Low host specificity of root-associated fungi at an Arctic site

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Abstract

In High Arctic ecosystems, plant growth and reproduction are limited by low soil moisture and nutrient availability, low soil and air temperatures, and a short growing season. Mycorrhizal associations facilitate plant nutrient acquisition and water uptake and may therefore be particularly ecologically important in nutrition-poor and dry environments, such as parts of the Arctic. Similarly, endophytic root associates are thought to play a protective role, increasing plants’ stress tolerance, and likely have an important ecosystem function. Despite the importance of these root-associated fungi, little is known about their host specificity in the Arctic. We investigated the host specificity of root-associated fungi in the common, widely distributed arctic plant species Bistorta vivipara, Salix polaris and Dryas octopetala in the High Arctic archipelago Svalbard. High-throughput sequencing of the internal transcribed spacer 1 (ITS1) amplified from whole root systems generated no evidence of host specificity and no spatial autocorrelation within two 3 m × 3 m sample plots. The lack of spatial structure at small spatial scales indicates that Common Mycelial Networks (CMNs) are rare in marginal arctic environments. Moreover, no significant differences in fungal OTU richness were observed across the three plant species, although their root system characteristics (size, biomass) differed considerably. Reasons for lack of host specificity could be that association with generalist fungi may allow arctic plants to more rapidly and easily colonize newly available habitats, and it may be favourable to establish symbiotic relationships with fungi possessing different physiological attributes.

Keywords: Bistorta vivipara, Dryas octopetala, ectomycorrhiza, host specificity, Salix polaris, Svalbard

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Introduction

Arctic environments are challenging for plant growth due to factors such as low moisture, low annual mean temperature, UV stress, extreme variation in radiation and seasonality (Thomas et al. 2008).

Symbiotic mycorrhizal associations are important in nutrient-poor environments (Smith & Read 2008) such as the Arctic. It has been estimated that mycorrhizal fungi supply as much as 61–86% of their host plant’s nitrogen in arctic environments (Hobbie & Hobbie 2006). In addition, endophytic fungi, such as dark septate fungal endophytes (DSE), could potentially enhance plant growth under defined conditions, especially when the majority of N is available in organic form (Jumpponen & Trappe 1998; Newsham et al. 2009; Newsham 2011; Yao et al. 2013).

In comparison with the low plant diversity typical of arctic and alpine habitats, the richness and heterogeneity of root-associated fungal communities are high (Newsham et al. 2009; Blaalid et al. 2011; Yao et al. 2013). Several mycorrhizal types are present in these habitats (Gardes & Dahlberg 1996; Bjorbaekmo et al. 2010; Geml et al. 2012; Timling & Taylor 2012), including arbuscular mycorrhizae (AM) that are primarily
associated with herbaceous plants, ericoid mycorrhizae (ERM) that are associated with ericaceous plants, and ectomycorrhizae (ECM) that are found in association with shrubs and some herbs (Väre et al. 1992; Gardes & Dahlberg 1996). In the Antarctic, dark septate fungi have similarly been shown to have an important role in nutrient uptake both in one of the two native flowering plants and in liverworts (Newsham et al. 2009; Upson et al. 2009; Hill et al. 2011).

The degree of host specificity of ectomycorrhizal fungi, here referred to as the ability to form ECM symbiosis with one (high specificity) or numerous (low specificity) host plant species, appears to vary between different habitats and seems to relate to numerous factors including competition, resource limitation and dispersal ability (Ishida et al. 2007; Morris et al. 2009; Diedhiou et al. 2010; Tedersoo et al. 2010b; Ding et al. 2011; Smith et al. 2011; Timling et al. 2012). Varying degrees of host specificity in ECM communities have been reported in a number of tropical and temperate ecosystems, ranging from highly specific to dominance by multihost fungi (Ishida et al. 2007; Diedhiou et al. 2010; Tedersoo et al. 2010b; Bent et al. 2011; Smith et al. 2011). In harsh alpine and arctic ecosystems, however, no significant host specificity has been found among ECM fungi (Ishida et al. 2007; Diedhiou et al. 2010; Tedersoo et al. 2010b; Bent et al. 2011; Smith et al. 2011). A study on three coexisting arctic plants with ericoid mycorrhizae drew the same conclusion (Walker et al. 2011). On the other hand, host identity influenced the root-associated fungal community in three alpine plant species not forming ECM or ERM (Becklin et al. 2012).

Common Mycelial Networks (CMNs), which are fungal mycelia that connect plant roots, may facilitate the growth and establishment of plants in harsh environments. Several studies have shown that carbon is transferred among adjacent host plants, and this transfer most likely occurs through CMNs (e.g. Simard et al. 1997). CMNs have also been suggested to play an important role in facilitating seedling growth, where the mycelium growing in symbiosis with larger plant roots serves as an inoculum for the seedling (Dickie et al. 2004; Simard & Durall 2004; Nara 2006; Hubert & Gehring 2008; Beiler et al. 2010; Bent et al. 2011). CMNs may be of particular importance for seedling establishment under stressful conditions (Bingham & Simard 2012). However, the formation of CMNs across different host plants demands the sharing of root-associated fungal species and, hence, assumes a lack of strong host specificity in the fungal mycelium. To what extent ECM plants in arctic habitats share the same root-associated fungal species and can build CMNs is largely unknown.

In community ecology studies of ECM fungi, only a small fraction of the fungal diversity is typically characterized by, for example, DNA sequencing of a small subset of ECM root tips. The Arctic provides a unique opportunity to investigate the ECM fungal communities because small-sized plants dominate, which have root systems that are easily dug up in their entirety, and the associated fungal communities can be characterized at the root system level. The application of high-throughput sequencing approaches allows a more in-depth analysis of the fungal communities and reduces the amount of fungal diversity that is missed due to insufficient sampling and sequencing effort, which may occur when using traditional morphtyping and Sanger sequencing approaches. In this study, we use high-throughput sequencing of entire root systems of the co-occurring and widespread arctic ectomycorrhizal plants Bistorta vivipara (L.) Delabre (Polygonaceae), Salix polaris Walenb. (Salicaceae) and Dryas octopetala L. (Rosaceae) to investigate ECM host specificity, the potential occurrence of CMNs and the effects of host root size on root-associated fungal communities.

Materials and methods

Host plant species

Svalbard is an archipelago situated in the High Arctic (74°-81°N, 10°-30°E). Of the 165 plant species currently reported from the archipelago, three of the common species are known to form ectomycorrhizae: Bistorta vivipara, Salix polaris and Dryas octopetala (Hesselman 1900; Elven & Elvebak 1996). These three species are all widely distributed, common in the Arctic tundra and are also known to host other root-associated fungi (Bjorbakmo et al. 2010; Blaalid et al. 2011).

Dryas octopetala and S. polaris are woody dwarf shrubs and may produce extensive root systems, including runners that may be used for vegetative reproduction, while the perennial B. vivipara has herbaceous growth form and a smaller root system and mainly reproduce asexually by bulbils (Elven 2005). All three host species produce flowers and may produce seeds. Despite this, S. polaris and B. vivipara have similar competitive capacity and colonization patterns, and both are, for example, important pioneer species in primary succession in recently deglaciated areas (Hesselman 1900; Väre et al. 1992; Elven 2005; Fujiyoshi et al. 2011).

Study site and sampling design

Two 3 m × 3 m plots located 47 m apart in Blomsterdalen, Svalbard (plot 1: N78°14′15.3″, E015°30′36.8″ and plot 2: N78°14′13.8″, 015°30′35.6″), were sampled in July 2011 (Fig. S1, Supporting Information). Blomsterdalen is
situated about 1 km northwest of the Svalbard Airport weather station, which reports a mean annual temperature of 6.7 °C and a mean annual precipitation of 190 mm (1961–1990; Eklima 2011). The two plot locations were selected to ensure inclusion of the focal ECM plants B. vivipara, D. octopetala and S. polaris. Ten separate plants of each species were collected per plot using the following criteria: ten focal individuals of D. octopetala <25 cm in diameter were marked, and then the closest specimen of S. polaris and B. vivipara (<5 cm away) were selected and sampled. We recognize the possibility that two plants (ramets) of the same species can belong to the same genet due to, for example, vegetative root growth. However, the sampled plants had distinct root systems that were dug carefully up to ensure that we did not cut off any parts of the root systems. In addition, plants of the same species were sampled at minimum 23 cm apart. The vegetation in the two plots was sparse and patchily distributed (see species list in Table S1, Supporting Information), and there was a low degree of direct physical contact between root systems of different higher plants. In total, 60 individual root systems were sampled. The collected plants were stored at 4 °C for a maximum of 36 h before further handling.

DNA extraction and sequencing

The whole plants with the root systems intact were rinsed in tap water and visible soil, plant debris and roots not attached to the plants were removed. The roots were then washed three times for 30 s in milli-Q water. Above-ground structures were removed from the plants; the root systems were placed in preweighed 50 mL BD Falcon™ tubes (BD Bioscience, San Jose, USA) and then weighed to give a measure of fresh root mass. Cetyltrimethylammonium bromide (CTAB) lysis buffer with mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA) was added to the tubes with root samples, which were then stored at −20 °C until DNA extraction. Seven 6.2-mm ceramic beads (M.P. Biomedicals, CA, USA) were added per falcon tube, and the 60 root samples were crushed at 4.0 m/s on a Fast Prep-24 beadbeater (M.P. Biomedicals) for 60 s and centrifuged for 30 s at 2000 rcf. After vortexing the samples, DNA was extracted from 600 μL of the aqueous phase, using a modified CTAB extraction protocol (Murray & Thompson 1980; Gardes & Bruns 1993) and cleaned with E.Z.N.A. soil DNA kit (Omega Bio-Tek, Norcross, USA) according to the manufacturer’s manual. PCR amplification with the fungal specific primers ITSIF and ITS4 (White et al. 1990; Gardes & Bruns 1993) was used to amplify the entire ITS region. The ITS1 region was further targeted using a nested PCR step using fusion primers consisting of the primers ITS5 and ITS2 (White et al. 1990) and manufacturer specified adaptors and tags for further 454-sequencing. Roche 454 MIDTs 16–31 were added at both the beginning and terminus of each amplicon. A nested PCR approach may lead to increased primer bias and chance of contamination. However, direct amplification using long fusion primers or post-PCR ligation of adapters may also be hampered by biases (Gillevet et al. 2010; Berry et al. 2011). Kauserud et al. (2012) achieved high consistency between replicate samples using the nested PCR approach. The reaction volumes in both PCRs were 20 μL including 4 μL 5× Phusion HF Buffer (Finnzymes Oy, Vantaa, Finland), 2 μL dNTP (0.16 mM), 1 μL of each of the primers (0.2 μM) and 0.2 μL of Phusion High Fidelity Polymerase (Finnzymes Oy). In the first run, 2 μL of DNA templates was added, and in the second PCR, 4 μL of 25× diluted template from the first PCR was added. Milli-Q water was added to achieve a total reaction volume of 20 μL. The following PCR conditions were used in both reactions: denaturation at 89 °C for 30 s; followed by 30 cycles of denaturation at 89 °C for 10 s, annealing at 53 °C for 20 s and extension at 72 °C for 20 s; ending with a final extension step at 72 °C for 7 min. All samples were run in triplicate and pooled before sequencing to reduce the effects of PCR stochasticity. The PCR products were cleaned using Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, USA) and normalized using the SequalPrep™ Normalization Plate Kit (Invitrogen Inc., CA, USA) following the manufacturer’s protocol. Three randomly picked samples were sequenced twice to test for sequencing consistency. One null mock extraction of milli-Q water was included as a negative control. The cleaned and standardized PCR products were pooled in equimolar amounts and sequenced in four of eight lanes of a plate using the Roche GS FLX Titanium Series 454 sequencing platform at the Norwegian High-Throughput Sequencing Centre (University of Oslo, Oslo, Norway). The order of the samples in the PCR and the placement on the sequencing plate were randomized using the statistical environment R (R Development Core Team 2010).

Bioinformatics and statistical analyses

Tag switching is a source of error in pyrosequencing studies (Carlsen et al. 2012), and therefore, sequences with nonmatching 5′ and 3′ tags were removed from the data set using a python script (available on request). For further quality filtering, denoising and clustering of sequences, QIME v. 1.5.0 (Caporaso et al. 2010) was used. Short reads (<200 bp), long reads (>550 bp) and reads with a quality score lower than 50 were discarded. Sequences with homopolymers of >10 bp and more than 1 ambiguous base (N) were removed from the data set. One base pair mismatch in both the forward and reverse primer was allowed. After denoising, the
sequences were clustered into operational taxonomic units (OTUs) at a 97% sequence identity level using the UCLUST algorithm (Edgar 2010) implemented in QIME (Caporaso et al. 2010). OTUs represented by only a single sequence across the entire data set were considered probable sequencing errors and removed (Quince et al. 2009; Tedersoo et al. 2010a). Any OTUs present in the negative control were removed. Samples with <400 reads were removed from the data set. Sequences identified by the perseus algorithm in MOTHUR v. 1.27.0 as chimeric (Schloss et al. 2009) were removed from the data set if they had a BLASTN-hit with <90% identity and <90% coverage to a known fungal sequence and/or were not present in more than one sample. The most abundant sequence of each OTU was subjected to a BLASTN search of the NCBI nr/nt database (November 2012). The best BLASTN match was recorded and taxonomy was assigned at order level. If the top hit was not taxonomically consistent with the remaining hits, the next best hit was selected. Sequences with a best BLASTN match to a nonfungal sequence, or <70% similarity and/or 50% coverage to a fungal sequence were removed.

The sample OTU matrix obtained was rarefied to 400 sequences per sample. G-tests of independence were conducted on all OTUs occurring in >4 samples to test whether some of them occurred more frequently with the different host species or plots using default settings in QIME (Caporaso et al. 2010). Read-abundance data have been suggested to provide reliable comparison within taxon (Amend et al. 2010), although they are not directly valid for between taxa comparisons without calibration. The abundance and frequencies of OTUs were not identical between sequencing replicates of the three samples PCR amplified and sequenced twice. However, they showed more intersample than intrasample differences (GNMDS with vector fitting; Fig. S2, Supporting Information). In addition, a paired t-test of abundance between the pairs of replicated samples showed no significant differences (P > 0.05) between the replicates, so within-taxon comparisons were carried out. To test whether some OTUs had higher relative abundance in any of the host species or plots, analyses of variances (ANOVA) were conducted using the default settings in QIME for all OTUs occurring in >4 samples. To test whether OTU richness in the samples was influenced by host species or root mass, an ANOVA, as implemented in R (R Development Core Team 2010), was performed. The plot number was also included in the model to test for difference in richness of OTUs across the two plots. The full ANOVA model was as follows: number of species ~ host plant + plot, where host plant and plot were treated as factor variables. GLM was used test whether the fresh plant root weight affected the OTU richness. ESTIMATES v. 7.5.2 (Colwell 2009) was used to calculate the pairwise number of shared species between samples.

Accumulation curves were constructed following the approach in Ugland et al. (2003) using the rarefied OTU matrix. Curves were constructed for each of the plant species, and separately for each of the plots. Estimates of total species richness were made in the statistical environment R (R Development Core Team 2010) using the package VEGAN v. 2.0.5 (Oksanen et al. 2012).

The nonrarefied OTU matrix was transformed using the Hellinger equation before further analyses, and all samples with <400 reads were omitted from the OTU matrix. This transformation was used to account for ‘blind sampling’ and numerous rare taxa and absences, as suggested by Ramette (2007) and implemented by Davey et al. (2013). Thereafter, detrended correspondence analysis (DCA; Hill 1979; Hill & Gauch 1980) and a Global Nonmetric Multidimensional Scaling (GNMDS; Kruskal 1964a,b; Minchin 1987) were used in parallel in the VEGAN package of R (R Development Core Team 2010; Oksanen et al. 2012). The DCA was performed using default settings. As suggested by Økland (1999) and Liu et al. (2008), the following settings were used for the GNMDS: distance measure – Bray–Curtis; maximum iterations – 200; dimensions – 2; initial configurations – 100. Ordinations were screened for outliers and checked for possible artefacts such as the tongue-effect in the DCA, or arch-effect in the GNMDS (Økland 1990; Økland & Elertsen 1993). Root weight and host plant were fitted into the GNMDS with the envfit function in VEGAN. Correlation tests between the axes in the two ordinations were conducted, using Kendall’s tau coefficient, and were used as an indicator that reliable gradients had been recovered. To investigate the potential for CMNs, a semivariogram was constructed with the geoR package in R (Ribeiro & Diggle 2001; Diggle & Ribeiro 2007; R Development Core Team 2010), using the GNMDS first dimension scores to assess spatial dependency between the samples. Furthermore, an OTU matrix containing only OTUs with affiliation to known ectomycorrhizal fungi was created, and the same analyses described above were conducted. As emphasized in Lindahl et al. (2013), molecular surveys of fungal communities are by no means standardized yet, but to a large extent, we follow recommendations given in Lindahl et al. (2013).

Results

Data characteristics

The raw data from all three host species consisted of 157 181 reads, of which 117 837 reads were retained...
after removal of sequences with different tags at each end, quality filtering and denoising. These reads clustered into 774 OTUs. Approximately 34% of the OTUs were singletons, but these accounted for <0.1% of the reads. After removing singletons (263), chimeric sequences (33), OTUs present in the negative sample (29), samples with <400 reads (14 OTUs, seven samples) and OTUs assigned to nontarget organisms or with unreliable BLAST matches (81), 354 root-associated fungal OTUs were included in the final matrix. There were on average 34.1 OTUs (range: 10–70) and 1492.8 reads (range: 429–3257) in each root system sample. The 354 OTUs occurred on average in 5.1 root systems (range: 1–36) and included an average of 223.5 reads (range: 2–13 756). Most of the OTUs occurred in <10 samples (84%), and 43% of the OTUs had fewer than 10 reads per sample. On average, 3.5 OTUs were shared across root systems, ranging from 0 to 15. In D. octopetala, the average number of OTUs per root system was 35.9, and the total number of OTUs was 249. The corresponding numbers for B. vivipara were 32.4 and 239 OTUs and for S. polaris 33.9 and 261 OTUs. After rarefaction of the matrix down to 400 sequences per sample, 315 OTUs in 53 samples were retained for further analyses.

**Richness across plant hosts**

Accumulation curves of OTU richness vs. sampling effort for each plant species indicated that the total fungal diversity in the study plots was not recovered (Fig. 1a). None of the curves reached an asymptote, but all had begun to level off. Estimates of total OTU richness associated with each plant ranged from 286 to 398 in B. vivipara, 303–422 in D. octopetala and 296–402 in S. polaris (Fig. 1b). No significant relationship was observed between the number of OTUs obtained per root system and host plant species, nor was any effect of root mass or a plot effect detected (all P-values >0.2).

**Host specificity of the fungal communities**

Neither the DCA (data not shown) nor the GNMDS analysis showed any structure supporting host specificity or effect of the root weights on the fungal community composition (Fig. 2). 72.7% of the OTUs were shared between at least two of the host plants (Fig. 3). Host plant individuals that were spatially close
to one another in the plots were randomly placed in the GNMDS (data not shown), and spatial autocorrelation was not evident in the semivariogram analyses of the plots (Fig. S4, Supporting Information). Moreover, the host plants showed no structure reflecting plot-related differences in the GNMDS analysis (Fig. S3, Supporting Information). Both the first and the second axes of the DCA and the GNMDS were strongly correlated (first axes: Kendall’s tau = −0.78; second axes: Kendall’s tau = 0.45). Although OTUs occurring exclusively in a single host species were recovered, all but one was recovered in fewer than four samples. Additionally, G-tests indicated that only one OTU occurring in >4 samples occurred significantly more frequently with a specific host species. This OTU was associated with B. vivipara and affiliated with Cortinariaceae. Further, the analyses revealed only one OTU that occurred more frequently within one of the two plots (plot 1). ANOVA suggested that three OTUs had higher relative abundance in one of the host plants (two in B. vivipara and one in S. polaris), and two OTUs had higher relative abundance in plot 1. Similar trends (i.e. no host specificity, plot effects, or spatial autocorrelation) were found in an OTU matrix containing only OTUs with taxonomic affinities to known ectomycorrhizal lineages (data not shown).

**Taxonomy**

Basidiomycota dominated the diversity recovered from all three host species. Of the 354 OTUs, 74.0% were assigned to Basidiomycota, and they accounted for more than 95% of the reads. Ascomycota represented an additional 20.6% of the OTUs, while Mucoromycotina and Chytridiomycota together accounted for approximately 4% of the OTUs. Only one OTU was assigned to the Glomeromycota. The most abundant order recovered was Agaricales followed by Thelephorales and Sebacinales (Table 1). The distributions of OTUs between orders were very similar for the three host species (Table 1). The two most common OTUs were assigned to the Agaricales. The most abundant was present in 36 samples and had a pairwise similarity of 100% to *Cortinarius parvannulatus* (GenBank Accession No. AY669664). The second most abundant OTU occurred in 31 of the samples and had a 99% pairwise similarity to *Hebeloma hiemale* (GenBank Accession No. GQ869528). *Inocybe* and *Cortinarius* were the two genera most commonly recovered, accounting for 39 and 35 of the OTUs, respectively. Twenty-two OTUs had their best BLASTN match to the genus *Mycelia*. Table 2 includes the best BLASTN match of the 25 most common OTUs, and information for the remaining OTUs is presented in Table S2 (Supporting Information). BLASTN match for OTUs present in the negative control sample can be found in Table S3 (Supporting Information).

**Discussion**

**Low host specificity**

No evidence for host specificity was detected in our analysis of the fungal communities associated with the three distantly related, ECM-forming arctic-alpine plant species, both in terms of the entire root-associated community as well as the ectomycorrhizal subset. This is contrary to recent findings that the primary factor structuring ECM communities on a global scale is host plant

<table>
<thead>
<tr>
<th>Taxonomic affinity</th>
<th>% of total OTUs</th>
<th>% of total reads</th>
<th>% of OTUs in B. vivipara</th>
<th>% of OTUs in D. octopetala</th>
<th>% of OTUs in S. polaris</th>
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lineage (Tedersoo et al. 2012) and also to a number of studies demonstrating host specificity of ECM fungi in boreal and temperate ecosystems (Ishida et al. 2007; Bent et al. 2011; Lang et al. 2011). However, many ECM fungi are known to associate with multiple host plant species (Molina et al. 1992). In line with our results, Timling et al. (2012) reported that the ectomycorrhizal fungal communities associated with two ECM host plants (Salix arctica and Dryas integrifolia) in the North American Arctic were not significantly different from one another. Similar observations have been made in Swedish alpine habitats (Ryberg et al. 2009, 2011). When it comes to fungal endophytes in non-ECM plants, distinct root-associated fungal communities have been identified in different host species (Becklin et al. 2012; Tejesvi et al. 2013).

What could be the reasons for the apparent low degree of host preference in the Arctic? Several factors might underlie the evolution of host specificity, or the lack of it, in root-associated fungi. First, it may be favourable for a host plant to form symbiotic relationships with fungi with many different physiological attributes as possible, in order to gain access to a broader range of nutrient pools (Molina et al. 1992). Fungi with a low degree of host specificity may be selected for and frequently represented in root-associated fungal communities. Second, association with generalist fungi may allow plants to more rapidly and easily colonize new areas or newly available habitats (Molina et al. 1992). In arctic environments with short growing seasons and scarce vegetation cover, the probability of ECM colonization is somewhat reduced, and a specialized requirement for one host plant may lead to a shortage of hosts and/or of available fungal partners and hence reduced survival.

**Fungal richness across host plants**

Although the accumulation curves showed that the entire fungal diversity in the plots was not recovered, the various estimates of total OTU richness indicate that the majority of the fungal diversity was accounted for (60–88%). In agreement with a similar study of S. arctica and D. integrifolia (Timling et al. 2012), we found no significant difference in fungal OTU richness between the three host plants. The host species with relatively large root systems, S. polaris and D. octopetala, did not include more fungal OTUs per root system than B. vivipara, nor did the root mass influence per root system OTU richness. Even if the root biomass differs between plant

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<table>
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<tr>
<th>Freq.</th>
<th>Reads</th>
<th>Description</th>
<th>Query cov. (%)</th>
<th>Max identity (%)</th>
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species, the number of rootlets and ECM root tips might not vary, because of relatively smaller lateral roots from the rhizome in, for example, *B. vieipara*.

It remains unclear whether interspecific competition occurs between belowground fungal mycelia in the Arctic or, if so, why it does not exclude more species from colonizing the roots. Some fungi clearly have different niches (e.g. endophytes vs. ECM fungi), but OTUs with seemingly similar niches (e.g. EMC fungi; Smith & Read 2008) and functions seem to cooccur in the same root systems. As discussed above, host plants might benefit from having numerous fungal partners with different physiological and functional attributes. We therefore speculate that strong fungal competitors that limit the diversity in the root-associated community may be selected against. Alternatively, a generally recognized feature of polar terrestrial ecosystems is that abiotic (physical environmental stresses) rather than biotic (e.g. competition) processes are important in the evolution of life history strategies (Convey 1996; Hogg et al. 2006). In general, competition is believed to select for an increase in ecological specialization (MacArthur & Wilson 1967; Southwood 1977, 1988; Grime 1988). Hence, if there is a low degree of competition, this may underlie the apparent absence of fungal host specificity in the analysed arctic fungal communities. Indeed, an analogous reduction in specificity has been reported in fungal-algal symbiosis in high latitude lichens (Romeike et al. 2002).

**Spatial structure**

No spatial structuring of the fungal communities between the two study plots was observed. ECM fungi are known to form CMNs, which can manifest as spatial autocorrelation across small distances. Spatial autocorrelation has been detected for ECM fungal communities on small spatial scales (<5 m) in forest ecosystems (Lilleskov et al. 2004; Pickles et al. 2012). The low similarity in fungal community composition between closely adjacent plant roots and lack of corresponding spatial autocorrelation suggests a low degree of CMNs across the arctic plant species in the study area. If CMNs were more widespread, the fungi associated with plants situated <5 cm apart would be predicted to be more similar than expected by chance, which was not the case.

**Taxonomy**

The studied root systems were dominated by basidiomycetes, both in terms of diversity and abundance. The most frequently identified orders, Agaricales, Thelephorales and Sebacinales, do all compromise both ECM fungi and non-ECM fungi. Numerous OTUs with taxonomic affiliation to *Inocybe* (39), *Cortinarius* (35), *Sebacina* (25) and *Tomentella* (23) were found, resembling the taxonomic composition of root-associated fungi observed in other studies of arctic-alpine ECM plants (Mühlmann et al. 2008; Bjorbaekmo et al. 2010; Blaalid et al. 2011; Fujiyoshi et al. 2011; Geml et al. 2012; Timling et al. 2012). Interestingly, numerous OTUs with taxonomic affinity to the assumed saprotrophic *Mycena* and *Morteriella* were detected. *Mycena* has frequently been detected in various plant root systems (Blaalid et al. 2011; Walker et al. 2011; Yao et al. 2013). Although we did not observe senescent or dead roots, we cannot exclude that some of these OTUs may be associated with unrecognized dead parts of the roots.

It is important to note that there could be some PCR, primer or sequencing bias against different taxonomic groups (Bellemain et al. 2010; Tedersoo et al. 2010a), and abundance measures in pyrosequencing may be of questionable reliability. For example, only one OTU had taxonomic affiliations to the Glomeromycota, despite that the host plants have previously been reported to form both ECM and AM (Blaalid et al. 2011; Kauserrud et al. 2012). Although there is known ITS primer bias against the Glomeromycota (Stockinger et al. 2010), few observations of Glomeromycota have been made in Svalbard (Väre et al. 1992) and in North-America, and AM fungi have been shown to decrease in frequency towards the poles (Olsson et al. 2004; Newsham et al. 2009). It is therefore not clear whether the low frequency of AM fungi in Svalbard is due to habitat filtering or a potential primer bias.

**Conclusions**

This study suggests a low degree of host specificity of ECM root-associated fungal communities in this High Arctic habitat. The lack of small-scale spatial structuring indicates that the ECM fungi in this area not usually form CMNs across plant roots. Under marginal arctic conditions, where the organismal energy budget is under pressure, resources are primarily allocated to survival and reproduction rather than competition, and therefore, host specialization and the production of CMNs may be too risky. The three ECM host plants analysed, despite having root systems of significantly different sizes, hosted a similar number of fungal root associates.

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remove sequences with incompatible tag combinations and three anonymous reviewers for valuable comments to the manuscript. The bioinformatics analyses were conducted on the Abel Computing Cluster at the University of Oslo.

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S.B. contributed to research design and partial funding, conducted fieldwork and laboratory analyses, analysed data and wrote the manuscript. U.V. contributed to research design, conducted fieldwork and contributed to analyses of the data and manuscript writing. T.C. contributed to research design, laboratory analyses and manuscript writing. P.B.E. secured partial funding and contributed to fieldwork and manuscript writing. M.L.D. contributed to analyses of the data and manuscript writing. H.K. secured funding, designed research and contributed to analyses of data and manuscript writing.

Data accessibility
The raw pyrosequences data with corresponding mapping files and R-scripts have been accessioned along with the final OTU matrix in Dryad (doi:10.5061/dryad.45pv2), as recommended by Nilsson et al. (2011).

Supporting information
Additional supporting information may be found in the online version of this article.

Table S1 Plant species other than the host plants present in the sampling plots.

Table S2 Overview of the OTUs found in the root systems of the three host plants Bistorta vivipara, Dryas octopetala and Salix polaris, including their frequency, number of reads and BLASTN top hits with accession numbers in GenBank.

Fig. S1 Localization of host plants (samples) within the two 3 m × 3 m plots (A and B) in Blomsterdalen, Svalbard.

Fig. S2 Global nonmetric multidimensional scaling (GNMDS) ordination of Hellinger transformed abundance of samples twice to check for consistency.

Fig. S3 Semivariograms showing no significant spatial autocorrelations.

Fig. S4 Global nonmetric multidimensional scaling (GNMDS) ordination based on Hellinger transformed abundance data of the operational taxonomic units.