S2 and S9).

Moss $\delta^{13}C$ was significantly higher in *R. lanuginosum* than in *R. ericoides* ($p < 0.001$) (Figure 2f, Appendix S1: Table S8). Moss-associated N_2-fixation rate (expressed as acetylene reduction rate) showed considerable variation along the chronosequence, but no significant trend with time since deglaciation (Figure 2h, Appendix S1: Tables S2 and S10). The average acetylene reduction rate in the forefield was 0.00769 μmol C_2H_2 kg^{-1} day^{-1}.

Moss and underlying soil bacterial community diversity and structure

There was no difference in soil or moss microbial diversity between the farthest and oldest edge of our sampling scheme and the closest and more recent land exposed by the glacier retreat (Appendix S1: Figure S1, Tables S11, S13, and S15). All of the diversity indicators were higher for the soil compared with the two moss species ($p < 0.001$ for both phylogenetic diversity, richness, and Shannon diversity; Appendix S1: Figure S1, Tables S12, S14, and S16).

Moss and soil bacterial community structure differed from each other (PERMANOVA $R^2 = 0.41$, $p < 0.001$; Figure 3a, Appendix S1: Table S17). The first two PCoA axes captured 74.4% of the total variation (Figure 3a). Part of the variation in the structure of the soil bacterial communities was related to moss C/N ratio (PERMANOVA

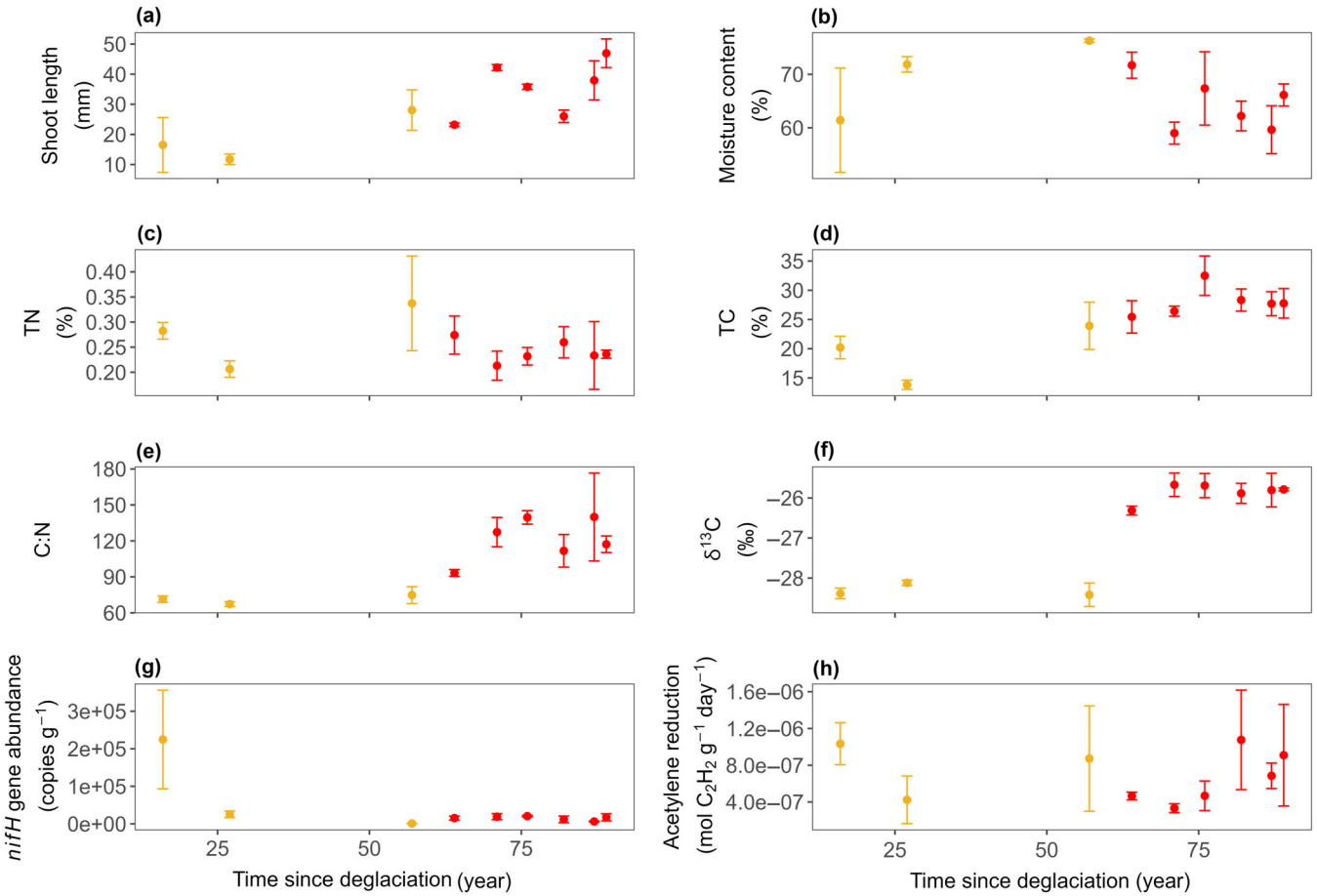

FIGURE 2 Variations in (a) moss shoot length, (b) moss moisture content, (c) total carbon (TC), (d) total nitrogen (TN), (e) C/N ratio, and (f) $\delta^{13}C$ content of moss shoots, (g) *nifH* gene abundance in the mosses, and (h) moss-associated N_2-fixation rates (measured by acetylene reduction) with time since deglaciation, in two moss species: *R. ericoides* (yellow) and *R. lanuginosum* (red). Shown are mean ± standard error of triplicate samples from each sampling location. *p* values and R^2 are shown in the top left corner of each plot when $p < 0.05$.

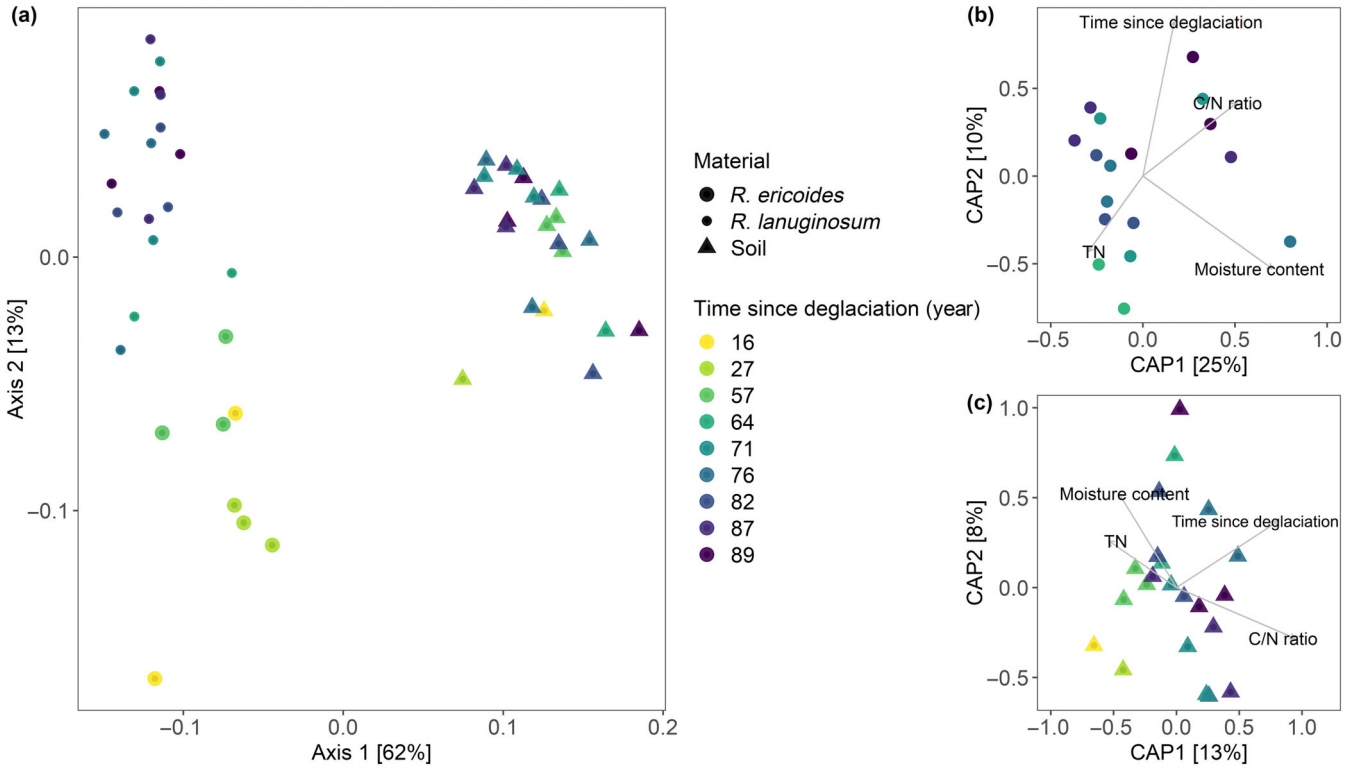

FIGURE 3 (a) Principal coordinate analysis biplot of the bacterial communities of the mosses *R. ericoides* and *R. lanuginosum* and the underlying soil on amplicon sequence variant level based on weighted UniFrac distances and (b) constrained analysis of principal coordinates (CAP) analysis of the bacterial community of the moss *R. lanuginosum* and environmental factors and (c) CAP analysis of the underlying soil bacterial community and environmental factors. TN, total nitrogen.

$R^2 = 0.06$, $p = 0.03$, Appendix S1: Table S18), also indicated by the length of the C/N ratio arrow in the CAP (Figure 3c). The moss bacterial community (regardless of moss species) was affected by time since deglaciation (PERMANOVA $R^2 = 0.15$, $p = 0.008$), moss moisture content (PERMANOVA $R^2 = 0.13$, $p < 0.001$, Appendix S1: Table S19), and C/N ratio (PERMANOVA $R^2 = 0.05$, $p = 0.01$, Appendix S1: Table S19).

When moss species were analyzed separately, the PERMANOVA showed that the *R. ericoides* bacterial community changed with time since deglaciation (PERMANOVA $R^2 = 0.22$, $p = 0.04$), but was also affected by moss TN (PERMANOVA $R^2 = 0.22$, $p = 0.03$). The *R. lanuginosum* bacterial community was not affected by time since deglaciation, but varied with moss moisture content (PERMANOVA $R^2 = 0.11$, $p = 0.02$, Appendix S1: Table S21), also indicated by the length of the arrow in the CAP plot (Figure 3b).

Bacterial community composition of *Racomitrium* mosses and underlying soil

Proteobacteria (36% in soil, and 35% and 28% on average in *R. ericoides* and *R. lanuginosum*, respectively), Acidobacteria (19%, and 15% and 23%), and Bacteroidetes (19%, and 25% and 24%) dominated the bacterial communities of soil and moss species in the Fláajökull glacier forefield (Figure 4).

Within the Alphaproteobacteria (Appendix S1: Figure S2), Acetobacteraceae, Beijerinckiaceae, and Caulobacteraceae were rarely found in soils, but in all locations in the moss samples. Xanthobacteraceae were on the other hand the dominating Alphaproteobacterial family in the soil samples. Sphingomonadaceae were more abundant in the moss samples, but were also detected in the soil samples. The families Solibacteraceae and Blastocatellaceae of the Acidobacteria were found in both the soil and moss samples (Appendix S1: Figure S3), while Acidobacteriaceae were more abundant in the mosses than in the soil and Pyrinomonadaceae were more abundant in the soil than in the mosses. Actinobacteria in soil and mosses were dominated by a large number of families with small relative abundances. The family Illumatobacteraceae was detected in soil in all sampling points except one (Appendix S1: Figure S4). The Chitinophagaceae was the most abundant family within the Bacteroidetes in the moss and soil samples (Appendix S1: Figure S5). Cyanobacteria never made up more than 5% of the total abundance of bacteria, but

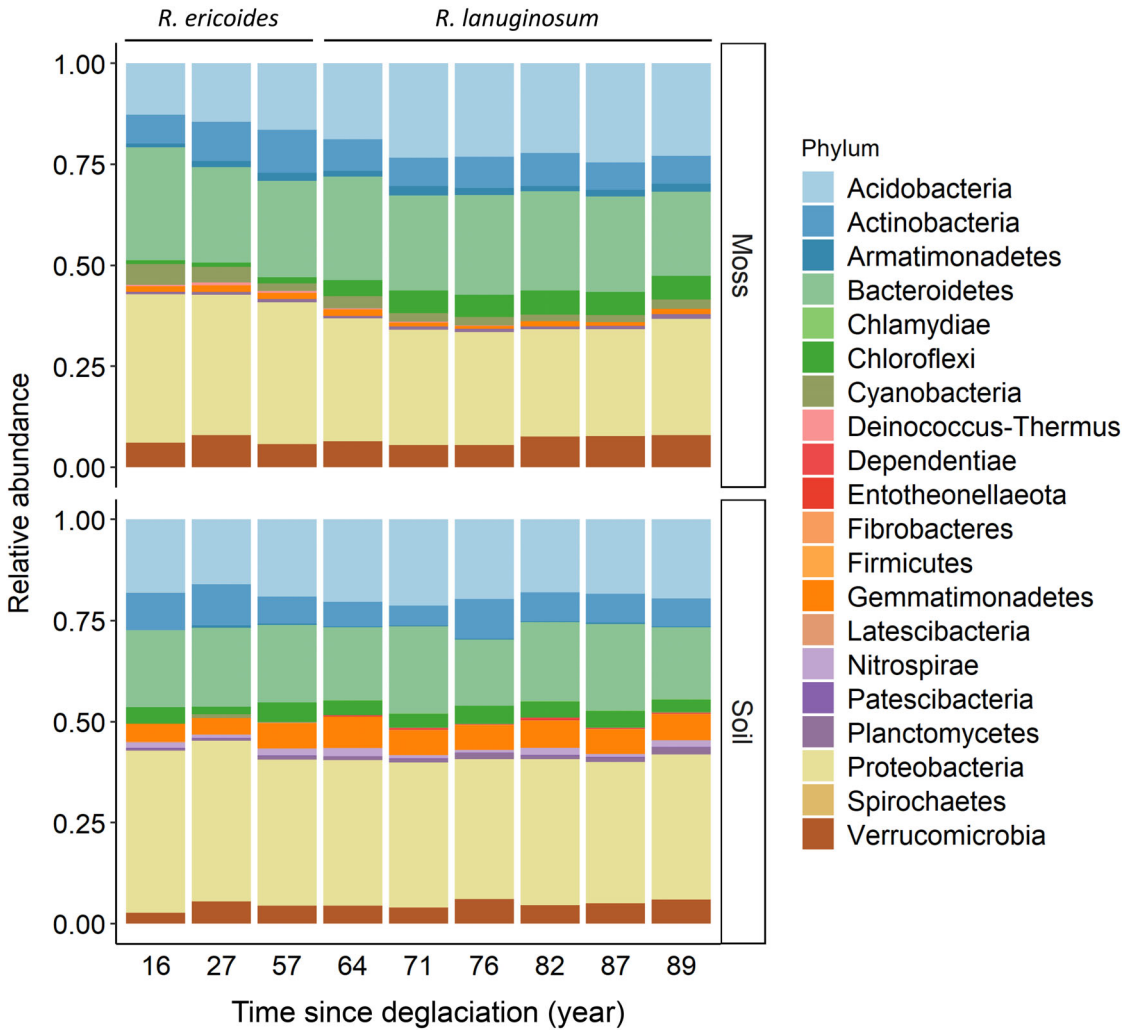

FIGURE 4 Phyla-level composition of the bacterial communities of *Racomitrium* mosses and underlying soil along a chronosequence in the Fláajökull glacier forefield. Shown are average relative abundance of the three replicates taken at each sampling point.

were more abundant in the mosses than in the soil (Appendix S1: Figure S6).

Changes in relative abundance of bacterial phyla and ASVs with time since deglaciation

On phylum level, the relative abundance of Chloroflexi increased across our chronosequence, both in moss and soil; while Proteobacteria, Cyanobacteria, and Bacteroidetes decreased in the moss (Figure 4, Appendix S1: Figures S8 and S9).

On the ASV level, we detected more ASVs changing in relative abundance with time since deglaciation in the soil than in the moss (Figure 5). All detected ASVs increased in relative abundance across the chronosequence. Most of these ASVs belonged to the Proteobacteria. The two ASVs that increased with time since deglaciation belonged to the candidate genus *Solibacter* and the family Acetobacteraceae. The ASVs showing the strongest increase in relative abundance with time since deglaciation in the soil belonged to the families Acetobacteraceae, Micropepsaceae, and Chinitophagaceae and the genera *Parafilimonas* and *Nocardioides*.

Linkages between N_2 fixation, time since deglaciation, moss moisture content, TN, and bacterial community structure

We used structural equation modeling to investigate the direct and indirect linkages between time since deglaciation, moss moisture content, moss TN and TC, the *R. lanuginosum*-associated bacterial community structure, *nifH* gene abundance, and N_2 fixation (Figure 6, Appendix S1: Table S22). Bacterial community structure represented by the position on the first PCoA axis was

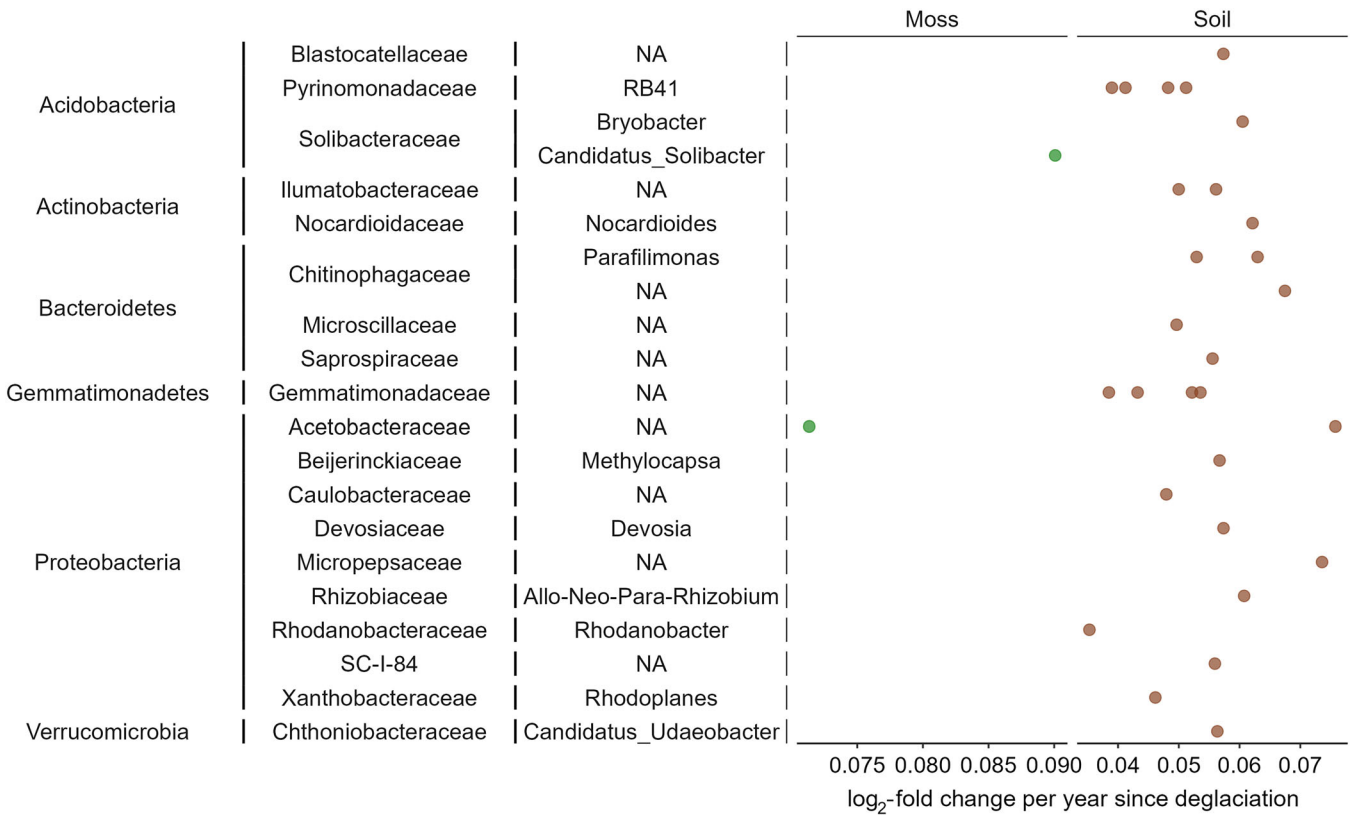

FIGURE 5 Log_2-fold changes per year since deglaciation in relative abundance of microbial groups (at amplicon sequence variant level) across the chronosequence in *Racomitrium* moss species (green) and underlying soil (brown). NA, not assigned (to genus level).

affected by TN (standardized path coefficient 0.41; $p < 0.01$) and moss moisture content (standardized path coefficient −0.63; $p < 0.001$). *nifH* gene abundance was negatively linked to the structure of the bacterial community (standardized path coefficient −0.87; $p < 0.001$) and N_2-fixation rate was negatively affected by *nifH* gene abundance (standardized path coefficient −0.68; $p = 0.01$). This indirect effect of the bacterial community structure on N_2-fixation rates via changes in *nifH* gene abundance was also significant (standardized path coefficient 0.60; $p = 0.047$; Appendix S1: Table S22).

DISCUSSION

Mosses are among the first colonizing plants on newly exposed soils following glacier retreat. Mosses and their bacterial communities play important roles in the C and N cycles, which are crucial during ecosystem development in glacier forefield. But it is unclear how moss bacterial communities develop during primary succession. Here, we studied moss traits, moss-associated bacterial communities, and N_2 fixation as well as the bacterial communities of the underlying soil along a chronosequence in the Fláajökull glacier forefield in Iceland. We found links between time since deglaciation and moss traits as well as bacterial community structure of the mosses. We also found that both soil and moss bacterial community structure are related to C/N ratio, while moss bacterial community structure was also linked to moss moisture content. N_2-fixation rates were linked to bacterial community structure, but not time since deglaciation. Our new dataset on primary succession as a driver of moss-associated bacterial community structure and associated processes contributes to the understanding of biogeochemical cycling in newly exposed ice-free soils.

Changes in moss functional traits with time since deglaciation

We hypothesized that moss TN and TC would increase with time since deglaciation. Most of the changes occurred in the earlier stages of the successional gradient and stabilized in the later stages. We expected TC to increase with succession, and the overall pattern pointed in that direction albeit not significantly, at least until 76 years after deglaciation. This TC trend in the moss partly agrees with the patterns of soil organic carbon

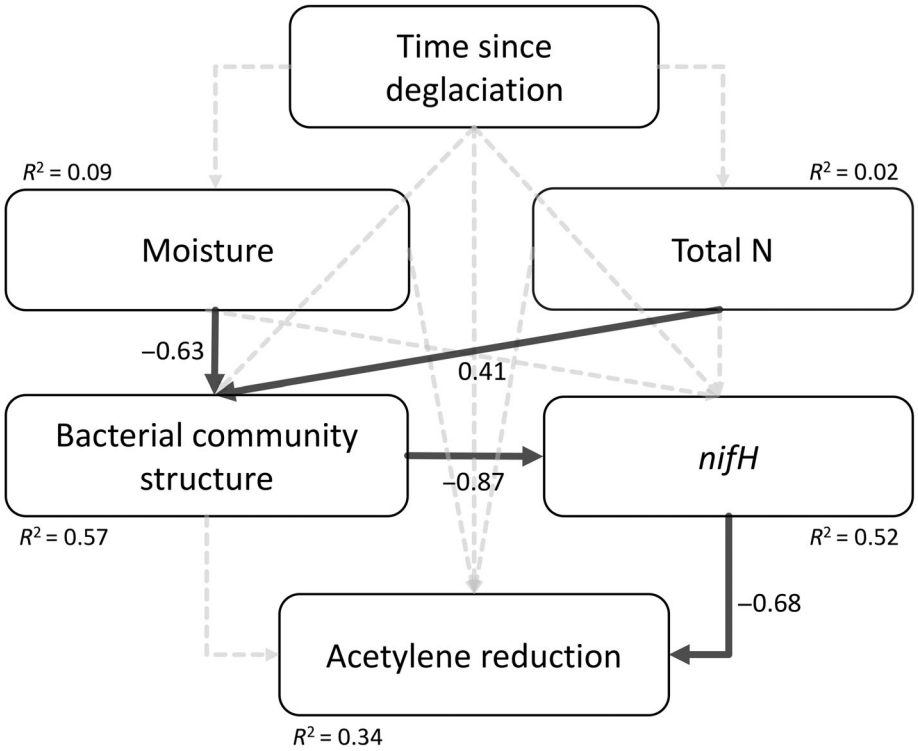

FIGURE 6 Structural equation model (SEM) showing linkages between time since deglaciation, moss moisture content, moss total nitrogen (N) and moss bacterial community, and N_2 fixation. Note that this SEM is only based on the moss *R. lanuginosum*. $\chi^2 = 2.59$, $p = 0.459$, df = 3.00, comparative fit index = 0.975, root mean square error of approximation = 0, and Tucker-Lewis index = 1.031. Significant effects are represented with black arrows and nonsignificant effects are indicated with dash-line arrows. The strength of the effect is indicated with the number next to the arrow for significant effects. The R^2 value represents the proportion of total variance explained for the specific dependent variable. Standardized path coefficients are presented in Appendix S1: Table S22.

(SOC) content in the same Fláajökull forefield in a parallel study by Wojcik et al. (2020), where the authors showed that TC content increased until the 1936 moraine and that TC was lower on the 1931 and 1929 moraines, probably due to soil disturbance via geomorphological events or due to the heterogeneous nature of the soil substrates in the Fláajökull forefield.

We also expected moss TN to increase with time since deglaciation, but our data did not show any successional trends in moss N content. In the early successional stages of the Fláajökull forefield, soil TN increases (Wojcik et al., 2020), but moss shoot TN did not correlate with the soil TN trend. There are several potential reasons for the discrepancy between moss and soil TN. Moss N may for instance be lost via denitrification or via leeching to deeper soil layers (Johnson et al., 2007). Moss N has also been found to be more rapidly lost from moss litter than C during decomposition (Philben et al., 2018). Nevertheless, as moss mat coverage and shoot length increased with succession, moss TN per square meter will increase. Overall moss C/N showed an increasing trend along the chronosequence, probably driven by the increasing C content, but again with lower values on the three oldest, potentially disturbed soils. The increase is similar to the increase in C/N found in the bryophytes with succession in glacier forefields in Tierra del Fuego in Chile (Arroniz-Crespo et al., 2014).

$\delta^{13}C$ can reflect the signal of multiple environmental factors (Waite & Sack, 2011) and often increases in moss tissue with ecosystem age (Bansal et al., 2012; Jonsson et al., 2015). Our data did show less negative values in moss shoot $\delta^{13}C$ with time since deglaciation. This however could also be due to differences between moss species, with lower values in *R. ericoides* (−28.3‰ ± 0.1) and more negative values in *R. lanuginosum* (−25.8‰ ± 0.1). As $\delta^{13}C$ is typically higher in dry environments, the difference between the two moss species could be a reflection of habitat preference. *R. ericoides* prefers moister sites with thicker snow cover and hence shows lower $\delta^{13}C$ values than *R. lanuginosum*, which is rather associated with drier, exposed sites (Frisvoll, 1983; Virtanen et al., 1997). This confirms the importance of moss species for $\delta^{13}C$ values (Bramley-Alves et al., 2015; Waite & Sack, 2011). Our average $\delta^{13}C$ value for *R. lanuginosum* (−25.8‰ ± 0.1) is comparable to those found in *R. lanuginosum* on Mauna Loa, Hawaii (−26.3% ± 0.4) (Waite & Sack, 2011).

Potential drivers of the moss-associated and underlying soil bacterial community structure

The bacterial community structure of the mosses and the soil were both affected by time since deglaciation. As moss species represents an important factor for the composition of the bacterial communities (Bragina et al., 2012; Holland-Moritz et al., 2018), the shift in moss species along the chronosequence may also have contributed to the effect of time since deglaciation on the moss bacterial communities.

Moss moisture content turned out to be an important factor contributing to variation in the moss bacterial community structure. Moisture content is an important driver of microbial decomposition (Schimel et al., 1999) and may thereby also affect bacterial community structure, especially in the decomposing part of the moss shoots. Interestingly, moisture has also been found to affect the occurrence of Antarctic moss-associated fungi (Hirose et al., 2016).

In our study, time since deglaciation and TN affected the bacterial community structure of *R. ericoides*, but not *R. lanuginosum*, which was only linked to moss moisture content. The discrepancy in factors structuring the bacterial communities of the two mosses may be caused by the smaller sample size of *R. ericoides* versus *R. lanuginosum*, but factors driving moss bacterial communities may also change with succession. Our results indicate that time since deglaciation and TN are important in the earlier stages of succession (e.g., in *R. ericoides*). C/N ratio was associated with the bacterial community structure of both moss species taken together.

The soil bacterial community structure below the mosses showed variation with succession, but less than the overall moss bacterial community. Interestingly, moss C/N ratio was also associated with the soil bacterial community structure. Moss C/N ratio may influence soil C/N ratio, without directly affecting the soil bacterial community, but plant traits such as leaf N can influence soil bacterial community structure (De Vries et al., 2012), and moss chemical traits may thus also affect bacterial community in the underlying soil.

The bacterial communities of *Racomitrium* moss species and underlying soil were clearly distinct at the ASV level, indicating that there might be little or no lateral transmission of the soil bacterial communities to the moss bacterial communities and/or vice versa. Instead, moss-associated bacterial community may originate from vertical transmission via the sporophyte (Bragina et al., 2012), vegetative reproduction of moss shoots, or aerial deposition (Morris, 2002). The bacterial community of *R. lanuginosum* in the Fláajökull glacier forefield was also similar to the bacterial community of *R. lanuginosum* from a subarctic-alpine heathland in northwest Iceland (Klarenberg et al., 2021). Many taxa are shared in similar proportions, such as the orders Acetobacterales, Acidobacterales, and Solibacterales, while Bacteroidetes were more abundant in the mosses in the glacier forefield and Planctomycetes more abundant in the heathland. Cyanobacteria were less abundant in the moss in the glacier forefield than in the heathland, potentially because the Fláajökull glacier forefield receives more N from deposition as the forefield is in the vicinity of farmlands that could provide the moss with N and reduce the need for Cyanobacteria as diazotrophic symbionts. Generally, the moss-associated bacterial communities are dominated by presumptively acidophilic bacteria often associated with ombrotrophic or other oligotrophic environments and are comparable to the bacterial communities of other moss species (Holland-Moritz et al., 2021).

Taxa specific trajectories in moss-associated and underlying soil bacterial communities with succession

Most of the bacterial phyla that shifted in relative abundance during succession were found in the moss. The phylum Chloroflexi increased in relative abundance with succession in both the soil and the mosses. An increase in Chloroflexi with succession has also been detected in the soil and rhizosphere of *Saxifraga oppositifolia* in a glacier forefield in the high Arctic (Mapelli et al., 2018). In the moss, we found decreasing abundance of Proteobacteria, Cyanobacteria, and Bacteroidetes. These taxa often become less abundant as succession progresses in glacier forefields in soils (Bajerski & Wagner, 2013; Bradley et al., 2016; Fernández-Martínez et al., 2017; Jiang et al., 2018) and our results show that similar patterns are found in the moss microbiome, but less so in the moss-covered soil.

On the ASV level, most changes with soil age were detected in the soil bacterial community. All of these ASVs increased in relative abundance with soil age. Many of them were classified as genera known to be able to degrade plant organic matter, such as *Ca. Solibacter* (Ward et al., 2009), *Nocardioides* (Guo et al., 2021), members of the Chitinophagaceae (Li et al., 2011), and Micropepsaceae (Harbison et al., 2016), indicating increased moss abundance with succession also increases the potential for degradation of dead moss material. While Cyanobacteria decreased in relative abundance with soil age in the moss, heterotrophic N_2 fixers became more abundant with soil age, for instance, *Devosia* (Rivas et al., 2002), Rhizobiaceae (Dobbelaere et al., 2003), *Methylocapsa* (Dedysh et al., 2002), and *Rhodoplanes*

(Buckley et al., 2007) in soil, and Acetobacteriaceae (Saravanan et al., 2008) in the mosses, probably because of increased substrate availability. An increase in potential denitrifiers (*Ca. Solibacter*; Ward et al., 2009 and *Rhodanobacter*; Kostka et al., 2012) suggests an increase in nitrates and/or nitrites with succession and loss of N via denitrification with succession. Some of the taxa increasing along the chronosequence are known to be acidophilic (Chitinophagaceae and Gemmatimonadaceae; Cline & Zak, 2015, Acetobacteraceae in moss; Kersters et al., 2006, and Micropepsaceae; Harbison et al., 2016) and may be linked to decreased soil pH with soil age in the Fláajökull glacier forefield (Wojcik et al., 2020 and Appendix S1: Table S24).

Moss N_2 fixation and diazotroph abundance during succession

We did not detect any trends in N_2-fixation rates with soil age. *nifH* gene abundance, however, showed an overall decrease with soil age, indicating a decreasing abundance of diazotrophs with succession. Moss-associated N_2-fixation rates were not affected by moss N content, soil age, or moss moisture, but rather by the abundance of diazotrophs and bacterial community structure, at least in *R. lanuginosum*. The negative link between *nifH* gene abundance and N_2-fixation rates could indicate that not all bacteria taxa possessing *nifH* genes are actively involved in N_2 fixation. In addition, the discrepancy between *nifH* gene abundance, N_2-fixation rates, and the relative abundance of diazotrophs could be due to the relatively low degeneracy of the *nifH* gene primer pair PolF/PolR (Gaby & Buckley, 2017). Nevertheless, the negative of *nifH* gene abundance with soil age is consistent with the decrease in the relative abundance of Cyanobacteria in the mosses with soil age. Additionally, past research has shown that shifts in *nifH* gene diversity with succession occur in soil in glacier forefields (Duc et al., 2009) and our study suggests that these shifts may also take place in mosses.

Moss N_2 fixation may be and stay an important source of N throughout glacier forefields, with increasing importance as moss cover increases with succession. However, without a conversion factor between acetylene reduction and N_2 fixation, it remains unclear how much N_2 is exactly fixed by the moss in the glacier forefield. This conversion factor may differ between moss species (Saiz et al., 2019) and can be affected by alternative nitrogenase activity (Bellenger et al., 2014). The relative importance of N_2 fixation and mineralization for the N content of the moss and the soil along the chronosequence may be better understood when ^{15}N depletion is taken into account in future studies.

CONCLUSION

We studied the development of *Racomitrium* moss bacterial communities as well as those of the underlying soil in relation to moss functional traits along a chronosequence in the glacier forefield of Fláajökull in southeast Iceland. While moss functional traits such as TN and moisture content did not show clear trends along the chronosequence, moss shoot length increased with succession. Time since deglaciation and moss C/N ratio and moss moisture content were related to moss bacterial community structure, showing for the first time how moss functional traits are important drivers for moss-associated bacterial communities. The bacterial communities of the underlying soil were also affected by time since deglaciation and by moss C/N ratio, highlighting the influence of moss traits on soil development. Moss and underlying soil bacterial communities differed strongly from each other, suggesting that little lateral transfer between them takes place. We did not detect any trends in moss-associated N_2-fixation rates with time since deglaciation or moss TN, but N_2-fixation rates were linked to bacterial community structure and negatively linked to *nifH* gene abundance. This may indicate a shift in diazotrophic taxa with different N_2-fixing efficiencies along the chronosequence and our data indeed show a proportional decrease in Cyanobacteria and an increase in heterotrophic N_2-fixing taxa.

Our study underlines the importance of moss functional traits as potential drivers for moss bacterial community structure, but also links moss functional traits to bacterial communities in the underlying soil. This is one way in which mosses can enhance soil development in glacier forefields, but our results also show that moss-associated N_2 fixation takes place along the whole chronosequence and thereby likely contributes to N availability. Our study contributes to the understanding of the role of mosses in ecosystem development, which will be increasingly important in a future warmer climate leading to increased glacier retreat.

AUTHOR CONTRIBUTIONS

Ingeborg J. Klarenberg designed the study, Oddur Vilhelmsson and Ingeborg J. Klarenberg collected the samples. Ingeborg J. Klarenberg, Christoph Keuschnig, and Alejandro Salazar performed the laboratory analysis. Ingeborg J. Klarenberg analyzed the data and wrote the paper with input from Oddur Vilhelmsson, Christoph Keuschnig, Alejandro Salazar, and Liane G. Benning.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Raw sequences are available in the European Nucleotide Archive under accession number PRJEB53628. Moss trait data, *nifH* gene abundance and acetylene reduction data, and soil pH and soil moisture data (Klarenberg, 2022) are available from Zenodo: https://doi.org/10.5281/zenodo.7313340.

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