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RESEARCH ARTICLE

Glacier retreat in the High Arctic: opportunity or threat for ectomycorrhizal diversity?

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One sentence summary: Climate change and deglaciation in the Arctic will be an opportunity for most early colonizing ectomycorrhizal fungi, but a threat for others; undescribed diversity and yet unknown functions will be lost.

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ABSTRACT

Climate change causes Arctic glaciers to retreat faster, exposing new areas for colonization. Several pioneer plants likely to colonize recent deglaciated, nutrient-poor areas depend on fungal partners for successful establishment. Little is known about general patterns or characteristics of facilitating fungal pioneers and how they vary with regional climate in the Arctic. The High Arctic Archipelago Svalbard represents an excellent study system to address these questions, as glaciers cover ~60% of the land surface and recent estimations suggest at least 7% reduction of glacier area since 1960s. Roots of two ectomycorrhizal (ECM) plants (*Salix polaris* and *Bistorta vivipara*) were sampled in eight glacier forelands. Associated ECM fungi were assessed using DNA metabarcoding. About 25% of the diversity was unknown at family level, indicating presence of undescribed species. Seven genera dominated based on richness and abundance, but their relative importance varied with local factors. The genus *Geopora* showed surprisingly high richness and abundance, particularly in dry, nutrient-poor forelands. Such forelands will diminish along with increasing temperature and precipitation, and faster succession. Our results support a taxonomical shift in pioneer ECM diversity with climate change, and we are likely to lose unknown fungal diversity, without knowing their identity or ecological importance.

Keywords: Arctic; early colonizing fungi; ectomycorrhiza; climate change; DNA metabarcoding; glacier foreland

INTRODUCTION

Climate change, with altered temperature and precipitation patterns, causes glaciers to retreat worldwide. These changes have been especially pronounced in the Arctic, where several glaciers are rapidly decreasing (Hansen *et al.* 2010; Parkinson and Comiso 2013; AMAP 2017; Bilt *et al.* 2019). Over the last 40–50 years, both temperature and precipitation have increased on the High Arctic Archipelago Svalbard, with an increase of 3–5°C and around 190 mm, respectively (Bilt *et al.* 2019). About 60% of Svalbard is covered by glaciers, but the dramatic changes in climate have resulted in accelerated glacier retreats (Martín-Moreno, Allende Álvarez and Hagen 2017; Bourriquen *et al.* 2018). Recent estimations show at least 7% reduction of glacier area since the 1960s (Bourriquen *et al.* 2018), rapidly exposing new land available for colonization of biota.

This new land may represent an opportunity for some species (Erschbamer 2007). Glacier forelands have, for example, been shown to represent possible refugia for cold-adapted vascular plants tracking their climatic niche under climate change (Müller *et al.* 2012). Two of the most important forage plants in

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Svalbard (Ims *et al.* 2013), *Bistorta vivipara* (L.) Delabre and *Salix polaris* Walenb, are among the earliest pioneer plant species in the Arctic (Hodkinson, Coulson and Webb 2003; Newsham 2011; Těšitel *et al.* 2014). Thus, they may benefit from glacier retreats. However, as they form ectomycorrhizal (ECM) associations with fungi (Hesselman 1900; Read and Haselwandter 1981), their colonization success will be related to the available ECM community.

Bistorta vivipara and *S. polaris* are both widespread species thriving in a range of different habitats, but the composition of their associated ECM community seems habitat dependent (Berg and Verhoef 1998; Taylor and Bruns 1999; Dickie *et al.* 2013; Mundra, Bahram and Eidesen 2016). The ECM genera *Lactarius* and *Russula* are, for instance, more abundant in nutrient-rich areas (Berg and Verhoef 1998; Taylor and Bruns 1999; Dickie *et al.* 2013; Mundra, Bahram and Eidesen 2016), whereas the tentatively more stress-tolerant *Laccaria* and *Hebeloma* are more abundant in nutrient-poor sites, such as mine tailings (Mundra, Bahram and Eidesen 2016), and have been identified as early colonizing ECM fungi (Cázares, Trappe and Jumpponen 2005; Fujiyoshi *et al.* 2011; Jumpponen *et al.* 2012; Davey *et al.* 2015). The most newly exposed forelands represent a very specific habitat (Dresch *et al.* 2019), and are likely to hold a habitat-specific ECM community. Fungal succession patterns after glacial retreat have previously been studied (Blaalid *et al.* 2012; Jumpponen *et al.* 2012; Dickie *et al.* 2013; Davey *et al.* 2015), but few focus on early colonizing ECM fungi. Furthermore, most previous studies have been from alpine areas and have focused on a single or a few glacier forelands. ECM communities in glacial forelands are, for instance, only characterized by high-throughput sequencing from one location in the High Arctic (Davey *et al* 2015). Hence, the general characteristics of early colonizing ECM fungi across Arctic glacial forelands are unknown. One can assume that these pioneer fungi play an important role as facilitators during the initial plant establishment.

Although the ongoing glacial retreat will leave more land available for colonization, the regional climate will change as well. The latter may be a threat for early colonizing ECM fungi in the High Arctic. In Arctic marginal environments, successional pattern deviates from the classical model for directional change and replacement of species (Matthews 1978; Svoboda and Henry 1987). Under high climatic stress competition is reduced, and directional, non-replacement succession becomes more common, where initial species remain, but new species are added through the succession (Svoboda and Henry 1987; Jones and Henry 2003). Previous studies from glacier forelands in Svalbard have suggested that colonization of both plants and root-associated fungi follows this directional, non-replacement succession model (Hodkinson, Coulson and Webb 2003; Davey *et al.* 2015), whereas soil fungi have been shown to follow a directional replacement model (Dong *et al.* 2016). With the steadily increasing temperatures, successional patterns in Svalbard may move toward the more classical directional-replacement pattern also for plants and root-associated fungi (Dong *et al.* 2016). This may in turn lead to early colonizers being outcompeted—and over time lead to risk of extinction, especially if the retreat is fast, and the habitat eventually disappears. Species loss may lead to a loss of interactions between organism groups, which can lead to cascading effects in the ecosystem (Cardinale *et al.* 2012). Further to this, the speed of succession is expected to increase; in temperate regions, secondary succession is shown to accelerate with increasing temperature (Fridley and Wright 2018). To understand effects of biodiversity loss, we must know what is already there, and how current biodiversity is related to the environment.

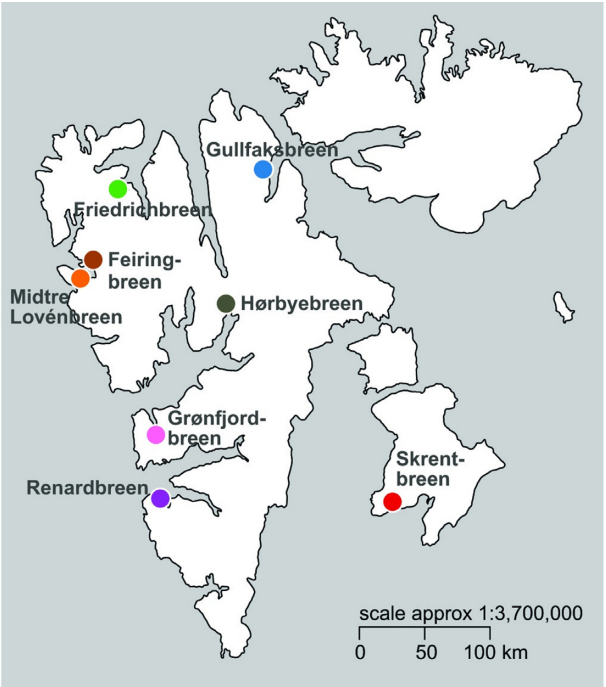

Figure 1. Map of the Arctic Archipelago Svalbard showing the different sampling locations. Each point represents one glacier foreland in one location.

To understand how the earliest pioneer communities of ECM fungi will be affected by the ongoing climate change and glacial retreat in the Arctic, we need to analyze several host species collected in the same successional stage, replicated from a sufficient number of locations along a regional climate gradient. In this study, we aimed to investigate which ECM fungi are present at the earliest successional stage during primary succession of glacier forelands in the High Arctic, and assess to what degree a core community of early colonizing ECM fungi is present in the High Arctic. Further, we aimed to identify the climatic and edaphic factors driving and structuring the communities of the early colonizing ECM fungi, hence making us better able to assess the consequences of ongoing climatic changes in the High Arctic. To address these questions, we investigated ECM fungi associated with the host plants *B. vivipara* and *S. polaris* during their very first establishment after glacial retreat in eight Arctic glacier forelands sampled across different bioclimatic zones (locations classified as bioclimatic zone C, C/B and B according to Elvebakk 1999). Characterization of the root mycobiome was done by extracting DNA from entire root systems followed by internal transcribed space (ITS) metabarcoding analyses.

MATERIALS AND METHODS

Study sites and sampling design

To characterize early colonizing ECM fungi, we sampled at eight different glacier forelands across the Svalbard High Arctic Archipelago (Table S1, Supporting Information; Fig. 1). The selected eight sampling locations were homogeneous in terms of successional stage and vegetation as much as possible, and represented three different regional climates (bioclimatic zones B, C and intermediate zone B/C; Table S1, Supporting Information; Elvebakk 1999). We did not include locations in the coldest bioclimatic zone (A—Polar dessert), as this is outside the range of

one of our host plants, *S. polaris*, which rarely occur in the coldest zone. All the locations were extremely sparsely vegetated and located at the youngest successional stage of glacier forelands where our host plants were growing. For three forelands (Renardbreen, Midtre Lovénbreen and Hørbyebreen), chronosequences were available (forelands where soil age history was known). Our host plants turned up in chronosequence 1960–36. By investigating the same habitat type (glacier forelands) with homogeneous environment at several locations, we aimed at minimizing the effect of local edaphic factors, surrounding vegetation and other potential effects of different habitat types. We sampled a total of 54 *S. polaris* (eight localities) and 49 *B. vivipara* (six localities) with their entire root systems. We were not able to find *B. vivipara* at two locations. At each site, samples were selected arbitrarily, but sampled at least 10 m apart to avoid small spatial autocorrelations in fungal communities. To validate that glacial foreland represents a coherent habitat that differs from established vegetation across locations, we additionally sampled 29 plant root systems (15 *B. vivipara* and 14 *S. polaris*) at two locations (Skrentbreen and Midtre Lovénbreen) from established vegetation outside the glacier foreland (Table S1, Supporting Information). Sampling was performed during the growing season (July–August) of 2012 and 2013. Soil samples were collected from the same spot where the plants were excavated. Soil samples were kept at −20°C until further handling. Plants were stored at 4°C for maximum 24 h before the roots were rinsed and washed as described in Botnen *et al.* (2014) and Mundra *et al.* (2016). In brief, after removing visible soil and plant debris, roots were rinsed in tap water, followed by washing with milliQ water for 5 min and storing them in 2% Cetyl Trimethyl Ammonium Bromide (CTAB) buffer [final concentration: Tris–HCl 100 mM (pH 8), NaCl 1.5 M, CTAB 2% (w/v), EDTA 50 mM (pH 7), β-mercaptoethanol 2% (w/v)] at −20°C until DNA extraction.

Abiotic factors, DNA extraction and Illumina sequencing

After removing the visible plant debris and roots, soil samples were dried, ground and sieved (2 mm mesh size). Soil pH was measured by shaking the dried soil in distilled water (1:2 volume ratios) and using a pH meter (Portable labTM, Mettler Toledo, with the In Lab 482 pH Sensor Module). Soil organic matter content was analyzed using loss of ignition as described in Eidesen *et al.* (2013). Total C and N contents of soil fractions were determined using a CHNS-O Elemental Analyzer 1110 (CE Instruments Ltd, United Kingdom). Monthly data on modeled precipitation, temperature and relative humidity in Svalbard at an ∼1 km scale from 2000 to 2013 were extracted from Schuler (2018), and annual and summer means over this period were calculated.

DNA was extracted from the entire plant root system using a modified CTAB extraction protocol (Murray and Thompson 1980), and further purified using the E.Z.N.A. Soil DNA kit (Omega Bio-tek, Norcross, Georgia, USA) following the manufacturer's protocol. A negative control was used during extraction procedure and included in PCR and sequencing. We amplified the internal transcribed space 2 (ITS2) region of the nuclear ribosomal rDNA using primers fITS7a (Ihrmark *et al.* 2012) and ITS4 (White *et al.* 1990). PCR procedures, library preparation and Multiplex Identification DNA-tags were as described in Mundra *et al.* (2016). Paired-end sequencing (2 × 300) was performed on an Illumina MiSeq sequencer and raw read data (doi:10.5061/dryad.7sqv9s4qw) were deposited in Dryad public sequence repository.

Bioinformatics

Bioinformatic workflow followed in this study has been described previously (Mundra *et al.* 2016). In brief, from a total of 11 451 758 sequencing reads, 9 400 594 reads were assembled using fastq-join (Aronesty 2013) and quality checked using FASTX-Toolkit; reads with per base quality scores >Q20, and >90% of bases with Q36 were kept, and sequence artifacts were removed, as implemented in Galaxy platform (https://usegalaxy.org/). A total of 8 283 858 reads were further demultiplexed and filtered using QIIME 1.8.0 (Caporaso *et al.* 2010) to remove reads <200 bp and >550 bp, homopolymers >8 bp, ambiguous base calls >0, >1 mismatch in the forward primer sequence and average quality score < 35 (50-bp sliding window was used to identify regions of low sequence quality). The 5 973 742 quality-filtered reads were checked for chimeras, using the usearch61 algorithm (Edgar 2010), and the remaining 5 804 420 reads were clustered into Operational Taxonomic Units (OTUs) at 97% similarity threshold using the UCLUST algorithm (Edgar 2010). The most abundant sequence of each cluster was designated as a representative sequence and further passed through ITS extractor (Bengtsson-Palme *et al.* 2013). Clusters represented by <5 reads were discarded as likely sequencing errors (Nguyen *et al.* 2015). Representative sequences of each cluster were subjected to BLASTn search against the UNITE+INSD fungal sequence database (Abarenkov *et al.* 2010). OTUs with no similarity to fungal sequences in the UNITE database and low bit score and coverage (score/length < 0.6) were removed, resulting in 1854 OTUs. OTUs were annotated functionally as ECM fungi based on genera information using FunGuild (Nguyen *et al.* 2016), and further confirmed as ECM according to Tedersoo, May and Smith (2010). The sampling depth was normalized to an even sampling depth of 2497 reads per sample, leaving a total number 1482 OTUs, and 948 ECM OTUs for further analyses. The % sequence similarity to the UNITE database of the representative sequences of the OTUs was compared to the similarity of the OTUs from non-glacier foreland samples in Svalbard (Blaalid *et al.* 2014; Botnen *et al.* 2019), Scotland, mainland Norway and the Alps (Botnen *et al.* 2019). We also calculated the proportion of OTUs shared by 2–8 locations. Since we observed a relatively large diversity of the poorly studied genus *Geopora,* some additional analyses (as described below) were performed using the representative sequences of the OTUs assigned to this genus.

Phylogenetic placement of Geopora reads

To obtain a deeper understanding of the phylogenetic diversity of the OTUs not identified at species level but assigned to the genus *Geopora*, we built a *Geopora* phylogeny based on known sequences. Fully identified ITS sequences of specimens in the genus *Geopora* were downloaded using emerencia (Nilsson *et al.* 2005; Ryberg *et al.* 2009), and aligned using the L-INS-i algorithm with default settings in MAFFT v.7.3 (Katoh and Standley 2013). A backbone tree for *Geopora* was constructed in RaxML (Stamatakis 2014) based on these sequences using a GTR gamma rate heterogeneity model with 666 random number of seeds. The representative sequences of the OTUs assigned to *Geopora* in this study were aligned with the ITS2 region of these reference sequences using MAFFT, and subsequently mapped to the reference tree using the Evolutionary Placement Algorithm (EPA) (Berger, Krompass and Stamatakis 2011) as implemented

in RAxML. The placement of the short reads was visualized using gappa (Czech, Barbera and Stamatakis 2019).

Statistical analyses

If not otherwise specified, the following analyses were conducted in the statistical environment R (R Development Core Team 2010), and based on the rarefied OTU matrix.

To assess the difference of the fungal OTU community composition related to environmental factors, and the different hosts, a global non-metric multidimensional scaling (GNMDS) or non-metric multidimensional scaling (NMDS) (Kruskal 1964; Minchin 1987) was performed using the vegan package (Oksanen *et al.* 2012) in R. The ordinations were performed with settings as recommended by Økland (1999) and Liu *et al.* (2008): distance measure = 'Bray–Curtis'; dimensions = 3; initial configurations = 100; and maximum iterations = 200. The GNMDSs were scaled in half change units, and subject to varimax rotation by PCA (principal components analyses) ordination, and the two best solutions were compared using Procrustes correlation with 999 permutations to confirm convergence. To ensure that an appropriate gradient structure was found, a detrended correspondence analysis (DCA) (Hill 1979; Hill and Gauch 1980), using default settings, was conducted in parallel. The three dimensions of the GNMDS were compared to the first three axes of the DCA by calculating Kendall's rank correlation coefficient τ (data not shown). Similar results from the two methods, and absence of visual artifacts, were interpreted as a strong indication of reliable gradient structures found [95].

To validate that glacial foreland represents a coherent habitat that differs from established vegetation across locations, NMDS analyses were performed on a subset of the OTU matrix including samples from Skrentbreen and Midtre Lovénbreen (Table S1, Supporting Information). Before ordination, OTUs with no occurrences in the subset were removed, and the reduced OTU matrix was square root transformed to adjust for high number of zeros. As expected, in correspondence with former studies (Blaalid *et al.* 2012; Davey *et al.* 2015), the ECM fungal community were highly distinct between glacier forelands and established vegetation (Figure S1, Supporting Information). We excluded samples from the established vegetation from further analyses.

We visualized the samples of the different host species using the GNMDS, and then we tested whether there was any difference in the community structure, by constrained correspondence analyses (CCA). We found no effect of host species at community level (Figure S2, Supporting Information; $P = 0.94$), congruent with previous studies from Arctic regions (Botnen *et al.* 2014; Timling *et al.* 2014). Thus, we continued with community analyses without taking host species into account.

The different locations were visualized in the GNMDS ordination by their standard error of the (weighted) centroids using the *ordiellipse* function in vegan, and by their standard deviation of the average scores using the *ordibar* function on the best GNMDS solution (as determined above). The numerical environmental variables were centered and scaled to gain numerical stability. Then, the number of reads/read abundance of ECM genera containing more than five OTUs (rarefied read numbers), as well as the environmental factors, were fitted to the site GNMDS axes using the squared correlation coefficient (R^2) as a goodness of fit statistic in the *envfit* function as implemented in vegan. Also, the species score axis from the GNMDS was extracted for OTUs belonging to ECM genera, and the species optima of the OTUs were visualized. To determine how much variation could be explained by the measured variables, a variation partitioning using CCA with forward selection was performed using the *cca* function in vegan.

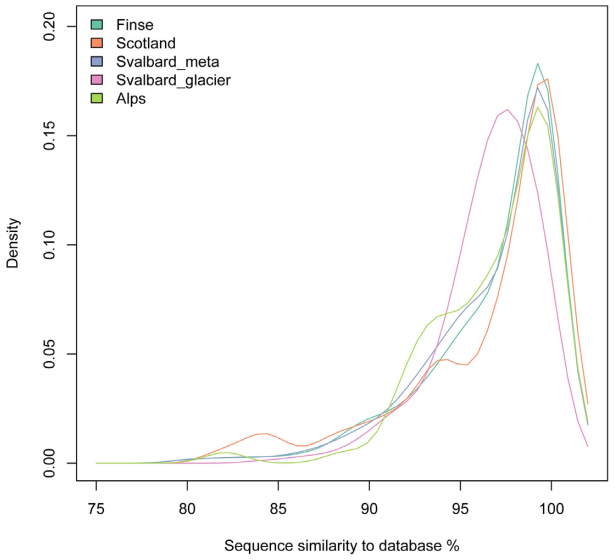

Figure 2. Density plot showing the obtained ITS2 sequence similarity of the representative sequences to known UNITE accessions. Different colors represent different locations; the pink line is from this study, whereas the other colored lines are data from Botnen *et al.* (2019). The same version of UNITE is used, and all root-associated fungal OTUs are included.

In order to relate the environmental variables to richness trends, i.e. number of OTUs per sample, general mixed effect models, assuming a negative binomial distribution using the glmmTMB package (Brooks *et al.* 2017), were applied. Sampling sites were included as a random contribution. We tested for richness difference between the host species, and found none ($P = 0.755$), and thus, continued further richness analyses without taking host species into account. To see whether different environmental factors were important when looking at the OTU diversity when taking read abundance into account, Shannon diversity index was calculated for each of the samples, and linear mixed effect models were applied, assuming a Gaussian distribution. Sampling site was included as a random contributor. Again, no change in the Shannon diversity index was observed between the host species. To find the optimal models, backward stepwise model selection based on Akaike information criterion values was performed.

RESULTS

Taxonomy and core community

A total of 948 ECM fungal OTUs were identified across eight glacier forelands in Svalbard. A comparison of all OTUs to reference sequences in UNITE revealed that the OTUs from glacier forelands in Svalbard in general obtained lower matches compared to root-associated fungi detected in other locations in Svalbard, mainland Norway, Scotland and the Alps (Fig. 2). In addition, several of the matching reference sequences in UNITE did not include taxonomic information below family (~25%) or genus level, making more specific taxonomic annotation impossible.

A major proportion of the OTUs (54.3%) was shared between at least two independent glacial forelands, whereas only 3.4% of the OTUs were shared among all glacial forelands. This limited

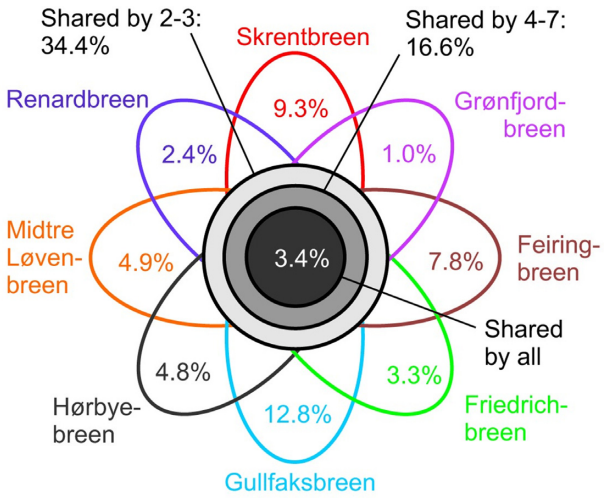

Figure 3. Diagram showing unique and shared OTUs between different glacier forelands. The different colors represent different locations.

core community was dominated by the genera *Geopora* and *Hebeloma* (Fig. 3; Table S2, Supporting Information). The overall most abundant ECM genera, both in terms of number of reads and number of OTUs, were ascomycetes of the genus *Geopora* and the basidiomycete genera *Alnicola, Cortinarius, Hebeloma, Inocybe, Sebacina* and *Tomentella* (Fig. 4). Among these seven most abundant genera, *Geopora* was the most abundant genus based on number of reads (Fig. 4A), whereas numbers of OTUs were more evenly distributed across genera (Fig. 4B). *Geopora* was especially abundant in five out of the eight glacier forelands, and it is noteworthy that there was a tendency that *Geopora* was relatively more abundant when *Hebeloma* was less frequent, and vice versa.

The number of OTUs and reads assigned to *Geopora* was surprisingly high, and to obtain a deeper understanding of their phylogenetic diversity, reads were mapped on to a *Geopora* ITS reference tree using the EPA (Fig. 5). The environmental reads distributed across the entire reference tree. Several reads mapped toward known *Geopora* morphospecies, especially toward the *G. arenicola* species complex. However, most reads mapped onto internal and not terminal branches (Fig. 5).

Community structure, diversity, and environmental characteristics

The fungal community structure from foreland root samples, as revealed by GNMDS, was related to both geographic and climatic factors (Fig. 6A and B). The factors pH, soil organic matter (OM) content, C:N ratio, temperature, precipitation, summer relative humidity (RH) and northing all correlated significantly to the ordination configuration (Table 1). Variation partitioning analysis revealed that location and soil OM content correlated most strongly to the community structure. However, only 14% of the compositional variation could be accounted for by the included factors: 11.1% by location, 2.1% by soil OM content and 0.8% by the interaction between them.

The ECM genera *Alnicola, Cortinarius, Geopora* and *Hebeloma* all showed some degree of sub-structuring in the GNMDS ordination (Fig. 6C–I). OTUs of *Cortinarius* and *Hebeloma* (Fig. 6E and F) were in general oppositely distributed to *Geopora* (Fig. 6C), indicating genus-level differences in niche preferences. *Geopora* OTUs were generally associated with northward locations,

Table 1. Significance and correlation between explanatory variables that were fitted to the GNMDS ordination by the envfit function.

Variables	GNMDS1	GNMDS2	r^2	Pr(>r)
pH	0.40835	−0.91283	0.1144	0.006
Soil organic matter %	−0.75150	0.65973	0.0626	0.045
C:N	0.56457	−0.82538	0.1479	0.001
Temperature	−0.53894	−0.84234	0.1329	0.003
Precipitation	−0.99708	−0.07638	0.1020	0.007
Summer RH	−0.07429	0.99724	0.0896	0.014
Northing	0.65799	0.75302	0.0921	0.014

Table 2. Results from the best model explaining difference in OTU richness and diversity (Shannon diversity index) across the samples. Sampling site was included in both models as a random factor. **Richness (presence):** Log-link fixed effects of a general linear mixed effect model, assuming a negative binomial distribution. OTU richness fitted with the scaled and centered variables 'pH' and 'C:N'. Intercept represents the mean OTU richness with mean pH and mean C:N. **Shannon diversity index:** Fixed effects of a linear mixed effect model, assuming a Gaussian distribution. OTU diversity fitted with the scaled and fitted variables 'Annual mean precipitation' and 'Annual temperature'. Intercept represents mean OTU richness with mean precipitation and temperature.

OTU richness

Variable	Estimate	Std. error	z-value	P
Intercept	3.65423	0.06068	60.23	<2e-16
pH	−0.09618	0.04866	−1.98	0.0481
C:N	−0.11372	0.05418	−2.10	0.0358

Shannon diversity

Variable	Estimate	Std. error	z-value	P
Intercept	1.64636	0.07967	20.664	<2e-16
Annual precipitation	0.20768	0.09092	2.284	0.0224
Annual temperature	−0.18603	0.08881	−2.095	0.0362

where both C:N ratio and soil pH were higher. *Cortinarius* and *Hebeloma* were associated with higher levels of precipitation and soil OM content. OTUs annotated as *Alnicola* (Fig. 6D) clustered closely together in one end of the GNMDS diagram, associated with higher levels of precipitation and higher temperatures. While for *Tomentella*, a weak trend was observed, somewhat associated with higher summer relative humidity. OTUs affiliated with *Inocybe and Sebacina* (Fig. 6G and H) were more widely dispersed in the GNMDS plot, not showing specific genus-level affinities to certain environmental conditions or locations.

The overall richness of ECM OTUs per plant root system was negatively related to increasing pH and C:N ratio (Table 2). On the other hand, the OTU diversity/evenness, measured as Shannon diversity index, was positively related to mean annual precipitation and negatively to temperature (Table 2).

DISCUSSION

The ECM communities associated with pioneer plants in newly exposed glacier forelands in Svalbard showed a varied composition of taxa, whereof many are poorly known. Only a small set of all OTUs (3.4%) was shared across all sampling locations in this study, mirroring results from previous studies suggesting a large turnover of species across sites (Bjorbækmo *et al.* 2010; Blaalid *et al.* 2014; Botnen *et al.* 2019). This means there is not

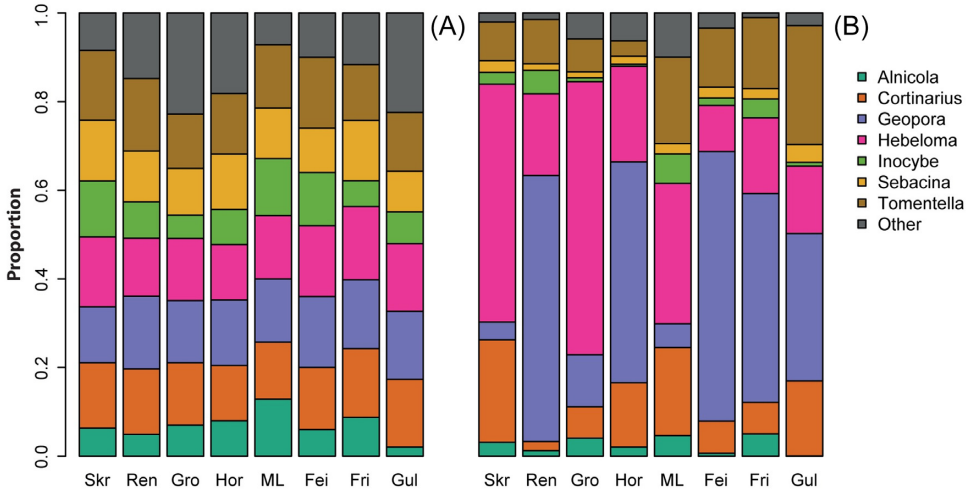

Figure 4. Taxonomic distribution of ectomycorrhizal fungal OTUs associated with *Salix polaris* and *Bistorta vivipara* in glacier forelands. **(A)** Frequency of OTUs (occurrences in samples). **(B)** Read abundance of OTUs.

a certain set of early colonizing core community of ECM fungi present in all glacier forelands, but rather that a sub-sample of fungi adapted to grow as early colonizers appears at each site. As discussed below, both climatic and soil edaphic factors are important for the fungal community composition in glacial forelands, filtering group of early colonizing ECM fungi that establish in different sites.

Who are the early colonizing ECM fungi?

Geopora was the most abundant genus based on number of reads and represented a high number of OTUs (125). These numbers represent a large mismatch to registered fruiting bodies of *Geopora* in Svalbard; only 10 collections of fruiting bodies from Svalbard are registered in the Norwegian Biodiversity Information Centre (artskart.artsdatabanken.no 2019), of which only two are identified at the species level. New species of this genus have been described over the last few years (Southworth and Frank 2011; Guevara-Guerrero *et al.* 2012; Flores-Rentería *et al.* 2014). Still, our results suggest the presence of several undescribed members of *Geopora*. Many of the reads mapped to internal and not terminal branches in the reference tree, suggesting these reads represent phylogenetically distinct entities. This may indicate that there are several undescribed species of *Geopora* in glacier forelands of Svalbard. Thus, the diversity and function of *Geopora* is likely much higher than what we currently know in Arctic environments.

For most fungi, dry and nutrient-poor conditions represent a highly stressful environment (Kubicek and Druzhinina 2007), and to thrive under such conditions require certain adaptations for survival (Jumpponen and Trappe 1998; Tibbett, Sanders and Cairney 2002; Kubicek and Druzhinina 2007; Tibbett and Cairney 2007; Newsham 2011; Dhakar and Pandey 2016; Pandey 2019). The ascomycete genus *Geopora* seems to belong to this group of specialists. Ectomycorrhizal ascomycetes, such as *Geopora*, are typically more stress tolerant than ectomycorrhizal basidiomycetes. Most of the *Geopora* OTUs we detected were associated with higher C:N and low soil OM (Fig. 6C), which is associated with undeveloped, nutrient-poor mineral soils (Yoshitake *et al.* 2007). In addition, the distribution of *Geopora* was related to lower precipitation. *Geopora* species have previously been found to dominate as early colonizer in post-fire succession (Fujimura *et al.* 2005), and to be common under drought stressed conditions (Gordon and Gehring 2011), e.g. on fly-ash, where the model species *Laccaria laccata* would not grow (Hrynkiewicz *et al.* 2009), and in costal sand dunes (Botnen *et al.* 2015). Similarly, previous studies have frequently found undescribed members of *Geopora* on ECM root-tips growing in different marginal habitats (Gehring *et al.* 1998; Fujimura *et al.* 2005; Hrynkiewicz *et al.* 2009; Ishida *et al.* 2009), including mine tailings in the Arctic (Mundra, Bahram and Eidesen 2016). Thus, *Geopora* are clearly a vital symbiont under extreme environmental conditions and may play an important role as facilitator of plant establishment in extreme, marginal environments, like in High Arctic glacier forelands studied here.

Geopora and also *Tomentella*, both abundant in our dataset, are characterized by species producing inconspicuous, semi-hypogeous fruit bodies. This is likely an adaptation to the extreme environment with irregular frost periods and limited precipitation. Although Svalbard has been surveyed by mycologists since the 1900s (Hesselman 1900), and accumulating literature based on fruit body collection and fungal cultivation have documented presence of ~750 macrofungi (Carlsen *et al.* 2013) (Elvebakk and Presterud 1996), the actual number is probably much higher. Current collections from Svalbard are mainly from two locations with logistic facilities (Longyearbyen and Ny-Ålesund), whereas Svalbard cover a land area of 65 000 km^2. Our study includes localities that very rarely have been visited due to the logistical challenges by accessing these locations. Furthermore, the belowground diversity, as indicated by DNA-based surveys (Blaalid *et al.* 2014; Botnen *et al.* 2014; Morgado *et al.* 2016; Mundra *et al.* 2016), are generally many times higher than what observed by macroscopic fruit bodies. Producing large fruiting bodies may be a haphazard strategy in the High Arctic, since they are vulnerable to both freezing and drought. Hence, a reason for the mismatch between registered macrofungi and DNA analyses could be that a larger proportion of species produce inconspicuous and cryptic fruiting bodies in the High Arctic, as a response to the extreme conditions. This speculative hypothesis remains to be properly tested.

Hebeloma was the second most abundant genus recovered in this study, and seemed to be more common when *Geopora* was less frequent (Fig. 4; Fig. 6C and F). Whereas *Geopora* was related to lower soil OM and lower precipitation, *Hebeloma* was

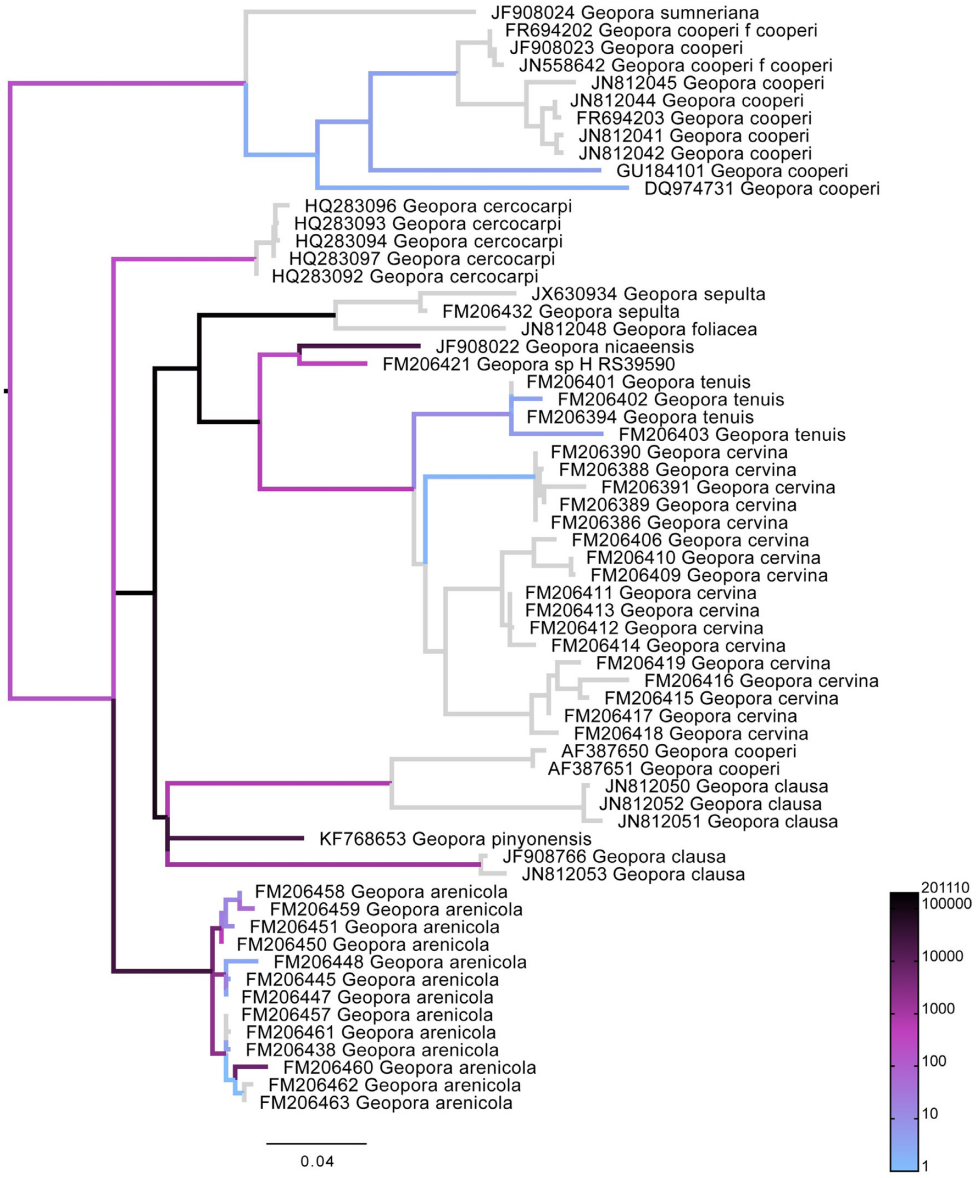

Figure 5. RAxML-generated backbone tree of *Geopora* species with midpoint branching showing the EPA-based placement of all Geopora reads. The number of reads placed on a branch is indicated in blue to purple to dark purple (ascending order), and grey branches represent branches on which no reads were placed.

associated with lower pH, higher soil OM and higher levels of precipitation. These results may suggest that *Hebeloma* has preference for crusted soil when colonizing glacier forelands; biological soil crusts (BSCs) promote soil formation and accumulation of organic matter in early stages of primary succession. Crusted surfaces also retain water, and have higher nutrient content than bare soil (Bliss and Gold 1999; Breen and Lévesque 2006, 2008). In addition, the extent of BSC depends on water availability. Regular rainfall (Büdel *et al.* 2009) or steady supply of glacier melt water promotes BSC development (Breen and Lévesque 2008). This is in line with *Hebeloma* being associated with higher levels of precipitation. *Cortinarius* showed a similar clustering pattern in the ordination as *Hebeloma*, indicating similarities in environmental preferences of these genera. Increased precipitation and temperatures are already registered as a response to climate change (Pachauri and Mayer 2015; AMAP 2017; Bilt *et al.* 2019), and accumulation of organic matter will probably increase as well. These genera may benefit from current changes and show increased abundance in glacier forelands in near future.

Alnicola may also become a more common ECM partner for pioneer plants in Svalbard in near future. A few OTUs, but a relatively high number of reads, were affiliated with *Alnicola*. Their species optima clustered closely together in the ordination structure and were associated with higher temperatures and precipitation. The distribution of the few *Alnicola* OTUs overlaps largely with *Hebeloma*, which may reflect their close phylogenetic relationship (Moreau, Peintner and Gardes 2006). Other groups, and especially *Inocybe* and *Sebacina*, distributed more widely in the ordination plot. These genera have earlier been identified as dominating members of ECM plant roots (Timling *et al.* 2012; Blaalid *et al.* 2014; Botnen *et al.* 2014) and in soil (Deslippe *et al.* 2012; Geml *et al.* 2012; Timling and Taylor 2012) of the High Arctic and might have a higher level of ecological plasticity to persist through extreme environmental changes.

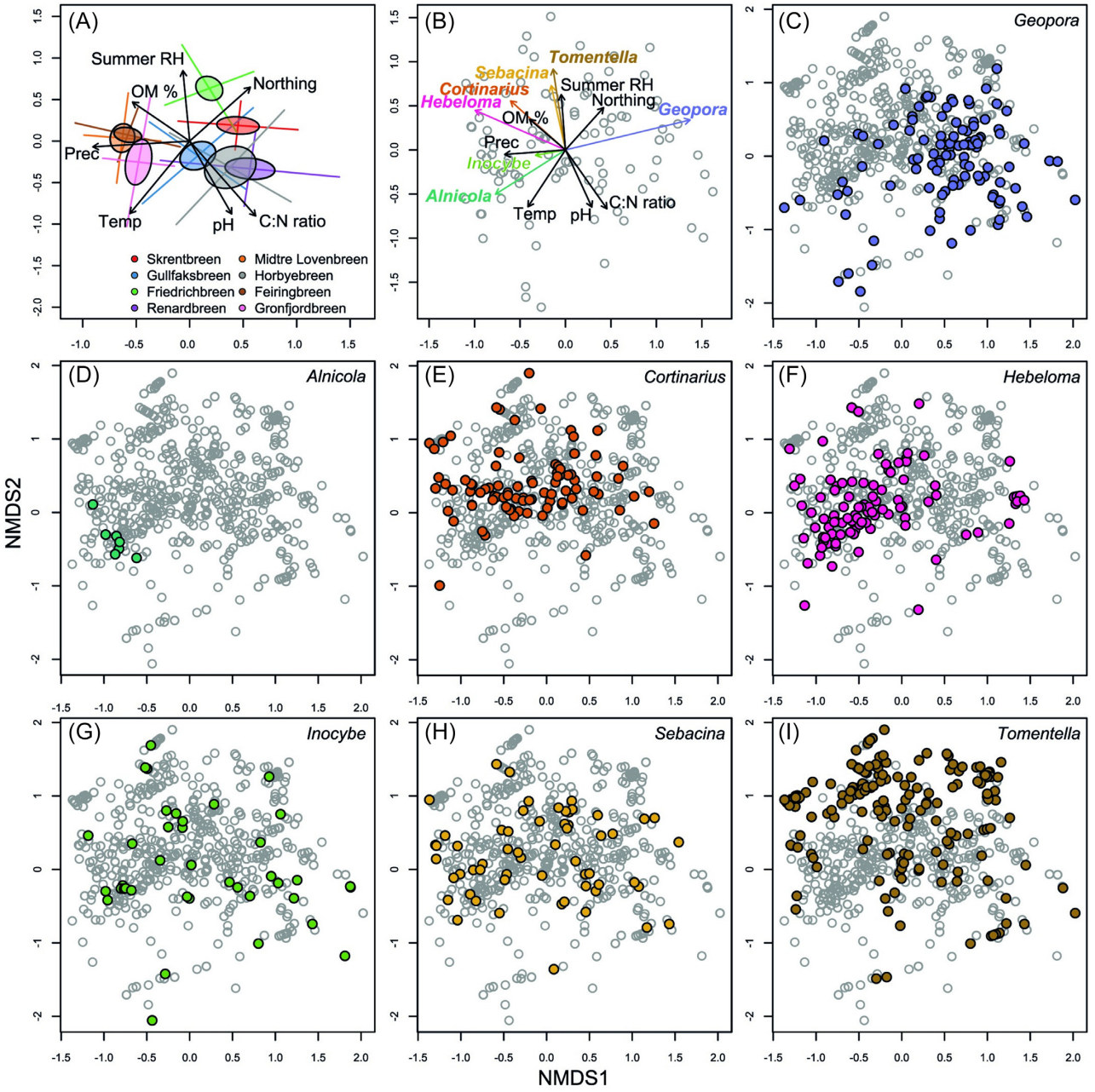

Figure 6. GNMDS ordinations of ECM fungal OTUs based on a rarefied OTU matrix. **(A)** The colored ellipses represent the standard errors (SE), and the colored lines represent the standard deviation (SD) of the centroids of the samples from the different glacier forelands. Arrows represent direction of maximum increase of annual precipitation and temperature, summer relative humidity (RH), soil organic matter (OM), soil pH, soil carbon:nitrogen (C:N) and northing. **(B)** Each point represents one root system, the black arrows represent the same as in (A), while the colored arrows represent the direction of maximum increase of reads in the genera *Geopora*, *Alnicola*, *Cortinarius*, *Hebeloma*, *Sebacina* and *Tomentella*. **(C–I)** Each point represents the species scores (weighted averages) of one OTU. Colored points represent OTUs with affiliation to the ECM genera: **C**: *Geopora*, **D**: *Alnicola*, **E**: *Cortinarius*, **F**: *Hebeloma*, **G**: *Inocybe*, **H**: *Sebacina* and **I**: *Tomentella*.

Drivers of community structure and diversity

Changes in the overall ECM community structure correlated with changes in several environmental and geographical factors. This confirms previous findings: at local scales community composition of root-associated fungi of ectomycorrhizal plants has been found to correlate with changes of several soil edaphic factors in alpine (Yao *et al.* 2013; Aas *et al.* 2019) and Arctic areas (Mundra *et al.* 2015); and at larger scales also with precipitation and temperature (Tedersoo et al. 2012, 2014; Timling *et al.* 2014; Botnen *et al.* 2019). The variation partitioning indicated sampling location and soil OM content to be the most important structuring factors. Thus, climatic factors were less pronounced looking at the community composition overall. However, it is important to note that some of the factors measured were somewhat correlated, and their effect on the fungal community structure are difficult to tease apart and combined effects might be important. The variation explained by location could, for example, mask some of the variation explained by the measured environmental factors. Still, a large fraction (~85%) of the variation in community composition could not be explained by our measured variables, including geography.

Our results indicate that pH and C:N ratio are the most important factors explaining differences in OTU richness across root systems. On a global scale, pH has been found to be one of the most important factors for predicting ECM fungal richness (Tedersoo *et al.* 2014). The negative correlation we observed between pH and richness is likely due to that high pH is typically associated with mineral soils with low OM content found in the harshest and climatically extreme localities (Yoshitake *et al.* 2007). On the other hand, when it comes to the Shannon diversity index, climatic factors seem to be more important. We observed an increase in diversity with higher precipitation, and a decrease with higher temperature. In general, allocation to roots is high in the Arctic, however, at very dry sites allocation decreases (Iversen *et al.* 2015). As such, the lower Shannon diversity associated with reduced precipitation could be explained by reduced resource availability.

The future and unknown diversity

The accelerated pace of glacier retreat, together with changes in the environmental conditions, such as warmer climate and less draught, will have profound effects on Arctic ecosystems. Models of plant succession in glacier forelands suggest that the effect of competition decreases with higher environmental stress (Svoboda and Henry 1987), and Davey *et al.* (2015) suggested a similar trade-off in root-associated fungal succession. Thus, with a reduction in environmental resistance, competition may become more important in successional patterns in the High Arctic. This may lead to changes toward a directional replacement successional pattern. As such, species prevalent in cold, dry and nutrient-poor environment with high soil disturbance may disappear. Many poorly studied *Geopora* species may be adapted to such environments and might be especially vulnerable to environmental change, due to faster plant succession and establishment of closed vegetation (Elven and Ryvarden 1975; Robbins and Matthews 2010).

However, for the other ECM fungi, such as *Cortinarius and Hebeloma* not bound to such marginal habitats, climate change might rather represent an opportunity to expand. Several studies show a decline in fungal richness toward the poles associated with changes in large-scale climatic factors (i.e. temperature and precipitation) (Tedersoo *et al.* 2014; Bahram *et al.* 2018; Tedersoo, Bahram and Zobel 2020). Thus, a warmer Arctic may support more fungal species than today, but based on our findings, the composition is likely to be different.

Sequences of the root-associated fungi retrieved from glacier forelands in Svalbard were clearly less documented and had lower taxonomic resolution in the UNITE reference database compared to datasets from other sites in Svalbard (Blaalid *et al.* 2014; Botnen *et al.* 2014), mainland Norway (Blaalid *et al.* 2012; Yao *et al.* 2013), Scotland and the Austrian alps (Botnen *et al.* 2019). Our investigations reveal a general knowledge gap connected to diversity of root-associated fungi in the extreme environments such as High Arctic glacier forelands, and suggest presence of undescribed species with yet unknown functions. Experiments simulating climate change in arctic environments, such as snow accumulation studies (Morgado *et al.* 2016; Mundra *et al.* 2016) and open top chambers studies (Morgado *et al.* 2015; Geml *et al.* 2016), have revealed that increasing temperature and more precipitation in winter can negatively affect the ECM fungal richness. Thus, with continued climate change we will likely lose unknown fungal diversity, without even knowing their identity or importance for the ecosystem.

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SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

DATA AVAILABILITY STATEMENT

Sequence data with corresponding mapping files are available at dryad.org: doi:10.5061/dryad.7sqv9s4qw.

AUTHOR CONTRIBUTIONS

All authors contributed to scientific ideas; PBE and SM designed sampling plan; HK, SM and PBE secured funding; all authors contributed to research design; SM, PBE and SSB conducted fieldwork; SM conducted labwork and did bioinformatical analyses; SSB performed phylogenetic and statistical analyses; and SM drafted parts of the manuscript related to fieldwork, labwork and bioinformatical analyses, while SSB drafted the rest with contribution from all authors. SSB and SM contributed equally to this paper, and are, as such, joint first authors.

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