RESEARCH ARTICLE

Community composition of arctic root-associated fungi mirrors host plant phylogeny

S. S. Botnen1,2,3,*†, E. Thoen1, P. B. Eidesen2, A. K. Krabberød1 and H. Kauserud1

1Section for Genetics and Evolutionary Biology (EVOGENE), Department of Biosciences, University of Oslo, PO Box 1066 Blindern, NO-0316 Oslo, Norway, 2The University Centre in Svalbard, PO Box 156, NO-9171 Longyearbyen, Norway and 3Oslo Metropolitan University, PO Box 4 St. Olavs plass, NO-0130 Oslo, Norway

*Corresponding author: Oslo Metropolitan University, PO Box 4 St. Olavs plass, NO-0130 Oslo. E-mail: s.s.botnen@ibv.uio.no; synnoves@oslomet.no

One sentence summary: Community composition of arctic root-associated fungi mirrors host plant phylogeny.

ABSTRACT

The number of plant species regarded as non-mycorrhizal increases at higher latitudes, and several plant species in the High-Arctic Archipelago Svalbard have been reported as non-mycorrhizal. We used the rRNA ITS2 and 18S gene markers to survey which fungi, as well as other micro-eukaryotes, were associated with roots of 31 arctic plant species not usually regarded as mycorrhizal in Svalbard. We assessed to what degree the root-associated fungi showed any host preference and whether the phylogeny of the plant hosts may mirror the composition of root-associated fungi. Fungal communities were largely structured according to host plant identity and to a less extent by environmental factors. We observed a positive relationship between the phylogenetic distance of host plants and the distance of fungal community composition between samples, indicating that the evolutionary history of the host plants plays a major role for which fungi colonize the plant roots. In contrast to the ITS2 marker, the 18S rRNA gene marker showed that chytrid fungi were prevalently associated with plant roots, together with a wide spectrum of amoeba-like protists and nematodes. Our study confirms that arbuscular mycorrhizal (AM) fungi are present also in arctic environments in low abundance.

Keywords: Arctic; host preference; microeukaryotes; root-associated fungi; Svalbard

INTRODUCTION

Most plants form root symbiosis with fungi, of which mycorrhizal associations are most common (Smith and Read 2008; Brundrett 2009). The ectomycorrhizal (ECM) and ericoid mycorrhizal (ErM) symbioses dominate in northern ecosystems, while arbuscular mycorrhiza (AM) is more widespread in the tropics (Smith and Read 2008; Steidinger et al. 2019). In arctic environments, ECM and ErM fungi are common but limited to a few geographically widespread plant species (Gardes and Dahlberg 1996), while AM has been assumed to be less common. Despite the importance of mycorrhizal symbiosis in comparable nutrient-poor temperate systems, a large portion of High Arctic plants have been reported as non-mycorrhizal (Väre, Vestberg and Eurola 1992; Kytöviita 2005; Newsham, Upson and Read 2009). Newsham, Upson and Read (2009) found evidence for an increase in plant species without mycorrhizal symbioses at higher latitudes. In a microscopy-based survey of root-associated fungi (RAF) of 76 plant species in the High-Arctic Archipelago Svalbard, Väre, Vestberg and Eurola (1992) observed only one single AM spore in a soil sample. Similar results have been reported from several other High-Arctic locations, with no
and alpine areas on both mycorrhizal and non-mycorrhizal
plant species (Newsham et al. 2014; Lindahl et al. 2015).
Several studies from arctic and alpine areas have shown that the dominant ECM plants typically share the same pool of ECM fungal symbionts (Ryberg, Larsson and Molau 2009; Ryberg, Andreasen and Björk 2011; Timling et al. 2012; Botnen et al. 2014; Linde et al. 2018). Several studies from arctic and alpine areas have shown that the dominant ECM plants typically share the same pool of ECM fungal symbionts. In this regard, we (5) investigate this diversity to get a broader picture and a better understanding of the ecosystem as a whole. From other areas, it is known that plant roots may host a rich diversity of various protists (including protists), Archeorhizomycetes and AM fungi (Rosling et al. 2011; Tedersoo et al. 2015; Dunthorn et al. 2017). However, the 18S rRNA gene is a far more conserved marker than ITS, and therefore the 18S rRNA gene data cannot be interpreted at lower taxonomic levels (i.e. species, genera).

In this study, we use both ITS2 and 18S rRNA gene markers to survey the diversity of fungi and other micro-eukaryotes in roots of 31 assumed non-mycorrhizal arctic plant species or plants species with unclear mycorrhizal status. We aim to (1) assess which types of fungi (taxonomically and functionally) are associated with roots in common arctic plant species not prevalently regarded as mycorrhizal, and to (2) obtain basic knowledge about tentative root symbionts and their mycorrhizal status. By using the 18S rRNA gene marker we also wanted to (3) obtain information about which other micro-eukaryotes are present, which has largely been neglected in the past. Further, we wanted to (4) assess whether the root-associated fungi are host generalist, as is typically the case for arctic ECM fungi, or whether they show some level of host specificity. In this regard, we (5) investigate whether the phylogenetic distance between the host plant species reflects which fungi are colonizing specific plant species.

MATERIALS AND METHODS

Sampling site

Fieldwork was conducted during July 2013 and 2014 in the High-Arctic Arcipelago Svalbard, Norway (Fig. 1 and Table S1, Supporting Information; RIS-ID: 6549 and RIS-ID: 6165). We sampled 31 plant species assumed to be non-mycorrhizal or having unclear mycorrhizal status (1-7 root systems per species) from 11 different locations across the archipelago (Fig. 1).

Sampling procedure, DNA extraction, PCR procedure and illumina sequencing

Whole root systems were dug up and cleaned within 24 h of sampling. The roots were first rinsed in tap water, removing all visible soil, debris and plant-roots not attached to the target plants. The roots were removed from the aboveground structure and finally rinsed in separate petri dishes in Milli-Q (MQ) H₂O three times for 30 s. The root samples were then stored at −20 °C until DNA extraction. Soil samples for determining soil characteristics were collected from the same hole as the plant roots. The roots were put in 50 ml Falcon tubes with 2000 µL CTAB and homogenized and extracted as described in Botnen et al. (2019). In short, a modified CTAB protocol was used (Murray and Thompson 1980; Gardes and Bruns 1993), and the DNA extracts were further cleaned using the E.Z.N.A soil kit (Omega Bio-Tek, Norcross, GA). The internal transcribed spacer 2 (ITS2)
of the rRNA gene was amplified using the forward primer fITS7a (Jhrmark et al. 2012) and the reverse primer ITS4 (White et al. 1990). Both primers were tagged with 12 base-pairs (bp) long molecular identifiers, and two bp (NN) was added to the 5’ end of both forward and reverse primers to achieve high multiplexing of samples. The PCR protocol and Illumina library preparations are described in detail in (Jacobsen et al. 2017). A total of 10 samples were sequenced twice for within-sample comparisons to ensure sequencing consistency. One library was sent to GATC Biotech for adaptor ligation and Illumina HiSeq Rapid Run 2 × 300 bp paired-end sequencing.

The V4 region of the eukaryotic 18S rRNA gene was amplified with the universal primers TAREuk454FWD1 and TAREukREV3 (Stoeck et al. 2010). The primers were tagged with a 6 bp long molecular identifier, and 1–3 bp (N) was added to the 5’ end of both forward and reverse primers to achieve high multiplexing of samples. PCRs were performed in 25 μL reaction containing: 15.6 μL MQ H2O, 2.5 μL GeneAmp PCR Buffer II, 2.5 μL MgCl2, 0.2 μL dNTPs (25 nM), 0.125 μL AmpliTaq Gold DNA polymerase, 1.5 μL reverse and forward primers (10 μM), 1 μL BSA (20 mg/mL) and 1.5 μL DNA template. The PCRs were run under the following conditions: 98°C for 7 min, then 15 cycles of 95°C for 30 s, 53°C for 30 s and 72°C for 45 s, followed by 15 cycles of 98°C for 30 s, 48°C for 30 s and 72°C for 45 s, ending with 72°C for 10 min. Amplicons were checked on 1.5% agarose gel for successful amplification and cleaned using the ZR-96 DNA Clean-up Kit (Zymo Research, Irvine, CA). The DNA-concentrations were then quantified using Qubit dsDNA BR Assay, and pooled in two equimolar libraries.

The libraries were then further purified and concentrated using DNA clean and concentrator (Zymo Research) before being shipped for 2 × 250 PE sequencing on an Illumina MiSeq sequencer at Fasteris (Switzerland).

Bioinformatics

Raw reads were demultiplexed using cutadapt (Martin 2011), with the following settings: anchored primers, and minimum length of 100 bp. Further quality filtering, error-correction (denoising), and merging of PE reads were conducted using DADA2 (Callahan et al. 2016) in the statistical environment R. Quality filtering was done with the following settings differing from default: maxEE = 2, truncQ = 11, minLen = 50. For the ITS2 data, a minimum of 50 bp overlap was required for merging of reads. For the longer V4 18S rRNA gene region, a minimum overlap of 15 bp was used. ITSx was used to extract the ITS2 region (Bengtsson-Palme et al. 2013). Because of the intraspecific variability in the ITS region, the ITS2 reads were clustered at a 97% similarity level into operational taxonomic units (OTUs) using VSEARCH v2.11.1 (Rognes et al. 2016). To achieve an improved OTU delimitation, LULU (Frøslev et al. 2017), was used to combined genetically similar OTUs with similar ecological signals in the resulting OTU table. Overview of the number of reads passing the different filtering steps can be found in S2. Taxonomy of the ITS2 OTUs was assigned in UNIVERS v8 (UNITe Community 2019), and their functional guilds and trophic modes were assigned using FUNGuild (Nguyen et al. 2016). For the OTUs assigned as possible ECM fungi other trophic mode (i.e. Ectomycorrhizal-Fungal Parasite-Plant Pathogen-Wood Saprotroph) in FUNGuild, the species hypotheses (SH) in UNITe was manually inspected. If the OTU was annotated to an ECM group (based on Teder-soo, May and Smith 2010), it was assigned as ECM, otherwise as dubious. OTUs not identified as fungi in UNITe was discarded from the dataset. For the 18S rRNA gene, we used the sequence variants from DADA2 (i.e. tentative haplotypes) as OTUs for further analyses. This is due to lower intraspecific variation in the 18S rRNA gene region. They were taxonomically assigned using a modified PR2 database (Guillou et al. 2013), were the V4 region matching the primers used had been extracted. Only 1.5% of the 18S rRNA gene reads were non-plant and 80% of those reads were from the mock fungal community, resulting in 33 677 reads for further analyses.

Phylogenetic analysis

Phylogenetic trees were constructed to verify the taxonomic assignment of the microeukaryotic OTUs (i.e. non-plants) obtained from the 18S rRNA gene V4 region. All the non-plant OTUs were blasted against the non-redundant nucleotide database at NCBI and PR2 and the top five hits for each OTU were added to a reference alignment of 365 full-length 18S rRNA gene sequences covering the major branches of eukaryotic diversity. The sequences were aligned using the L-INS-i algorithm in MAFFT v7.427 (Katoh and Standley 2016) and a preliminary tree was built with FastTree2 (Price, Dehal and Arkin 2010) implemented in Geneious Prime 2019 (https://geneious.com). Based on the preliminary tree the alignment was split
into separate subsets for each of the main branches of eukaryotes: Alveolata, Rhizaria, Stramenopiles, Hacrobia, Fungi, Metazoa and other Podiata (i.e. without Metazoa and Fungi). Fungi and the other podiates are hard to get good results with 18S rRNA gene on a species level, which can be related to several of the groups being polyphyletic (e.g. Amoebozoa and Apusozoa), poor taxon resolution, or potential incongruence with other common phylogenetic markers. Each of the subsets was realigned with L-INS-i in MAFFT v7.427 (Katoh and Standley 2016), and ambiguously aligned characters removed with trimAl v1.2rev59 ([Capella-Gutiérrez, Silla-Martínez and Gabaldón 2009], setting the gap threshold (-gt) to 0.3 and the minimum average similarity allowed (-st) to 0.001). Finally, maximum likelihood phylogenetic trees were constructed for each group separately with RAxML v8.0.26 (Stamatakis 2014) using the rapid bootstrapping and subsequent ML search and the GTR-GAMMA model. The number of bootstraps was automatically calculated based on the MRE bootstrapping criterion (Pattengale et al. 2010). Trees were visualized with FigTree 1.4.4 (Rambaut 2018).

Statistical analyses

If not otherwise specified, the statistical analyses were conducted in R (R Development Core Team 2010). For 18S rRNA gene OTUs, some of the technical replicates (i.e. samples sequenced twice) were slightly divergent, and some samples included only plant reads. Due to this, as well as the low amount of 18S rRNA gene data obtained, compositional analyses of the 18S rRNA gene data were not deemed valid. However, the included mock communities were identical in the 18S rRNA gene data, as well as close to identical in proportion of reads (0–1.3% differences). Thus, we only report overall 18S rRNA gene taxonomical composition and proportional non-plant reads for 18S rRNA gene data. In the ITS2 data, the technical replicates were similar in abundance and frequencies, and though not identical, they showed more inter-sample than intra-sample differences (GNMDS, Figure S1, Supporting Information). Additionally, paired t-tests of abundances were conducted on each of the replicated sample pairs (raw reads), and no statistically significant differences in between repeat abundance measures of individual OTUs (P > 0.05) were found. Supported by this initial evaluation, we conducted compositional analyses of the ITS2 data, including comparisons of abundance between samples.

To ensure that the abundance data did not significantly affect statistical conclusions, both abundance and occurrence data were analysed in the form of: (1) a matrix rarefied to 1527 reads per samples transformed to presence/absence data and (2) a matrix of abundance data transformed to relative abundance (proportions) of samples with > 1500 reads. Procrustes analyses showed consistent results (P = 0.001) of the ordinations structures derived from the two different matrices.

The community structure of the root-associated fungi was analysed using global non-metric multidimensional scaling (GNMDS). Detrended correspondence analyses (DCA) were performed in parallel to ensure the reliability of the results. Correspondence in the results between the GNMDS and DCA and absence of artefacts, such as arch-effect, tongue-effect and extreme outliers, were interpreted as reliable gradients. The GNMDS settings were as recommended by Liu et al., (2008), and the DCA was run using default settings, both using the vegan package (Oksanen et al. 2012). The envfit function in vegan was used to fit vectors of the environmental variables: host plant species; host plant family; host plant order; longitude; latitude; mean July temperature and precipitation; pH and sampling locations on the ordination. To confirm the expected taxonomical structure, the potential clustering of plant families was visualized by standard error (SE) and standard deviation (SD) of their centroids using the ordiellipse and ordispider functions in vegan. To quantify the components of variation in the community composition explained by the variables mentioned above, variation partitioning, with forward selection, was performed using canonical correspondence analyses (CCA) with 999 permutations, as implemented in vegan.

To test for correlation between genetic relationships of hosts and the fungal community, distance matrices of the host DNA were constructed. The ribulose bisphosphate carboxylase large chain (rcBLA) region of the investigated host plants was downloaded from The Barcode of Life Data System (BOLD; Ratnasingham and Hebert 2007), and chloroplast trnL intron sequences were downloaded from NCBI (NCBI Resource Coordinators 2018) based on Sanstebe et al. (2010). The sequences were then aligned using the L-INS-i algorithm in MAFFT (Katoh and Standley 2013). Genetic distance matrices were constructed using the ape and adegenet package in R (Jombart 2008; Popescu, Huber and Paradis 2012). Correlation was tested by a Procrustes correlation test with 999 permutations between the distance matrix of the hosts DNA and the distance matrix of the fungal communities (Bray-Curtis distances, used in GNMDS analyses above).

RESULTS

Data characteristics, taxonomy and functional roles

A total of 847 fungal OTUs appeared in the ITS2 dataset, distributed across the 31 plant species root systems. Each root systems hosted on average 22.9 fungal ITS2-based OTUs, ranging from 6 in Eriophorum scheuchzeri to 54 in Carex rupestris (Fig. 2). Helotiales was the most abundant order, both in terms of number of reads (Fig. 3A) and OTUs (Figure S2, Supporting Information), followed by Pleosporales and Agaricales. Within the Helotiales, the majority of the ITS2 reads could not be assigned with confidence at a lower taxonomical level.

The 18S rRNA gene data was highly dominated by DNA sequences from the host plant; in all but one sample the host plant sequences made up from 96.2 to 100% of the reads (one outlier sample of Juncus biglumis had 83.9% plant DNA), which resulted in 248 non-plant OTUs. Of the non-plant reads, Fungi, Metazoa, Cercozoa and Stramenopiles made up most of the sequences (Fig. 4). Within Fungi, chytrids were the most abundant subgroup, largely missed out in the ITS2 dataset. Within Metazoa, nematodes were highly prevalent in the 18S rRNA gene data (~12% of the non-host 18S rRNA gene OTUs), while Oomyco was the dominating stramenopile group. The groups with highest phylogenetic diversity were the cercozoan subphyllum Filosa (35 OTUs), Amoebozoa (29 OTUs), nematodes (30 OTUs) followed by ciliates (19 OTUs) and oomycetes (18 OTUs) (Figures S3–S9, Supporting Information).

In the ITS2 dataset, reads annotated as fungal saprotrophs or pathogens dominated in most plants (46.7%; Fig. 3B). The most common ITS2 OTU both in terms of reads and occurrences (266 774 reads, 87 occurrences), was affiliated with Rhizocerosporidium panici (97.9% identity), a plant root pathogen. Likewise, a root system of Draba cf. corymbosa was dominated by one ITS2 OTU with the closest hit (96%) to the plant pathogen Olpidium brassicae, which may indicate an infection. The proportion of reads assigned as mycorrhizal fungi varied greatly, from absence in Festuca and Eriophorum scheuchzeri to 17.7% in Oxycia digyna.
Figure 2. Table showing number of samples, mean number of OTUs/reads and proportion of reads assigned as mycorrhizal for ITS2, mean number of ASVs and proportion of non-plant reads. The different colors represent different plant families. Schematic phylogenetic tree showing relationships between sampled hosts is based on: Saarela et al. 2018 (within Poaceae); Tkach et al. 2015 (within Saxifragaceae); Jordon-Thaden et al. 2010 (within Draba) and Soltis et al. 2011 (families within Angiosperms).

In total, 16 ITS2 OTUs with affiliations to Glomeromycota appeared across 18 samples and eight locations in roots of the known AM hosts: Taraxacum arcticum, Ranunculus sp. (all but one sample), Potentilla puchella (all but one sample), and Micranthes nivalis (one sample). The five 18S rRNA gene OTUs with affiliation to Glomeromycota were detected in the same plant genera as for the ITS2 OTUs. Glomeromycota represented 2% of the ITS2 OTUs, and 6.6% of the fungal 18S rRNA gene OTUs. A total of 97 ITS2 OTUs were assigned as ECM fungi. The plant species hosting most potential ECM fungi, in terms of proportional read abundance, were O. digyna, Luzula confusa and Carex rupestris. There were also some ITS2 OTUs classified as orchid mycorrhizal (OM), and they all had closest hit to Serendipita sp. (86–100% identity). Only nine ITS2 OTUs were classified as DSE. The most common OTU found in Deshampsia alpina had its closest hit to Cadophora malrum (93.8%), which was classified as an endophyte.

Host identity and drivers of community structure

The composition of the fungal communities (based on ITS2), was highly structured by the host plant identity, both at species, family and order level (Fig. 5 and Figure S10, Supporting Information). In correspondence with this, we observed a correlation between phylogenetic distance among host plants, assessed both by the rbcLa (Procrustes correlation: 0.41, P = 0.001) and trnL (Procrustes correlation: 0.37, P = 0.001) cpDNA markers, and fungal community distance between the host plants. Furthermore, the variation partitioning analysis indicated that host species explained 33% of the total inertia (TI). The families Poaceae (six species) and Polygonaceae (one species), formed particularly clear clusters with no overlap in SE with other families (Fig. 5). A certain degree of clustering was also observed for Orobanchaceae (Pedicularis sp.), although some overlap with other families was found. The families Caryophyllaceae (mainly Cerastium) and Juncaceae clustered closely together. The rest of the families cluster together on one side of the ordination, with little difference separated along the first ordination axis (Fig. 5).

In addition to host plant identity, we also observed that the recovered community structure was related to certain climatic factors (July temperature and precipitation), soil pH, sampling location, longitude and latitude (Table 1). The variation partitioning indicated that sampling location explained 13.5% of TI while interaction effects between different factors accounted for a further 5.5% of TI.
Figure 3. Taxonomy (A) and functional guilds (B) on a genus level based on read abundance data of the ITS2 OTUs.

Figure 4. Overview of the total taxonomy based on the 18S OTUs. Number of OTUs are indicated in the circle around the pies. The left-hand pie represents approx. kingdom level taxonomy, while the right-hand pie approx. phylum.
Their corresponding coefficient of determination ($R^2$) and omyvectors fitted to the GNMDS by the envfit function in vegan, with their corresponding coefficient of determination ($R^2$) and $P$-value.

| Variable               | $R^2$   | $P$-value |
|------------------------|---------|-----------|
| Plant genus            | 0.4529  | 0.001     |
| Plant species          | 0.5460  | 0.001     |
| Plant family           | 0.3109  | 0.001     |
| Plant order            | 0.1611  | 0.015     |
| Latitude               | 0.1087  | 0.015     |
| Longitude              | 0.1716  | 0.001     |
| Mean July temp         | 0.2632  | 0.001     |
| Mean July prec         | 0.1052  | 0.008     |
| Soil pH                | 0.4213  | 0.001     |
| Soil N%                | 0.0883  | 0.028     |
| Sampling location      | 0.3035  | 0.001     |

**DISCUSSION**

Our study revealed a rich diversity of fungal groups associated with roots of arctic plants species expected to be non-mycorrhizal or with unclear mycorrhizal associations. The community composition of these fungal groups was strongly related to plant host identity. Although we obtained limited non-plant sequence data from the 18S rRNA gene marker, these data indicate that ITS2 data provide a highly biased view of the fungal communities, especially missing out Chytridiomycota and Mucoromycota, which were among the most abundant fungal groups in the 18S rRNA gene data.

As revealed by the ITS2 marker, different ascomycetes orders were dominant in the investigated plant roots. These results are in stark contrast to what has been observed associated with arctic ECM plants at Svalbard, where ECM basidiomycetes typically dominate (Bjørbaekmo et al. 2010; Blaalid et al. 2014; Botnen et al. 2014; Davey et al. 2015; Mundra et al. 2015a, b, 2016). The observed taxonomic composition is more similar to what has been observed as aboveground endophytes (Zhang and Yao 2015) in Svalbard. We observed relatively few DSE. DSE are frequently reported from plants in alpine and polar areas, where they have been shown to play an important function in nutrient uptake (Jumpponen, Mattson and Trappe 1998; Upson, Read and Newsham 2009; Hill et al. 2019). The low proportion of DSE could partly be due to insufficiently annotated reference sequences in the databases; several of the Ascomycetes annotated with unknown functions may be DSE.

While the ascomycete orders Helotiales, Pleosporales, Chaetothyriales and Sordariales dominated in most of the 31 plant species in the ITS2 data, O. digyna represented an exception. There was a relatively high abundance of ECM basidiomycetes associated with O. digyna roots, such as Thelephorales (Tomentella), Inocybe and Cortinarius of Agaricales. Oxyporhinus digyna belongs to the same family as Bistorta vivipara, which is a widespread ECM forming plant in the Arctic (Hesselman 1900; Gardes and Dahlberg 1996) and is well known for its extensive root structures (Maessen et al. 1983). Cripps and Eddington (2005) did not observe any signs of mycorrhiza in alpine O. digyna. ECM fungi have earlier been observed inside roots of non-ECM-forming plant species without typical ECM structures (Vrålstad, Schumacher and Taylor 2002; Vrålstad 2004; Smith and Read 2008; Schneider-Maunoury et al. 2020). Thus, many ECM fungi may live inside the plant roots without forming ECM symbiosis. Alternatively, based on our DNA data, we may hypothesize that O. digyna forms ECM-like symbiosis in the Arctic. This must be confirmed with re-synthesis experiments and microscopy. Still, the majority of the ITS2 reads in O. digyna were classified as belonging to Pathotroph–Saprotroph fungi by FUNguild. Hence, if a mycorrhizal association is present, it is likely not the dominating root symbiosis in this species.

In the ITS2 data, we also observed 19 OTUs (26 occurrences) affiliated with Serendipita vermifera, which was functionally annotated as orchid mycorrhizal (OM) by FUNguild. However, in agreement with our results, Serendipita has been observed with a global distribution in numerous plant species not belonging to Orchidaceae (Oberwinkler et al. 2013; Botnen et al. 2014; Ray et al. 2018; Sarkar et al. 2019; Thoen et al. 2019), suggesting that different types of root associations and mycorrhiza exist in this species complex. This highlights that the automatic functional annotation through FUNguild must be critically evaluated.

We further observed AM fungi (Glomeromycota) in seven plant species. This includes some of the plant species Newsham et al. (2017) recently found to form AM in Svalbard, including the genera Taraxacum and Ranunculus. Still, a varying degree of AM has been observed in the Arctic; from no sign of AM in the plant roots studied by Väre, Vestberg and Eurola (1992) and Bledsoe, Klein and Bliss (1990) to being prevalent in other studies/sites (Dalpé and Aiken 1998; Olsson, Eriksen and Dahlberg 2004; Allen et al. 2006). Allen et al. (2006) argues that the difference observed might reflect microclimate, or a combination of biotic and abiotic factors. We did, however, detect Glomeromycota in eight of 11 locations, suggesting the AM fungi are relatively widespread in Svalbard, but likely in low abundance.

Ordination analyses of the ITS2 data clearly showed that host plant identity was the most important factor structuring the root-associated fungal communities. In this respect, our results...
contrast previous studies of ECM and ErM plants in the Arctic, where a low degree of host preference has been observed (Ryberg, Larsson and Molau 2009; Ryberg, Andreasen and Björk 2011; Walker et al. 2011; Timling et al. 2012; Botnen et al. 2014). The strong host preference revealed in this study may be linked to function; a large part of the OTUs was classified as putative biotrophs such as endophytes and parasites. Biotrophs might have evolved a stronger host-specificity compared to ECM and ErM fungi due to the host defence mechanisms they must encounter. Likewise, in a recent study of endophytic fungi in aboveground structures of arctic plant species, a high degree of host preference was observed (Zhang and Yao 2015). In correspondence with the ordination analyses, we observed a strong positive relationship between the phylogenetic distance of the plant hosts and the fungal community distances of the ITS2 data. Hence, closely related plants host more similar fungal communities than more distantly related plant. Hoksema et al. (2018) observed a similar pattern: They studied mycorrhizal associations in a large meta-study and concluded that the evolutionary history of the plant hosts impacts the strength of the mutualism.

Although host identity accounted for a large part of the compositional variation, the community structure was also related to climatic factors, pH and N. This was expected since previous studies have identified both climate, pH and N as important structuring factors for RAF diversity (Tedersoo et al. 2014; Timling et al. 2014; Steidinger et al. 2019).

The ITS2-primers might provide a skewed picture of the fungal diversity due to primer biases (Bellemain et al. 2010; Rosling et al. 2011; Tedersoo et al. 2015; Nilsson et al. 2019). We therefore implemented the 18S rRNA gene marker using primers assumed to amplify all eukaryotes (Hadziavdic et al. 2014). As expected, host plant sequences made up a dominant proportion of the sequences. Unfortunately, as the plant sequences typically made up 99% of the data, we obtained a rather restricted insight into the micro-eukaryotic and fungal diversity based on the 18S rRNA gene data. In further 18S rRNA gene-based studies of root-associated fungi/micro-eukaryotes, blocking-primers for plant DNA (Arenz et al. 2015) should be considered to avoid dominance of host sequences. Despite this limitation, it is interesting to see that the non-host 18S rRNA gene sequences provided a markedly different picture of the fungal diversity compared to the ITS2 data. Most strikingly, the assumed less biased 18S rRNA gene data suggest that Chytridiomyctota were at least prevalent in the arctic plant roots as Dikarya, followed by Mucormycota. In correspondence to our 18S rRNA gene results, a relatively high abundance of Chytridiomycota has earlier been reported in arctic tundra when using 18S rRNA gene primers (Shi et al. 2015). Members of the Chytridiomycota are mainly known as decomposers, and a few are known to act as plant pathogens (Longcore and Simmons 2012). Still, this phylum probably consists of a high diversity of unknown species with unknown functions. Mucormycota was previously thought to mainly include saprotrophic fungi (Moore, Robson and Trinci 2011). However, recent studies have shown that Mucormycota may form mycorrhizal or mycorrhizal-like mutualism with both liverworts (Field et al. 2015; Orchard et al. 2017), and early-diverging vascular plants (Hoysted et al. 2019). We may speculate that they also can form mutualistic associations with the arctic plants studied here, which should be an interesting topic for further investigations.

We expected to find a higher diversity of Glomeromycota in the 18S rRNA gene data compared to the ITS2 data, but this expectation was not met. This might be related to the low sequencing depth: while Glomeromyctota represented 2% of the ITS2 OTUs, it represented 6.6% of the fungal 18S rRNA gene OTUs. The overall mismatch between the ITS2 and 18S rRNA gene data on higher-order taxonomic composition of fungi is probably due to primer mismatches in the ITS2 primers towards the more basal fungal lineages (Bellemain et al. 2010; Rosling et al. 2011; Tedersoo et al. 2015). Although the 18S rRNA gene primers are considered eukaryote-general, we cannot exclude that mismatches also are present in these primer sites.

The 18S rRNA gene data also revealed a high diversity of other microeukaryotes and invertebrates associated with arctic plant roots. We observed e.g. a relatively high diversity of Oomycetes, which consists of several known plant pathogens (Bourke 1964; Govers, Drenth and Pieterse 1997; Thines 2014). Oomycota, with 29 OTUs, and Cercozoa, with 39 OTUs, were also relatively prevalent in this study, both of which contain several species commonly observed in soil, e.g. Glissomonadida (Howe et al. 2009), and Cercomonads (Flues et al. 2018) from Cercozoa; and Tubulinea in Amoebozoa (Anderson 2012, 2017). Members of Glissomonadida have been suggested to be the dominant predators of soil bacteria (Howe et al. 2009). Since plant roots can also host a large diversity of bacteria, we may speculate that some of the Glissomonadidae observed here may feed on root bacteria. Notably, experimental studies have found that presence of mycophagous amoeba (members of Tubulina) have resulted in reduced mycorrhizal colonization of plant roots (Chakraborty, Theodorou and Bowen 1985). From Metazoa, we observed a high diversity of nematodes, which are known to parasitize plant roots (Ingham et al. 1985; Dickie et al. 2011; Kynadt et al. 2013), graze on fungi (Ingham et al. 1985), or be ‘grazed’/trapped by fungi (Bordallo et al. 2002; Yang et al. 2019). It has been experimentally shown that presence of fungal grazing nematodes affects plant host nutrient uptake (Ingham et al. 1985).

A large part of both the ITS2 and 18S rRNA gene OTUs could not be taxonomically assigned at a low taxonomic level (i.e. below order level). Poor taxonomic resolution is in general common in DNA metabarcoding studies of microbes, which is also the case here, indicating presence of many poorly studied fungi and other micro-eukaryotes associated with arctic plant roots. In the mainly desolated and environmentally stressful arctic habitats, where 80% of the plant biomass is allocated to roots (Mokany, Raison and Prokushkin 2006), plant roots represent hotspots of available C and other nutrients and much of the terrestrial diversity may therefore be centred around plant roots. The arctic plant roots might be considered as isolated ecosystems, where complex interactions between diverse communities of organisms occur. As such, arctic plant roots may represent a future avenue for research on complex biotic interactions and ecosystem functions (Iversen et al. 2015).

DATA AVAILABILITY STATEMENT
Sequence data with corresponding mapping files are available at dryad.org: https://doi.org/10.5061/dryad.6wwpzgmwr

AUTHOR CONTRIBUTIONS
SSB, HK and PBE designed main research ideas; SSB, HK and PBE secured funding; SSB and ET did fieldwork; SSB did parts of the labwork, performed bioinformatics and statistical analyses with input from HK; ET did parts of labwork; AKK gave input on the microeukaryotic diversity, constructed 18S rRNA gene phylogenetic trees and drafted text related to this; SSB lead the writing process with contributions from all authors.
ACKNOWLEDGMENTS

We thank Kelsey Lorberou and Jacky Hess for field assistance in 2014, Peter Convey and Mildrid Elvik Svoen for root-washing help in 2013 and Cecile Mathiesen and Eivind Ronold for help in the lab. We thank Tor Carlsen for research ideas, Reidar Elven and Anne Brysting for help in identifying host plants. Svalbard Science Forum and the Norwegian Research council are acknowledged for providing ‘Arctic Field Grant’, ‘Jan Chistensens legat til fremme av studier og forskning ved Universitetsenteret på Svalbard’, The University of Oslo and ConocoPhillips and Lundin Petroleum through The Northern Area Program for funding.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

CONFLICTS OF INTEREST. None declared.

REFERENCES

Allen N, Nordlander M, Mcgonigle T et al. Arbuscular mycorrhizae on Axel Heiberg Island (80°N) and at Sattakeo (52°N) Canada. Can J Bot 2006;84:1094–100.
Anderson OR. Amoebozoan lobose amoebae (Tubulinea, Flabellinae, and others). In: Archibald JM, Simpson AGB, Slammaals CH (eds). Handbook of the Protists. Cham: Springer International Publishing, 2017, 1279–309.
Anderson OR. The fate of organic sources of carbon in moss-rich tundra soil microbial communities: a laboratory experimental study. J Eukaryot Microbiol 2012;59:564–70.
Arenz BE, Schlatter DC, Bradeen JM et al. Blocking primers reduce co-amplification of plant DNA when studying bacterial endophyte communities. J Microbiol Methods 2015;117:1–3.
Becklin KM, Hertweck KL, Jumpoonen A. Host identity impacts rhizosphere fungal communities associated with three alpine plant species. Microb Ecol 2012;63:682–93.
Bellemain E, Carlsen T, Brochmann C et al. ITS as an environmental DNA barcode for fungi: an in silico approach reveals potential PCR biases. BMC Microbiol 2010;10:189.
Bengtsson-Palme J, Ryberg M, Hartmann M et al. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Method Ecol Evolut 2013;4:914–9.
Berthelot C, Leyval C, Foulon J et al. Plant growth promotion, metabolite production and metal tolerance of dark septate endophytes isolated from metal-polluted poplar phytomanagement sites. FEMS Microbiol Ecol 2016;92:fw144.
Björbakemo MFM, Carlsen T, Brysting A et al. High diversity of root associated fungi in both alpine and arctic Dryas octopetala. BMC Plant Biol 2010;10:244–.
Blaalid R, Davey ML, Kaueraud H et al. Arctic root-associated fungal community composition reflects environmental filtering. Mol Ecol 2014;23:649–59.
Bledsoe C, Klein P, Bliss LC. A survey of mycorrhizal plants on Truelove Lowland, Devon Island, N.W.T., Canada. Can J Bot 1990;68:1848–56.
Bonito G, Reynolds H, Robeson MS et al. Plant host and soil origin influence fungal and bacterial assemblages in the roots of woody plants. Mol Ecol 2014;23:3356–70.
Bordallo JJ, Lopez-Llorca LV, Jansson H-B et al. Colonization of plant roots by egg-parasitic and nematode-trapping fungi. New Phyto1 2002;154:491–9.

Botnen S, Vik U, Carlsen T et al. Low host specificity of root-associated fungi at an Arctic site. Mol Ecol 2014;23:975–85.
Botnen SS, Davey ML, Aas AB et al. Biogeography of plant root-associated fungal communities in the North Atlantic region mirrors climatic variability. J Biogeogr 2019;46:1532–46.
Bourke PMA. Emergence of potato blight, 1843–46. Nature 1964;203:805–8.
Brundrett M. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 2009;320:37–77.
Callahan BJ, McMurdie PJ, Rosen MJ et al. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 2016;13:581–3.
Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 2009;25:1972–3.
Chakraborty S, Theodorou C, Bowen GD. The reduction of root colonization by mycorrhizal fungi by mycophagous amoebae. Can J Microbiol 1985;31:295–7.
Cripps CL, Eddington LH. Distribution of mycorrhizal types among alpine vascular plant families on the beetroot plateau, rocky mountains, U.S.A., in reference to large-scale patterns in arctic-alpine habitats. Arct Antarct Alp Res 2005;37:177–88.
Dalpé Y, Aiken SG. Arbuscular mycorrhizal fungi associated with Festuca species in the Canadian High Arctic. Can J Bot 1998;76:1930–8.
Davey M, Blaalid R, Vik U et al. Primary succession of Bistorta vivipara (L.) Delablé (Polygonaceae) root-associated fungi mirrors plant succession in two glacial chronosequences. Environ Microbiol 2015;17:2777–90.
Dickie IA, Yeates GW, John MGS et al. Ecosystem service and biodiversity trade-offs in two woody successions. J Appl Ecol 2011;48:926–34.
Dunthorn M, Kaueraud H, Bass D et al. Yeasts dominate soil fungal communities in three lowland Neotropical rainforests. Environ Microbiol Rep 2017;9:668–75.
Durán P, Thiergart T, Garrido-Oter R et al. Microbial interkingdom interactions in roots promote arabidopsis survival. Cell 2018;175:973–83. e14.
Field KJ, Rimington WR, Bidartondo MI et al. First evidence of mutualism between ancient plant lineages (Haplomitriopsis liverworts) and Mucoromycotina fungi and its response to simulated Palaeozoic changes in atmospheric CO2. New Phyto1 2015;205:743–56.
Flues S, Blokker M, Dumack K et al. Diversity of cercomonad species in the phyloplankton and rhizosphere of different plant species with a description of Necercromonas epiphylla (Cercozoa, Rhizaria) a leaf-associated protist. J Eukaryot Microbiol 2018;65:587–99.
Frøslev TG, Kjøller R, Bruun HH et al. Algorithm for post-clustering curvature of DNA amplicon data yields reliable biodiversity estimates. Nat Commun 2017;8:1–11.
Fujimura KE, Egger KN. Host plant and environment influence community assembly of High Arctic root-associated fungal communities. Fung Ecol 2012;5:409–18.
Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol Ecol 1993;2:113–8.
Gardes M, Dahlberg A. Mycorrhizal diversity in arctic and alpine tundra: an open question. New Phyto1 1996;133:147–57.
Gittel A, Bártta J, Kohoutová I et al. Site- and horizon-specific patterns of microbial community structure and enzyme activities in permafrost-affected soils of Greenland. Front Microbiol 2014;5:541.

Govers F, Drenth A, Pieterse CMJ. The potato late blight pathogen Phytophthora infestans and other pathogenic oomycota. In: Carroll GC, Tuzunyski P (eds), Plant Relationships Part B: Part B. The Mycota. Berlin, Heidelberg: Springer, 1997, 17–36.

Guillou L, Bachar D, Audic S et al. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small-subunit rRNA sequences with curated taxonomy. Nucleic Acids Res 2013;41:D597–604.

Hadarziavdic K, Lekang K, Lanzen A et al. Characterization of the 18S rRNA gene for designing universal eukaryote specific primers. PLoS One 2014;9:e87624.

Hassani MA, Durán P, Hacquard S. Microbial interactions within the plant holobiont. Microbiome 2018;6:58.

Hesselman H. Om mykorrhizabildeingar hos arktiska växter. Bilag Till Kongl Svenska Vetenskaps-Akademiens Handlingar 1900;26:1–46.

Hill PW, Broughton R, Bougoure J et al. Angiosperm symbioses with non-mycorrhizal fungal partners enhance N acquisition from ancient organic matter in a warming maritime Antarctic. Ecol Lett 2019;22:2111–9.

Hoeksema JD, Bever JD, Chakraborty S et al. Evolutionary history of plant hosts and fungal symbionts predicts the strength of Mycorrhizal mutualism. Commun Biol 2018;1:116.

Howe AT, Bass D, Vickerman K et al. Phylogenomy, taxonomy, and astounding genetic diversity of Glissomonadida ord. nov., the dominant gliding zooflagellates in soil (Protozoa: Cercozoa). Protist 2009;160:159–89.

Hoysted GA, Jacob AS, Kowal J et al. Mucoromycotina fine root endophyte fungi form nutritional mutualisms with vascular plants. Plant Physiol 2019;181:565–77.

Ihrmark K, Bödeker ITM, Cruz-Martinez K et al. New primers to amplify the fungal ITS2 region – evaluation by 454 sequencing of artificial and natural communities. FEMS Microbiol Ecol 2012;82:666–77.

Ingham RE, Trofytmow JA, Ingham ER et al. Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. Ecol Monogr 1985;55:119–40.

Ishida TA, Nara K, Hogetsu T. Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer-broadleaf forests. New Phytol 2007;174:430–40.

Iversen CM, Sloan VI, Sullivan PF et al. The unseen iceberg: plant roots in arctic tundra. New Phytol 2015;205:34–58.

Jacobsen RM, Kauerud H, Sverdrup-Thygeson A et al. Wood-inhabiting insects can function as targeted vectors for decomposer fungi. Fung Ecol 2017;29:76–84.

Jombart T. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 2008;24:1403–5.

Jordon-Thaden I, Hase I, Al-Shehbaz I et al. Molecular phylogeny and systematics of the genus Draba (Brassicaceae) and identification of its most closely related genera. Mol Phylogenet Evol 2010;55:524–40.

Jumpponen A, Mattsson KG, Trappe JM. Mycorrhizal functioning of Phialocephala fortinii with Pinus contorta on glacier forefront soil: interactions with soil nitrogen and organic matter. Mycorrhiza 1998;7:261–5.

Jumpponen A, Trappe JM. Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol 1998;140:295–310.

Katoh K, Standley DM. A simple method to control over-alignment in the MAFFT multiple sequence alignment program. Bioinformatics 2013;32:1933–42.

Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 2013;30:772–80.

Kohn LM, Stasovski E. The mycorrhizal status of plants at Alexandra Fiord, Ellesmere Island, Canada, a high arctic site. Mycologia 1990;82:23–35.

Kyndt T, Vieira P, Gheyssen G et al. Nematode feeding sites: unique organs in plant roots. Plants 2013;238:807–18.

Kytöviiita M. Asymmetric symbiont adaptation to Arctic conditions could explain why high Arctic plants are non-mycorrhizal. FEMS Microbiol Ecol 2005;53:27–32.

Lindahl BD, Nilsson RH, Tedersoo L et al. Fungal community analysis by high-throughput sequencing of amplified markers – a user’s guide. New Phytol 2013;199:288–99.

Linde S, Suz LM, Orme CDL et al. Environment and host as large-scale controls of ectomycorrhizal fungi. Nature 2018;558:243–8.

Liu H, Økland T, Halvorsen R et al. Gradients analyses of forests ground vegetation and its relationships to environmental variables in five subtropical forest areas, S and SW China. Sommerfeltia 2008;32:3–196.

Longcore JE, Simmons DR. Chytriomyctyma. In eLS. American Cancer Society, 2012.

Maessen O, Freedman B, Nams MLN et al. Resource allocation in high-arctic vascular plants of differing growth form. Can J Bot 1983;61:1680–91.

Malam LA, Pearce DA. Microbial diversity and biogeography in Arctic soils. Environ Microbiol Rep 2018;10:611–25.

Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnetjournal 2011;17:10–12.

Mokany K, Raison RJ, Prokushkin AS. Critical analysis of root: shoot ratios in terrestrial biomes. Global Change Biol 2006;12:84–96.

Moore D, Robson GD, Trinci AP. Chapter 3. Natural classifications of fungi. In 21st Century Guidebook to Fungi. Cambridge University Press, 2011, 52.

Morgado LN, Semenova TA, Welker JM et al. Long-term increase in snow depth leads to compositional changes in arctic ectomycorrhizal fungal communities. Global Change Biol 2016;22:3080–96.

Mundra S, Bahram M, Tedersoo L et al. Temporal variation of Bistorta vivipara-associated ectomycorrhizal fungal communities in the High Arctic. Mol Ecol 2015a;24:6289–302.

Mundra S, Halvorsen R, Kauerud H et al. Arctic fungal communities associated with roots of Bistorta vivipara do not respond to the same fine-scale edaphic gradients as the aboveground vegetation. New Phytol 2015b;205:1587–97.

Mundra S, Halvorsen R, Kauerud H et al. Ectomycorrhizal and saprotrophic fungi respond differently to long-term experimentally increased snow depth in the High Arctic. Microbiol Open 2016;5:856–69.

Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 1980;8:4321–5.

NCBI Resource Coordinators. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 2018;46:D8–D13.

Newsham KK, Eidesen PB, Davey ML et al. Arbuscular mycorrhizas are present on Spitsbergen. Mycorrhiza 2017;27:725–31.

Newsham KK, Upsom R, Read DJ. Mycorrhizas and dark septate root endophytes in polar regions. Fung Ecol 2009;2:10–20.
Newsham KK. A meta-analysis of plant responses to dark septate root endophytes. New Phytol 2011;190:783–93.

Nguyen NH, Song Z, Bates ST et al. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Funct Ecol 2016;20:241–8.

Nilsson RH, Anslan S, Bahram M et al. Mycobiome diversity: high-throughput sequencing and identification of fungi. Nat Rev Microbiol 2019;17:95–111.

Oberwinkler F, Ries K, Bauer R et al. Enigmatic Sebacinales. Mycol Progress 2013;12:1–27.

Oksanen J, Blanchet FG, Kindt R et al. vegan: community ecology package. R package version 2.0-5., http://CRAN.R-project.org/package=vegan, 2012.

Olson PA, Eriksen B, Dahlberg A. Colonization by Arbuscular mycorrhizal and fine endophytic fungi in herbaceous vegetation in the Canadian High Arctic. Can J Bot 2004;82:1547–56.

Orchard S, Hilton S, Bending GD et al. Fine endophytes (Clomus tenue) are related to Mucoromycotina, not Glomeromycota. New Phytol 2017;213:481–6.

Pattengale ND, Alipour M, Bininda-Emonds ORP et al. How many bootstrap replicates are necessary? J Comput Biol 2010;17:337–54.

Price MN, Dehal PS, Arkin AP. FastTree 2—Approximately maximum-likelihood trees for large alignments. PLoS One 2010;5:e9490.

Rambaut A. FigTree v1. 4. 4, a graphical viewer of phylogenetic trees. Java, 2018.

Ratnasingham S, Hebert PDN. bold: the barcode of life data system (http://www.barcodinglife.org). Mol Ecol Notes 2007;7:355–64.

Ray P, Chi M-H, Guo Y et al. Genome sequence of the plant growth promoting fungus Serendipita vermisfera subsp. bescii: the first native strain from North America. Phytobiomes J 2018;2:62–3.

R Development Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria: R Foundation for Statistical Computing, 2010.

Read DJ, Haselwandter K. Observations on the mycorrhizal status of some alpine plant communities. New Phytol 1981;88:341–52.

Rognes T, Flouri T, Nichols B et al. VSEARCH: a versatile open source tool for metagenomics. PeerJ 2016;4:e2584.

Rosling A, Cox F, Cruz-Martinez K et al. Archaeorhizomycetes: unearthing an ancient class of ubiquitous soil fungi. Science 2011;333:876–9.

Ruotsalainen A, Väre H, Vestberg M. Seasonality of root fungal colonization in low-alpine herbs. Mycorrhiza 2002;12:29–36.

Ryberg M, Andreasen M, Björk RG. Weak habitat specificity in ectomycorrhizal communities associated with Salix herbacea and Salix polaris in alpine tundra. Mycorrhiza 2011;21:289–96.

Ryberg M, Larsson E, Molau U. Ectomycorrhizal diversity on Dryas octopetala and Salix reticulata in an alpine cliff ecosystem. Arct Antarct Alp Res 2009;41:506–14.

Saarela JM, Burke SV, Wysoki WP et al. A 250 plastome phylogeny of the grass family (Poaceae): topological support under different data partitions. PeerJ 2018;6:e4299.

Sarkar D, Rovenich H, Jeena G et al. The inconspicuous gate-keeper: endophytic Serendipita vermifera acts as extended plant protection barrier in the rhizosphere. New Phytol 2019;224:886–901.

Schneider-Maunoury L, Devseau A, Moreno M et al. Two ectomycorrhizal truffles, Tuber melanosporum and T. aestivum, endophytically colonise roots of non-ectomycorrhizal plants in natural environments. New Phytol 2020;225:2542–56.

Schoch CL, Seifert KA, Huhndorf S et al. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci 2012;109:6241–6.

Shi Y, Xiang X, Shen C et al. Vegetation-associated impacts on arctic tundra bacterial and microeukaryotic communities. Appl Environ Microbiol 2015;81:492–501.

Smith SE, Read DJ. Mycorrhizal Symbioses, 3 edn. London, UK: Academic Press, 2008.

Soltis DE, Smith SA, Cellinese N et al. Angiosperm phylogeny: 17 genes, 640 taxa. Am J Bot 2011;98:704–30.

Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 2014;30:1312–3.

Steidinger BS, Crowther TW, Liang J et al. Climatic controls of decomposition drive the global biogeography of forest-tree symbioses. Nature 2019;569:404.

Stoeck T, Bass D, Nebel M et al. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol Ecol 2010;19:21–31.

Sønstebo KH, Gielly L, Brysting AK et al. Using next-generation sequencing for molecular reconstruction of past Arctic vegetation and climate. Mol Ecol Resour 2010;10:1009–18.

Tedersoo L, Anslan S, Bahram M et al. Shotgun metagenomes and multiple primer pair-barcode combinations of amplicons reveal biases in metabarcoding analyses of fungi. MycoKeys 2015;10:1–43.

Tedersoo L, Bahram M, Polme S et al. Global diversity and geography of soil fungi. Science 2014;346:1052–3.

Tedersoo L, May TW, Smith ME. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 2010;20:217–63.

Thines M. Phylogeny and evolution of plant pathogenic oomycetes—a global overview. Eur J Plant Pathol 2014;138:431–47.

Thoen E, Aas AB, Vik U et al. A single ectomycorrhizal plant root system includes a diverse and spatially structured fungal community. Mycorrhiza 2019;29:167–80.

Timling I, Dahlberg A, Walker DA et al. Distribution and drivers of ectomycorrhizal fungal communities across the North American Arctic. Ecosphere 2012;3:1–25.

Timling I, Walker DA, Nusbaum C et al. Rich and cold: diversity, distribution and drivers of fungal communities in patterned-ground ecosystems of the North American Arctic. Mol Ecol 2014;23:3258–72.

Tkach N, Röser M, Miehe G et al. Molecular phylogenetics, morphology and a revised classification of the complex genus Saxifraga (Saxifragaceae). Taxon 2015;64:1159–87.

UNITE Community. Full UNITE+INSD dataset for Fungi, UNITE Community, 2019.

Upson R, Read DJ, Newsham KK. Nitrogen form influences the response of Deschampsia antarctica to deep septate root endophytes. Mycorrhiza 2009;20:1–11.

Vrålstad T, Schumacher T, Taylor AFS. Ectomycorrhizal synthesis between fungal strains of the Hymenoscyphus ericae aggregate and potential ectomycorrhizal and ericoid hosts. New Phytol 2002;153:143–52.
Vrålstad T. Are ericoid and ectomycorrhizal fungi part of a common guild? New Phytol 2004;164:7–10.
Väre H, Vestberg M, Eurola S. Mycorrhiza and root-associated fungi in Spitsbergen. Mycorrhiza 1992;1:93–104.
Walker JF, Aldrich-Wolfe L, Riffel A et al. Diverse Helotiales associated with the roots of three species of Arctic Ericaceae provide no evidence for host specificity. New Phytol 2011;191:515–27.
White TJ, Bruns T, Lee S et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols: A Guide to Methods and Applications. Innis MA, Gelfand DH, Sninski JJ et al. (eds). San Diego: Academic Press, 1990.
Yang C-T, Ulzurrun GV-D, Gonçalves AP et al. Natural diversity in the predatory behavior facilitates the establishment of a new robust model strain for nematode-trapping fungi. bioRxiv 843698, 2019, DOI: 10.1073/pnas.1919726117.
Zhang T, Yao Y-F. Endophytic fungal communities associated with vascular plants in the high arctic zone are highly diverse and host-plant specific. PLoS One 2015;10:e0130051.