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ORIGINAL PAPER

Weak habitat specificity in ectomycorrhizal communities associated with *Salix herbacea* and *Salix polaris* in alpine tundra

Martin Ryberg · Mathias Andreasen · Robert G. Björk

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Abstract This study explores mid-alpine ectomycorrhizal communities on *Salix herbacea* and *Salix polaris* in plant communities differing in nutrient status and snow conditions. Plant species were identified by tracking roots back to above ground structures while fungal species were identified using molecular methods. The fungi were identified to 34 molecular operational taxonomic units (MOTUs)/species but species accumulation curves indicated that the communities were only partially sampled. The estimated total species richness was 49 (±9 SD) MOTUs/species. No significant ectomycorrhizal community specificity was found between the two plant species and only weak specificity between different plant communities. Furthermore, no difference in proportion of colonized root tips could be demonstrated between plant communities. However, some fungal taxa showed tendencies to associate with specific environmental conditions. Sebacinaceae, *Inocybe egenula*, *Russula* cf. *emetica*, and a *Tomentella* sp. were found in meadow communities but not in the heath communities. *Sistotrema* cf. *alboluteum* and *Tomentella* cf. *terrestris* were only found in the dry and mesic heath communities. Classifications into exploration types showed that the contact type is more abundant in the dry heath community than the other communities. *Cenococcum geophilum* was the most common species but *Cortinarius* spp., *Russula* spp., *Tomentella* spp., and *Lactarius* spp. were also common. This study confirms that alpine communities are rich in ectomycorrhizal fungi including species from a wide variety of fungal lineages and also show that many dominant species have wide ecological amplitude.

Keywords Alpine · Arctic · Ectomycorrhiza · Environmental gradient · Exploration types · *Salix*

Introduction

Alpine and arctic environments cover about 8% of the surface of the Earth and represent rather extreme environments considering the low winter temperatures and the short growing season. They are remarkable in the high abundance of dwarf shrubs and herbs in genera such as *Salix*, *Betula*, *Dryas*, *Kobresia*, and *Bistorta* forming ectomycorrhizal associations (Cripps and Eddington 2005). Despite this, the ectomycorrhizal communities of this biome are less studied than those of, for example, temperate forests. As alpine and arctic ectomycorrhizal communities are species rich, with about half of the species unique to this environment (Gardes and Dahlberg 1996), it is important to increase our knowledge of these systems especially when considering threats to this biome from climate change.

It is well known that different ectomycorrhizal fungal species have differing habitat preferences, regarding for example soil condition and plant species association (e.g., Knudsen and Vesterholt 2008). In addition, different plants may benefit most from association with specific fungi

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M. Ryberg · M. Andreasen · R. G. Björk
Department of Plant and Environmental Sciences,
University of Gothenburg,
P.O. Box 461, 405 30 Gothenburg, Sweden

M. Ryberg (✉)
Department of Ecology and Evolutionary Biology,
University of Tennessee,
Hesler 334,
Knoxville, TN 37996-1610, USA
e-mail: kryberg@utk.edu

depending on the enzymatic capabilities of the fungal species (Smith and Read 2008) and what soil conditions the plants are most commonly associated with and the biochemical composition of that soil. For instance, Read (1991) and Read and Perez-Moreno (2003) hypothesized that the dominant form of mycorrhiza in a community is correlated with the relative concentration of different forms of nitrogen (N). Soils dominated by NO_3^- are proposed to be dominated by arbuscular mycorrhiza, whereas soils dominated by NH_4^+ should be dominated by ectomycorrhiza (Smith and Read 2008). Giesler et al. (1998) and Björk et al. (2007a) found the model applicable and relevant to natural small-scale gradients in a boreal forest and a tundra landscape, respectively. However, few studies exist investigating how the mycorrhizal communities associated with a few or a single plant species change within natural gradients. A study of alpine tundra (Hrynkiewicz et al. 2009) found a decreased diversity in the mycorrhizal community with increased N supply for *Salix polaris*. For another arctic willow, *Salix arctica*, Fujimura et al. (2008) found a strong association between the ectomycorrhizal fungal communities and site-specific soil conditions in high arctic Canada. Furthermore, numerous fertilization studies (e.g., Wallenda and Kottke 1998; Urcelay et al. 2003; Treseder 2008; Clemmensen and Michelsen 2006; Clemmensen et al. 2008) in a variety of ecosystems have found changes in the ectomycorrhizal community after addition of N. It was therefore expected that the mycorrhizal communities would differ between plant communities differing in soil properties.

In this study, we investigate the ectomycorrhizal fungal communities, and their exploration types (Agerer 2001), associated with *Salix herbacea* and *S. polaris* in five plant communities that differ in pH, carbon (C), and N ratio, dominant N form, soil moisture content, and duration and thickness of snow cover. We also investigate if there is any difference in the proportion of root tips colonized by ectomycorrhiza between the different plant communities. In addition we compare fungal communities between *S. herbacea* and *S. polaris* to investigate if there is any specificity in plant–fungi association.

Materials and methods

Study site and plots

The fieldwork was conducted at Latnjajaure Field Station in northern Sweden (68°20′ N, 18°30′ E). The field station is situated in a mid-alpine U shaped valley at an altitude of about 980 ma.s.l. The annual mean temperature is −1.9°C (1993–2008), the warmest month is July, with a mean temperature of +8.6°C, and the coldest month is February, with a mean temperature of −9.4°C. The mean annual precipitation is 847 mm (1990–2008), of which 200 mm falls during the growth season (approximately June–August). The vegetation is of low arctic character (Molau et al. 2003; Lindblad et al. 2006).

Five plant communities were studied; mesic meadow, meadow snowbed, dry heath, mesic heath, and heath snowbed. The heath plant communities have a pH ranging between 3.4 and 4.1 while the meadow plant communities range between pH 4.4 and 5.3 (Björk et al. 2007a). The net N mineralization rate is more than three times higher in the meadow ecosystems than in the heath ecosystems. The sample areas are located within 250 m from each other, with a maximum difference in altitude of 20 m. *S. herbacea* and *S. polaris* have a plant coverage ranging from 7% to 18% (total plant coverage 27% to 74%). *S. herbacea* has wider ecological amplitude than *S. polaris*, but preferentially grow in more acidic sites, whereas *S. polaris* grow under more calcareous conditions and is common in snowbeds. For a more complete description of the vegetation and soil properties of the studied plant communities see Björk et al. (2007a). The root biomass distribution of the plants is mostly concentrated in the shallow organic soil layer in the studied plant communities (from <1 cm to ca. 6 cm deep; Björk et al. 2007a, b).

Collection of root tips

Between July 15 and 30, 2008, six soil cores (10×10 cm) were randomly collected from a 10×10 m square within each plant community, except for the mesic meadow where five samples were collected. All samples were taken to a depth of 5 cm, or until hitting stone, but to a minimum depth of 3 cm. The samples were submerged in water overnight to facilitate separation of roots from the soil. All samples were processed within 2 days of collection. *S. herbacea* and *S. polaris* plants were identified and roots were tracked from above ground structures. The roots were examined under a dissection microscope, and root tips colonized with ectomycorrhiza, as judged from the presence of a fungal mantle layer, were assigned to morphotypes and the number of tips for each morphotype was counted. Non-colonized root tips were also counted. One to four healthy and well-developed ectomycorrhizal tips from each morphotype and sample were selected for sequencing and stored in 1% CTAB (cetyltrimethylammonium bromide) buffer.

Generation of sequences and species identification

DNA extraction was performed using the CTAB protocol of Larsson and Jacobsson (2004). PCR reactions were carried out using illustra^{TM} PuReTaq ReadyToGo PCR Beads (GE

Healthcare BioSciences AB). The primers ITS1F (Gardes and Bruns 1993) and LR21 (Hopple and Vilgalys 1999) were used to amplify the complete ITS region and about 375 bp of the 5′ end of the LSU region. Amplified products were purified using Qiaquick spin columns (Qiagen) applying 35 µl elution buffer instead of 100 µl to increase the final concentration. Sequencing was conducted by Macrogen Inc. (Seoul, South Korea). The ITS3 primer (White et al. 1990) was used for sequencing to obtain sequences of the ITS2 region and the 5′ part of the LSU region. For samples where no sequence was obtained, an additional PCR was performed on the previous PCR product using the ITS1 and ITS4 primer (White et al. 1990). These were then purified and sequenced as above.

To provide a taxonomic affinity each obtained sequence was searched against the GenBank (Benson et al. 2010), and UNITE (Kõljalg et al. 2005) databases for similar sequences using BLAST (Altschul et al. 1997). Sequences best matched by ectomycorrhizal species (as given in Rinaldi et al. 2008 and Tedersoo et al. 2010) were divided into taxonomic groups based on the results of the BLAST output. The sequences best matched by species of other nutritional modes or with dubious taxonomic affiliation were excluded from further analysis. For each taxonomic group similar sequences from the BLAST outputs and sequences from phylogenetic studies (Shimono et al. 2004; Garnica et al. 2005; Douhan and Rizzo 2005; Matheny 2005; Osmundson et al. 2005; Nilsson et al. 2006; Ryberg et al. 2008) were selected to provide a phylogenetic context. Each taxonomic group, including the additional sequences, were aligned in ClustalW (Thompson et al. 1994) as implemented in BioEdit (version 7.0.9.0; Hall 1999) and then manually adjusted in SeaView (Gouy et al. 2010). *Cortinarius* was divided into two matrices: subgenus *Telamonia* and remaining *Cortinarius*.

The alignments were used to estimate phylogenies under the maximum likelihood criteria using RAxML 7.0.4 (Stamatakis 2006). For each alignment a rapid search for the best scoring maximum likelihood tree was done in combination with 1,000 bootstrap replicas (TreeBase: 10400). In addition to the phylogenetic analysis a sequence similarity of 97% (Hamming distances; Swofford et al. 1996) in the ITS2 region was used to delimit molecular operational taxonomic units (MOTUs)/species.

Statistical analysis

Correspondence analysis (CA) for the ectomycorrhizal communities and Detrended correspondence analysis (DCA) for exploration types were conducted using CANOCO 4.5 to investigate differences between plant communities. Multi-response permutation procedure (MRPP) using Euclidean and Bray-Curtis (Bray and Curtis 1957) distances was also used to test the hypothesis of no difference between the ectomycorrhizal communities, both regarding species and exploration types, using the vegan package (Oksanen et al. 2009) in R (R development core team 2009). Euclidean distances is more in accordance with the ordination analysis but it have no upper limit, it may give higher similarities between samples based on shared missing species, and it depends on the scale of the variables. Bray-Curtis distances do not suffer from these issues but they do not consider abundances and thus give equal weight to both rare and common species. *P* value for the MRPP was estimated from 999 permutations. Root colonization and species densities (species/area) were analyzed using a one-way ANOVA, with plant community as the fixed factor. All data were, after addition of a constant, log-transformed and concomitantly scaled to unit variance to achieve a normal distribution, to eliminate skewness, and to ensure homogeneity of variances according to Økland et al. (2001). The one-way ANOVAs were conducted using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). EstimateS 8.2.0 (Colwell 2009) was used to construct species accumulation curves and to estimate the number of species in the communities using the Chao 2 estimator. Species specificity between the two *Salix* spp. was evaluated using Fisher's exact test (following Tedersoo et al. 2008).

Results and discussion

This study investigated 7,457 root tips of which about 50% were ectomycorrhizal. Despite their preference for different soil conditions, we did not find any support for *S. herbacea* and *S. polaris* to be associated with different ectomycorrhizal fungal partners when compared in the same vegetation types ($P=0.94$). Specificity in plant association has been demonstrated in several other communities (Ishida et al. 2007; Tedersoo et al. 2008) but the occurrence of preference in plant association is considered to be less pronounced in tundra environments (Kernaghan and Harper 2001; Ryberg et al. 2009). This may be due to the extreme environmental features of the tundra that promote fungi adapted to these conditions rather than plant-specific specialists. Another explanation for the lack of species specificity may be the close phylogenetic relationship between *S. herbacea* and *S. polaris.* Since no specificity was indicated, all samples from the two plant species were pooled together in the rest of the analyses.

Eleven different ectomycorrhizal morphotypes were found, which is in the midrange of what has previously been reported for *S. herbacea* and *S. polaris* (four to 21 morphotypes, Dhillion 1994; Clemmensen and Michelsen 2006; Mühlmann and Peintner 2008; Hrynkiewicz et al.

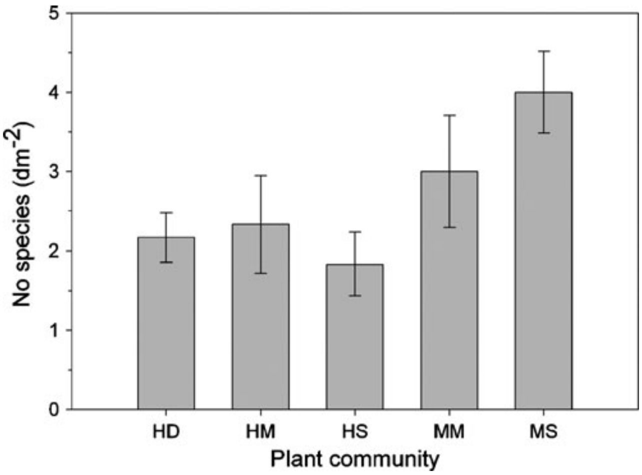

Fig. 1 Mean number of species per square decimeter for the different plant communities: dry heath (*HD*), mesic heath (*HM*), heath snowbed (*HS*), mesic meadow (*MM*), and meadow snowbed (*MS*). *Error bars* represent standard errors of the means

2009). From these 154 sequences of ectomycorrhizal taxa were obtained which were classified into 34 MOTUs/species (Online Resource 1). Since we determined ectomycorrhizal colonization from presence of a mantle layer, we may have missed some morphotypes with poorly developed mantles, such as some Ascomycetes. Sequences from the same morphotype within each sample generated the same MOTUs/species except for in a few cases attributable to dual colonization or secondary colonization by saprotrophic fungi. In such cases the morphotype was attributed to the MOTU/species that was indicated by the most sequences. The total MOTUs/species richness in our study is somewhat higher but in the same range as that of other tundra studies (Fujimura et al. 2008; Mühlmann and Peintner 2008), but lower than in a cliff ecosystem at the same site (Ryberg et al. 2009). We found a tendency towards higher species density in the meadow communities (P=0.058; Fig. 1). However, as no species accumulation curves leveled off (Fig. 2) the real species richness of these communities is probably higher and the Chao 2 estimates of minimum species richness for each plant community were: 11 (±5 SD) mesic heath, 14 (±8 SD) heath snowbed, 25 (±8 SD) meadow snowbed, 40 (±21 SD) dry heath, and 47 (±24 SD) for the mesic meadow, respectively. The minimum total richness was estimated to 49 MOTUs/species (±9 SD) but is probably an underestimation since the samples in each community are correlated compared to between communities. Fujimura et al. (2008) found higher species density in the more nutrient-rich communities in high-arctic tundra, which is in line with the tendency found in our study. Thus, even though the relative cover of ectomycorrhizal plants is higher in heath communities, these communities may have lower species richness and consequently Read's (1991; Read and Perez-Moreno 2003) model may not hold for small-scale gradients as previously suggested by Björk et al. (2007a).

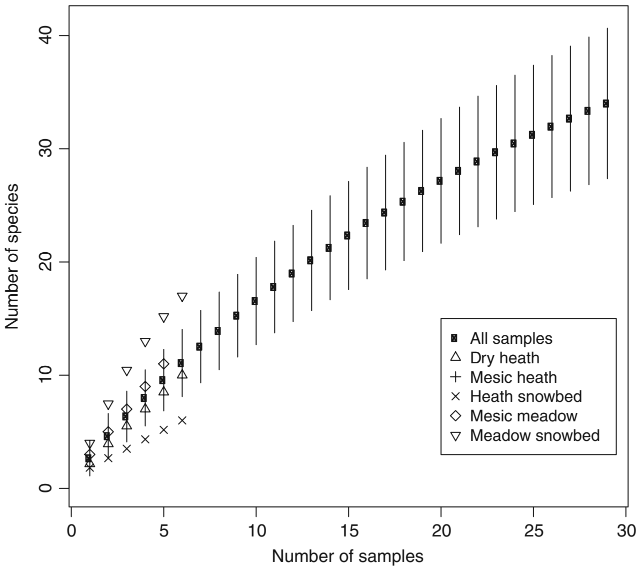

Fig. 2 Species accumulation curves for each plant community separately and for all combined. For all combined the 95% confidence interval is indicated

Unexpectedly, we found only a trend toward differences between the ectomycorrhizal communities in the plots of different plant communities using CA (Fig. 3) or Euclidean distances in MRPP (P=0.06; A=0.06). However, using Bray-Curtis distances the difference was significant (P=0.013; A=0.06). The CA uses only the difference along the two most prominent axes in the multidimensional space of the ordination while the MRPP uses the actual differences. The fact that we did not observe any difference

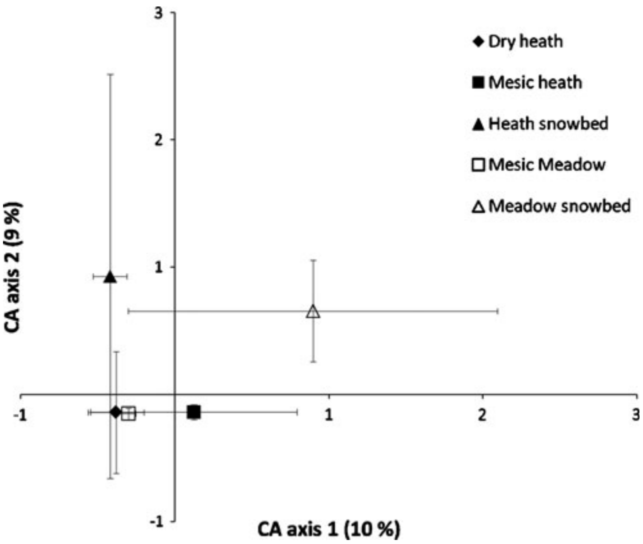

Fig. 3 Mean values (±85% confidence interval corresponding to a α= 0.05 test; see Payton et al. 2000, 2003) of sample scores from the correspondence analysis (*CA*) of the ectomycorrhizal communities within the different plant communities

between the plant communities in the CA and using Euclidean distances in the MRPP is due to that many of the dominating species such as *Cenococcum geophilum*, *Cortinarius* cf. *saniosus*, *Cortinarius* cf. *croseus*, *Lactarius* cf. *trivialis*, and a *Russula* sp. were found in several vegetation types and across vegetation series. However, 53% of the MOTUs/species were found in only one sample and consequently only in one plant community (Online Resource 1). *C. geophilum* was the most abundant species, both regarding the number of samples (79%) it was found in and regarding the number of root tips (24%) it was collected from (Fig. 4). This corroborates findings in other tundra soils (Dhillion 1994; Clemmensen and Michelsen 2006; Mühlmann and Peintner 2008; Hrynkiewicz et al. 2009). The dominance of *C. geophilum* in our study (relative abundance 58%) was also higher than found by Hrynkiewicz et al. (2009; relative abundance ca. 32%), which in addition reported differences in ectomycorrhizal communities between plant communities. *C. geophilum* possesses proteolytic enzymes and may have higher ligninolytic capacity in comparison to other ectomycorrhizal fungi (Bending and Read 1997), which make them able to mobilize organically bounded nitrogen (Dahlberg et al. 1997) favoring them in nutrient poor environments such as tundra soils. Even though the CA showed no support for habitat specificity in the ectomycorrhizal community, we found some taxa that were specific to either heath or meadow communities. For instance, Sebacinaceae spp., *Inocybe egenula*, *Russula* cf. *emetica*, and one of the *Tomentella* species were only found in the two meadow communities, whereas *Sistotrema* cf. *alboluteum* and *Tomentella* cf. *terrestris* were found only in the dry and mesic heath. The fact that the dominating ectomycorrhizal fungal species were found in several plant communities and that there was no significant difference in the CA analysis and the MRPP using Euclidean distances show that the community specificity is low in these ecosystems. However, the MRPP using Bray-Curtis distances and the tendency towards higher species richness in the meadow communities indicate that there are some differences between communities regarding less common species.

Unlike Hrynkiewicz et al. (2009), we did not find any significant difference in the proportion of colonized root tips between the different plant communities (Fig. 5). The difference between our studies implores further exploration of this pattern. The proportion of colonized root tips in our study is slightly higher than in the study of Hrynkiewicz et al. (2009), but lower than what was found in nearby fellfield vegetation (Michelsen et al. 1996; Clemmensen and Michelsen 2006) and a glacier forefront in the Austrian Alps (Mühlmann and Peintner 2008). It has been proposed that root colonization by mycorrhizal fungi decreases with increasing nutrient availability in the soil, because plants can meet their nutrient requirements through direct uptake by roots without the extra C expenditure required to support mycorrhizal fungi (Aerts and Chapin 2000). However,

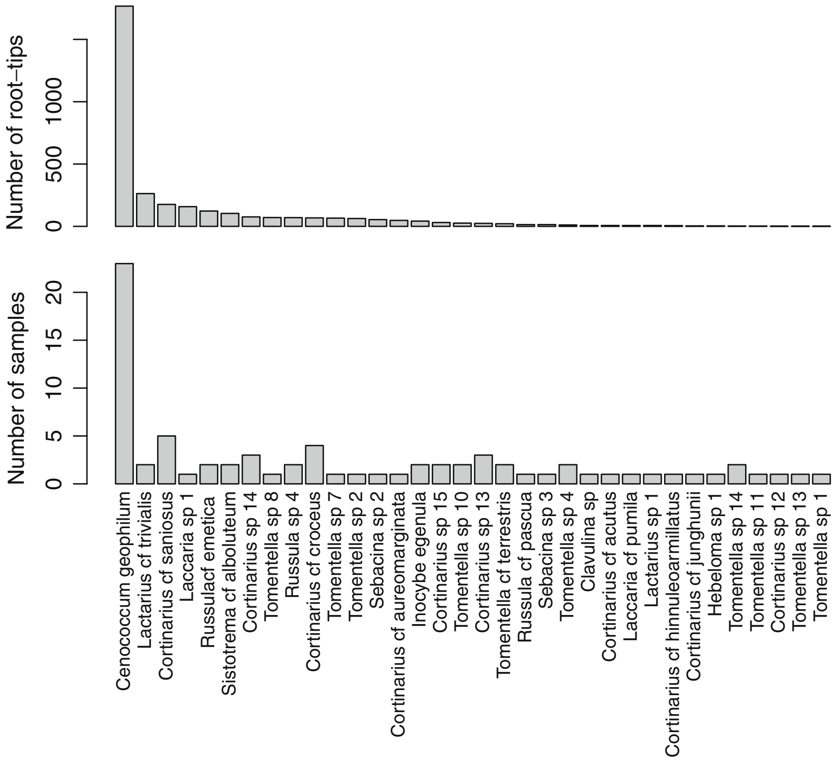

Fig. 4 Number of root tips per species/MOTU (*above*) and number of samples each species/MOTU were found in (*below*)

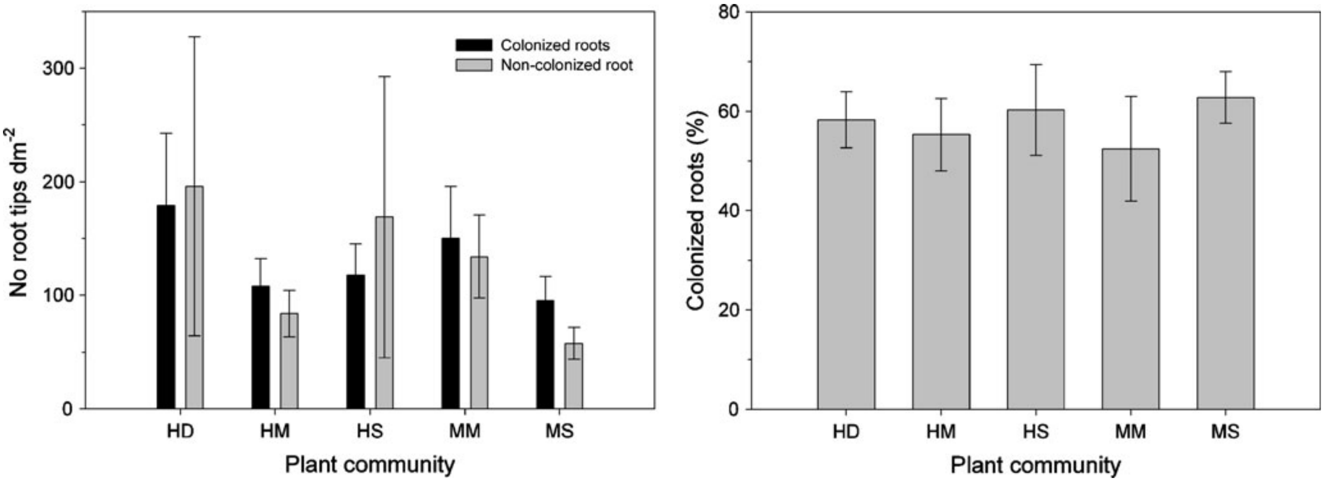

Fig. 5 Mean number of root tips colonized with ectomycorrhiza. To the left is number of root tips per square decimeter and to the right is proportion of colonized roots. *Error bars* represent standard errors of the means

while short-term N fertilization studies (e.g., Urcelay et al. 2003; Treseder 2008) show a decrease in the root colonization rate long-term studies do not (reviewed in Wallenda and Kottke 1998; Clemmensen and Michelsen 2006). Clemmensen and Michelsen (2006) suggested that the differences between short- and long-term studies were a time effect of the adjustment of the ectomycorrhizal community to the altered environmental conditions. However, we did not find any differences in colonization rate and only a weak difference in ectomycorrhizal communities between the different natural occurring N conditions, which may suggest that short-term effects may be due to ecosystem disturbance rather than an N fertilization effect.

Three different exploration types were found, contact, short-distance, and medium-distance fringe type (Agerer 2001). In general, the short-distance was the most common exploration type (2,842 root tips) followed by the contact type (818 root tips) and medium-distance fringe type (65 root tips). However, in the dry heath the contact type was the most common, this difference was significant in the CA (Fig. 6) but not in the MRPP (Euclidean distances P=0.08, A=0.06; Bray-Curtis P=0.09, A=0.07). Studies in mountainous Norway spruce stands (Baier et al. 2006; Scattolin et al. 2008) have found the short-distance type to be related to the organic layer and high N content, and the contact type to the mineral soil layer. These studies hypothesized that since the mineral soil is characterized by organic matter that is hard to degrade, stabilized by calcium–magnesium–humic compounds, the contact types might be able to utilize these sources because they are often hydrophilic and can degrade lignin to increase access to nitrogen complexed to phenolic substances (Agerer et al. 2000; Agerer 2001). The preference for the short-distance type to the organic layer was ascribed to their dense cover of emanating hyphae (Baier et al. 2006; Scattolin et al. 2008). Hence, they are probably more suitable to explore the larger volume of the looser and more porous litter and organic layers of the soil (Agerer 2001). Kranabetter et al. (2009) found an increase of contact and medium-distance types with increased soil fertility along a productivity gradient in a boreal forest. Our data does not support any of the above alternatives, although the dry heath has very thin organic layer (as do the snowbeds), it is also very poor in N compared to the meadows. However, the dry heath has the shallowest snow cover (maximum 10 cm thick), and it is therefore frequently subjected to freeze-thaw events, which may interrupt longer hyphae and rhizomorphs and consequently favor the contact

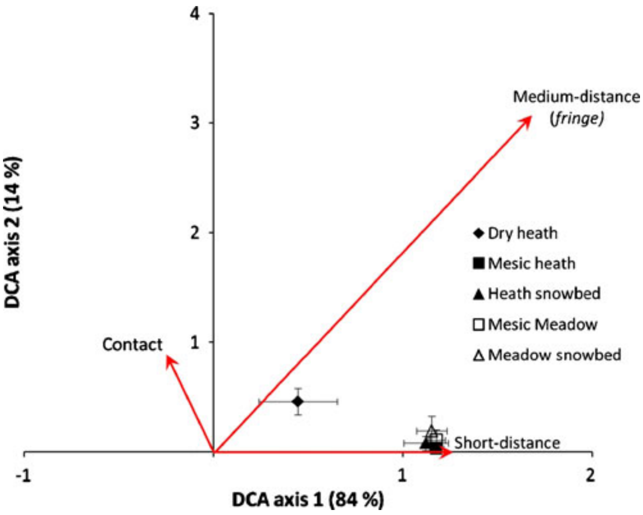

Fig. 6 Mean values (±85% confidence interval corresponding to a α= 0.05 test; see Payton et al. 2000, 2003) of sample scores from the detrended correspondence analysis (*DCA*) of the exploration types within the different plant communities together with the species scores outline as vectors illustrating the increase of an exploration type within the ordination space

type in this community. However, there may be a bias towards the contact and short-distance type as hyphae and rhizomorphs signifying medium- and long-distance types may be lost when extracting the root tips from the soil.

Alpine and arctic plant communities with dwarf shrubs and herbs form unique environments for ectomycorrhizal fungi. We show that several common fungi in these environments have wide ecological amplitude and can occur in several plant communities. However, soil factors may still be important for many of the rarer species. Information on habitat specificity will be important to evaluate the threat to the species diversity of the alpine and arctic biome as these retract and decrease in area.

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