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Long-term increase in snow depth leads to compositional changes in arctic ectomycorrhizal fungal communities

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Abstract

Many arctic ecological processes are regulated by soil temperature that is tightly interconnected with snow cover distribution and persistence. Recently, various climate-induced changes have been observed in arctic tundra ecosystems, e.g. shrub expansion, resulting in reduction in albedo and greater C fixation in aboveground vegetation as well as increased rates of soil C mobilization by microbes. Importantly, the net effects of these shifts are unknown, in part because our understanding of belowground processes is limited. Here, we focus on the effects of increased snow depth, and as a consequence, increased winter soil temperature on ectomycorrhizal (ECM) fungal communities in dry and moist tundra. We analyzed deep DNA sequence data from soil samples taken at a long-term snow fence experiment in Northern Alaska. Our results indicate that, in contrast with previously observed responses of plants to increased snow depth at the same experimental site, the ECM fungal community of the dry tundra was more affected by deeper snow than the moist tundra community. In the dry tundra, both community richness and composition were significantly altered while in the moist tundra, only community composition changed significantly while richness did not. We observed a decrease in richness of *Tomentella*, *Inocybe* and other taxa adapted to scavenge the soil for labile N forms. On the other hand, richness of *Cortinarius*, and species with the ability to scavenge the soil for recalcitrant N forms, did not change. We further link ECM fungal traits with C soil pools. If future warmer atmospheric conditions lead to greater winter snow fall, changes in the ECM fungal community will likely influence C emissions and C fixation through altering N plant availability, fungal biomass and soil-plant C-N dynamics, ultimately determining important future interactions between the tundra biosphere and atmosphere.

Keywords: arctic ecology, climate changes, fungal ecology, ITEX, snow fence, snow pack, Toolik Lake

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Introduction

Artic ecosystems are beginning to exhibit significant shifts in ecosystem structure and function induced by changes in climatic conditions (Elmendorf *et al*., 2012; Tape *et al*., 2012). Despite interannual and regional variability, global mean surface temperature have consistently increased since the late 19th century (Collins *et al*., 2013). In the Arctic, temperatures have risen between 0.06 to 0.1 °C per yr, while the global average increase has been ca. 0.017 °C per yr during the past 30 years (Comiso & Hall, 2014). These temperature increases have already had major consequences, including accelerated summer ice loss, extended periods of open water in the Arctic Ocean and delayed autumn freeze up (Stroeve *et al*., 2014). At the same time, precipitation in the Arctic has increased (greatly exceeding the global average increase), especially during the cold season, where most precipitation falls as snow (Kattsov & Walsh, 2000; Screen & Simmonds, 2012). Additionally, state-of-the-art models predict further increases in the twenty-first-century, possibly by more than 50% of the current precipitation, leading to thicker snow cover (Collins *et al*., 2013; Bintanja & Selten, 2014). Deeper snow would have a suite of consequences for tundra ecosystems. These include protection from the abrasive wind (Liston *et al*., 2002; Sturm *et al*., 2005; Blok *et al*., 2015), warmer winter soil temperatures and increased soil moisture with subsequent effects on thaw depth and C storage (Natali *et al*., 2012, 2014), N turnover (Schimel *et al*., 2004; DeMarco *et al*., 2011), plant phenology and mineral nutrition (Borner *et al*., 2008; Leffler

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& Welker, 2013; Pattison & Welker, 2014), vegetation composition (Wahren *et al*., 2005; Welker *et al*., 2005) and soil microbial respiration (Aanderud *et al*., 2013; Natali *et al*., 2014). However, with the exception of Buckeridge & Grogan (2008) that compared bacterial and fungal biomass growth responses, how arctic soil fungal communities may respond to changes in winter snow depth conditions is still largely unknown.

Microbial activity in the Arctic has been shown to increase due to higher winter soil temperatures inducing changes in the nitrogen (N) cycle dynamics, particularly in moist tussock tundra and less so in dry heath tundra in Arctic Alaska (Schimel *et al*., 2004; DeMarco *et al*., 2011; Natali *et al*., 2014; Pattison & Welker, 2014). In the Arctic, fungi are considered to constitute the bulk of soil microorganisms biomass (Callaghan *et al*., 2005) and have a key ecological role. Hobbie & Hobbie (2006) estimated that up to 86% of the N obtained by tundra plants is via mycorrhizal fungi, in exchange, plants can allocate between 10 to 20% of their photosynthate-derived C to their fungal partners (Harley, 1971; Hobbie, 2006), constituting an important pool of soil C. Additionally, these exchanges might be positively correlated, i.e., increased allocation of plant C to the mycorrhizal partner might lead to increased uptake of N from the soil pool and subsequent delivery to the plant host (Talbot & Treseder, 2010). The limiting step in soil N cycling is the breakdown of macromolecular organic compounds, particularly the depolymerization of proteins (Schimel & Bennet, 2004; Jones & Kielland, 2012) that in high-latitude ecosystems has been correlated with fungal biomass (Wild *et al*., 2013), and is particularly attributed to ectomycorrhizal (ECM) and ericoid mycorrhizal fungi (Read & Perez-Moreno, 2003). Recently, several studies reported major changes in the arctic fungal mycorrhizal communities in response to summer warming (Deslippe *et al*., 2011; Geml *et al*., 2015; Morgado *et al*., 2015; Semenova *et al*., 2015), with the fungal community of moist tussock tundra typically showing more pronounced response than the dry heath tundra, including potential shifts in functional traits and the subsequent ecosystem processes. However, possible effects of increased winter soil temperatures on the richness and compositional structure of soil fungal communities have not yet been investigated.

Tundra plant community responses to increased winter snow depth include a combination of shifts in community composition as well as increases in net plant productivity (Borner *et al*., 2008; Natali *et al*., 2014) and plant N tissue concentrations (Leffler & Welker, 2013). At the community level, the general trends are increases in shrub coverage and litter layer and, decrease in lichens, bryophytes, and leaf C:N ratio (Welker *et al*., 2005; Wipf & Rixen, 2010; Pattison & Welker, 2014). Wahren *et al*. (2005) and Mercado-Díaz (2011) reported (from the same experimental plots that we used in our study) an increased coverage of several species of deciduous and evergreen shrubs, and a sedge species. Although most of these plants are highly dependent on root-associated fungi, especially ECM fungi, to acquire soil nutrients, how soil fungal community changes in response to deeper snow remains uncertain. Here, we focus on ECM fungal community responses to long-term increased snow depth and the associated warming soil temperatures across the dry heath and moist acidic tussock tundra.

Ectomycorrhizal fungi represent the most taxonomically diverse fungal guild in the Arctic tundra (Gardes & Dahlberg, 1996; Geml *et al*., 2012; Timling & Taylor, 2012), and provide crucial roles in soil-root interaction, particularly in plant N uptake (Read *et al*., 2004; Ekblad *et al*., 2013) and in soil C dynamics (Clemmensen *et al*., 2013; Averill *et al*., 2014). Recently, an increasing amount of studies on fungal functional traits are amassing valuable insights into the role of the community structure in potential ecosystem functions (e.g. reviewed in Fernandez & Koide, 2014; Treseder & Lennon, 2015). For example, Hobbie & Agerer (2010), gathered evidences from $\delta^{15}N$ patterns and argued that ECM fungi have two main strategies for growth and nitrogen acquisition that match the extramatrical mycelium (EMM) characteristics of the ectomycorrhizae. ECM fungi with low abundance of EMM and hydrophilic mycorrhizae with contact, short-distance and medium-distance smooth hyphal exploration types (ETs) were argued to focus on uptake of labile nitrogen (N) forms, such as amino acids, ammonium, and nitrate. Supporting this hypothesis, many taxa composing this group showed limited growth in media of protein culture conditions (Lilleskov *et al*., 2011). On the other hand, the ECM fungi with high abundance of EMM with medium-distance fringe, medium-distance mat and long-distance ETs with hydrophobic rhizomorphs (or mycelial cords), likely focus on widely dispersed and spatially concentrated soil resources requiring efficient long-distance translocation. Such investment in exploratory hyphae is unlikely to rely on labile substrates under low nutrient availability; therefore, this group of taxa likely requires enzymes with hydrolytic and oxidative capabilities in order to access nonlabile N forms, such as proteins (Hobbie & Agerer, 2010). Supporting this hypothesis some studies pointed to increased exoenzyme activity in ECM fungi with abundant EMM (Tedersoo *et al*., 2012; Talbot *et al*., 2013). Another example of a fungal trait and a potential ecosystem function is the presence of melanin in hyphal cell walls, which was thoroughly discussed by Koide *et al*. (2014). Therefore, it seems reasonable to com-

pare community composition of fungi with certain functional traits in light of contrasting environmental conditions which in turn might feedback to altered ecosystem functions.

This research focuses on the effects of long-term increased snow depth on ECM basidiomycete communities. Based on the evidence previously stated, we hypothesize that: 1) ECM fungal community composition is strongly affected by increased snow depth, and that the response will be more pronounced on the moist tundra community; and 2) changes in ECM fungal community composition will reflect altered patterns in vegetation, soil nutrient pools and moisture, induced by the increased snow depth. Therefore, we expect altered patterns in the ECM fungal community functional traits.

Material and methods

Study site and experimental design

The International Tundra Experiment (ITEX) (Henry & Molau, 1997; Welker *et al*., 1997) study site that we sampled is located on the northern foothills of the Brooks Range, at the Toolik Lake Field Station. The area lies in the Arctic tundra biome within the bioclimatic subzone E, which covers approximately 36% of the Arctic dry land surface (Walker *et al*., 2005). The mean air annual temperature is −7 °C and annual precipitation ranges between 200 mm and 400 mm with approximately 50% falling as snow. The average snow depth is 50 cm (DeMarco *et al*., 2011). The snow fence experiment was established in the summer of 1994 in moist tussock and dry heath tundra (Jones *et al*., 1998; Walker *et al*., 1999; Welker *et al*., 2000). The snow fences are 2.8 m high and 60 m long, inducing leeward drifts of ca. 60 m long (Walker *et al*., 1999; Pattison & Welker, 2014). Our sampling was focused on the intermediate zone near the center of the experimental setup, corresponding to ca. 1–1.5 m winter snow depth. Although the deeper snow slightly shortens the growing season by ca. 5–8 days, this does not affect plant phenology significantly (Borner *et al*., 2008). The average winter soil temperatures at 2 cm depth were −2.9 °C (±0.2) and −4.7 °C (±0.2) in the increased snow depth plots and in the control plots, respectively (Pattison & Welker, 2014).

We sampled soil at the end of July 2012 from two tundra types, the dry heath and moist tussock tundra experimental sites. In each tundra type, we sampled five plots/replicates with increased snow depth and five plots with ambient snow depth ('control plots'). The control plots were located adjacent to the snow fences (the experimental setup was described in detail by Walker *et al*., 1999). Each replicate consisted of five soil cores of 2 cm diameter and 20 cm depth. Both organic and mineral layers were included in the soil cores, while undecomposed litter, moss, and coarse roots were removed. For each replicate the soil cores were thoroughly mixed and kept frozen until lyophilization. In total we sampled 100 soil cores across 20 plots of ca. 1 m^2 each.

The vegetation of the dry heath tundra is characterized by *Dryas octopetala*, *Salix polaris*, *Vaccinium* spp. and fruticose-lichens, while the moist tussock tundra is dominated by *Betula nana*, *Salix pulchra*, and the sedge *Eriophorum vaginatum*. Detailed descriptions of the plant communities can be found in Walker *et al*. (1999) and Kade *et al*. (2005), and their detailed response to the altered snow depths in Walker *et al*. (1999), Wahren *et al*. (2005), Welker *et al*. (2005), Mercado-Díaz (2011), Pattison & Welker (2014).

Molecular work and sequence quality control

The DNA extraction, PCR protocol, Ion Torrent sequencing and data clean-up procedures were described in detail elsewhere (Geml *et al*., 2014a). Briefly, for each sample we carried out two independent DNA extractions, using ca. 1 ml of lyophilized soil and pooled them to optimize extraction homogenization. In the PCR we targeted the ITS2 region of the nuclear ribosomal internal transcribed spacer that is currently accepted as the universal barcode marker for fungi (Schoch *et al*., 2012). We used primers fITS7 (Ihrmark *et al*., 2012) and ITS4 (White *et al*., 1990). The ITS4 primer was labeled with sample-specific Multiplex Identification DNA-tags (MIDs). The amplicon library was sequenced using an Ion 318™ Chip by an Ion Torrent Personal Genome Machine (PGM; Life Technologies, Guilford, CT, U.S.A.) at Naturalis Biodiversity Center. For the initial clean-up of the raw sequence data we used the online platform Galaxy (https://main.g2.bx.psu.edu/root), in which the sequences were sorted according to samples. Primers and adapters were removed. We used a parallel version of MOTHUR v. 1.32.1 (Schloss *et al*., 2009) for subsequent sequence analyses. Sequences shorter than 150 bp and longer than 400 bp were removed following Blaalid *et al*. (2013), Geml *et al*. (2014a) Geml *et al*. (2014b), Morgado *et al*. (2015) and Semenova *et al*. (2015), because these tend to be low-quality reads. The quality-filtered sequences were normalized following Gihring *et al*. (2012) by random subsampling so that each sample contained equal number of sequences. We then clustered the sequences into operational taxonomic units (OTUs) using OTUpipe (Edgar, 2010) with the simultaneous removal of putatively chimeric sequences using *de novo* and reference-based filtering using the curated dataset of fungal ITS sequences of Nilsson *et al*. (2011), with the default settings. We used a 97% sequence similarity clustering threshold following many other fungal ecology studies (e.g. O'Brien *et al*., 2005; Higgins *et al*., 2007; Geml *et al*., 2008; Geml *et al*., 2009; Amend *et al*., 2010; Tedersoo *et al*., 2010; Geml *et al*., 2012; Kauserud *et al*., 2012; Brown *et al*., 2013; Blaalid *et al*., 2013; Geml *et al*., 2014b; Davey *et al*., 2015). Global singletons were discarded from further analysis. The reference database published by Kõljalg *et al*. (2013) was used to determine the taxonomic affinity of the OTUs using USEARCH v7 (Edgar, 2010). OTUs with less than 80% similarity to any identified fungal sequence were excluded from the final analysis due to unreliable classification, and/ or uncertainty regarding their ecological role. A representative sequence of each OTU was deposited in GenBank under the accession numbers KP827673 – KP828017. Because GenBank

only accepts sequences with more than 199 bp, and we included sequences ranging 150 to 400 bp in our dataset, we include a representative sequence of each OTU as Supporting Information (Data S1).

ECM fungal database and EMM determination

We followed Tedersoo & Smith (2013) to determine ECM basidiomycete genera. For most OTUs we used a ≥ 90% sequence similarity to determine genera. Because Sebacinales have a diverse ecology we selected ECM OTUs based on their supported phylogenetic placement (with ≥70% bootstrap and/or ≥0.95 posterior probability) among sequences of taxa that were morphologically confirmed as ECM published by Glen *et al*. (2002), Urban *et al*. (2003), Ryberg *et al*. (2009) and Tedersoo & Smith (2013).

To determine the EMM characteristics, we followed the work of Agerer (2006), Tedersoo & Smith (2013) and consulted the DEEMY database (http://deemy.de, accessed in November, 2014 – an information system for the characterization and determination of ectomycorrhizae). In the genus *Russula*, if no EMM information was available for the species of interest, we assumed the EMM characteristics based on the closest species with known characteristics. To determine the closest species, we followed the phylogenetic study by Miller & Buyck (2002). Similarly, for OTUs of the genus *Hebeloma*, we followed the phylogenetic study by Boyle *et al*. (2006).

Statistical analysis

For each replicate, we calculated rarefied OTU accumulation curves using the R package Vegan (Oksanen *et al*., 2012) and determined the Good's coverage (complement of the ratio between the number of local singletons and the total sequence counts) for the fungal OTUs to estimate the exhaustiveness of our deep sequencing effort. Beta diversity, for all ECM basidiomycetes OTUs and for the ECM genera with the highest OTU richness, in each tundra type and treatment combination was calculated following Baselga (2010) with the R package betapart (Baselga & Orme, 2012), with the function *beta.multi* using a Sørenson dissimilarity matrix. This function computes community dissimilarity accounting for the spatial turnover and nestedness. OTU presence was defined as more than four sequences on a per sample basis following the suggestion of Lindahl *et al*. (2013) to minimize false positives (e.g. OTUs that are common in one sample, but may be low-abundant contaminants in others). Due to uncertainty of sequence abundance as indicator of species abundance in the samples (Amend *et al*., 2010), we carried out analyses with two types of data transformations. First, we transformed the data into presence–absence matrix. However, given that the most abundant and the rarest OTUs have equal weight in presence–absence datasets, we also wanted to see if taking read counts in consideration influences the results. Therefore, as a second dataset, we used square-root transformed sequence abundance to moderate the influence of OTUs with extremely high sequence counts, while maintaining some approximation of template abundance that may reflect ecological significance. We used PC-ORD v. 5.32 (McCune & Grace, 2002) to run nonmetric multidimensional scaling (NMDS) on a primary matrix of experimental plots by OTUs and a secondary matrix of plots by OTU richness per taxon, EMM characteristics and sequence counts. The dataset was subjected to 500 iterations per run using the Sørensen similarity (Bray-Curtis index) and a random starting number. We also calculated the Pearson's correlation coefficient (r) values between relative OTU richness per taxon and axes 1 and 2. We tested whether fungal communities were statistically different across the treatments using a multiresponse permutation procedure (MRPP) and determined any preferences of individual OTUs for either control or increased snow depth plots in dry and moist tundra using Indicator Species Analyses (Dufrêne & Legendre, 1997) as implemented in PC-ORD v. 5.32. We also tested for significant differences in OTU richness across the dry and moist tundra control and deeper snow plots, per taxa (genera) and EMM characteristics using Student's t-test. Sequence abundance was also compared between each tundra type and treatment combination using Student's t-test. Correlation coefficients were calculated as implemented in Microsoft Excel v. 2010 between the most OTU-rich genera and the hyphal exploration types that had been combined in two functional groups: (I) contact, short-distance, medium-distance smooth, and (II) medium-distance fringe and long-distance ETs. The Venn diagram for the whole community and genera with higher OTU richness was also calculated, using the online version of the publication by Oliveros (2007).

Results

Through the pipeline: from raw data to taxonomic diversity

We obtained 3 960 925 sequences with a median length of 268 bp. After quality control and random subsampling we retained 1 161 160 sequences with a mean length and standard deviation of 255.1 ± 52.7 bp. Clustering the sequences at 97% similarity generated 7015 OTUs, excluding global singletons and putative chimeric OTUs, of which 459 ECM basidiomycete OTUs were retained for further analyses (Data S2). Across all treatments, ECM fungi were represented by 23 genera classified in seven orders (Table 1, Fig. 1). Overall, *Cortinarius* and *Tomentella* were the most OTU-rich genera, with 125 (ca. 27%) and 124 OTUs (ca. 27%), respectively, followed by *Inocybe* (79 OTUs, 17%) and *Russula* (40 OTUs, 9%), with the remaining genera having less than 5% of the OTUs per genus. The order Agaricales had by far the highest OTU richness (224 OTUs, ca. 49%), followed by Thelephorales (128 OTUs, ca. 28%), Russulales (57 OTUs, ca. 12%), Cantharellales (33 OTUs, ca. 7%), Sebacinales (11 OTUs, ca. 2%), Boletales (4 OTUs, ca. 1%), and Atheliales (2 OTUs, ca. 1%). The analysis with sequence abundance resulted in similar patterns as the OTU richness analysis (Data S3). The

recovered OTU richness was higher than in previous publications that used similar methods to investigate arctic ECM fungal communities, but genera diversity and patterns of genera richness were in general agreement (Bjorbækmo *et al*., 2010; Timling *et al*., 2012; Geml *et al*., 2012; Morgado *et al*., 2015). The asymptotic rarefaction curves (Fig. 2a) and estimated Good's coverage (Fig. 2b) indicate that the deep sequencing allowed a very high OTU coverage and that most fungi in the samples were sequenced. It is important to note that OTU delimitation based on sequence similarity cut-off is a proxy for species delimitation with some inherent uncertainties. While the methodology we used is routine practice in fungal ecology and 2–3% ITS sequence divergence usually delimit different species in many basidiomycete lineages (Hughes *et al*., 2009), deep sequencing may slightly overestimate the number of species. Therefore, we consider the estimated absolute number of OTUs of secondary importance; rather, we think that the extent and direction of changes in richness among treatments (discussed below) are of primary importance, because these give us insights into trends that the fungal groups exhibit in response to experimental manipulations.

Overall results

The NMDS analyses of the presence–absence and square-root sequence abundance matrices gave similar results. However, the final stress values were somewhat lower in the square-root abundance ordinations, therefore, we continued with these for the main interpretations, while the presence–absence ordinations are shown in Supporting Information (Data S4). For the total dataset, the square-root abundance NMDS analysis resulted in a 2-dimensional solution with a final stress and instability of 0.1062 and <0.00001 respectively. The results of the Monte Carlo test indicated that the two dimensional solutions using the real data were significantly better than occurrences by chance ($P < 0.01$). The coefficients of determination for the correlations between ordination distances and distances in the original n-dimensional space were axis 1: $r^2 = 0.599$; axis 2: $r^2 = 0.240$; total $r^2 = 0.839$; orthogonality = 88.3%. The NMDS ordination plot was orthogonally rotated by the treatment to visualize correlations between snow depth effect and fungal community composition in general (Fig. 3a). The MRPP analysis indicated a clear distinction between dry and moist ECM community composition ($P < 0.0000001$, $A = 0.14601$). The NMDS and MRPP analysis with the presence–absence matrix results were similar (Data S4a). Beta diversity values in the dry tundra were similar among control and treatment plots and among plots per treatment; while in the moist tundra the values were generally higher among control and treatment plots than among plots per treatment. Species turnover was substantial in all comparisons (Table 2).

Across the ambient snow plots of both dry and moist tundra, *Tomentella* had the higher OTU richness with 103 OTU (ca. 29%), followed by *Cortinarius* with 78 OTUs (ca. 22%), *Inocybe* with 66 OTUs (ca. 19%) and *Russula* with 28 OTUs (ca. 8%). All the other genera had less than 5% of the OTUs per taxa and combined solely represented ca. 22% of the OTUs. On the other hand, across the deeper snow plots, *Cortinarius* had the higher OTU richness with 78 OTUs (ca. 36% of all OTUS), followed by *Tomentella* with 45 OTUs (ca. 20%), *Inocybe* with 28 OTUs (ca. 13%) and *Russula* with 24 OTUs (11%). All the other genera had less than 5% of the OTUs per taxa (Fig. 1). Differences between the ambient and deeper snow plots were also evident at the order level. Agaricales and Russulales had an increased OTU richness in deeper snow areas, while Thelephorales and Cantharellales had a decrease. Approximately 53% of the OTUs were only present in the ambient snow plots, ca. 24% were solely found in the deeper snow plots, and the remainder 23% present in both (data not shown). There was a significant decrease in OTU richness from the ambient to the deeper snow plots ($P = 0.0377$), with the control plots having on average 66.2 ± 24.5 OTUs, while the deep snow plots had 43.8 ± 28.4 OTUs (Table 1). Together the contact, short-distance and medium-distance smooth ET represented the group with highest OTU richness in the control plots with an average of 46.1 ± 17.9 OTUs per plot, while the medium-distance fringe and long-distance ETs group had an average of 16.6 ± 7.09 OTUs per plot. Comparing with the OTU richness values of the deep snow plots, the first group had a significant decrease ($P = 0.0042$, 24.6 ± 14.05 average OTUs per plot); whilst the later did not change significantly ($P = 0.36$, 15.2 ± 12.71 average OTUs per plot) (Data S5). The overall patterns of changes in functional traits were also depicted when comparing the OTUs in the ambient with the deeper snow plots (Fig. 1).

Dry heath tundra

The NMDS analysis of the square-root sequence abundance matrix resulted in a 2-dimensional solution with a final stress of 0.0763 and a final instability < 0.00001. The results of the Monte Carlo test indicated that the two dimensional solutions using the real data were significantly better than occurrences by chance ($P < 0.01$). The coefficients of determination for the correlations between ordination distances and distances in the original n-dimensional space were axis 1: $r^2 = 0.667$, axis 2:

Table 1 Average and standard deviation OTU richness *per* genus and extramatrical mycelium features

	Dry heath tundra			Moist acidic tussock tundra		
	DC	DS	*P*	MC	MS	*P*
Tomentella	21 ± 13.55	3.4 ± 2.07	0.02*	20.6 ± 7.40	12.6 ± 8.33	0.07
Cortinarius	9 ± 4.12	9.4 ± 6.80	0.46	16.6 ± 7.13	17.2 ± 16.44	0.47
Inocybe	10.4 ± 5.32	5.2 ± 1.92	0.05*	8.6 ± 4.28	3.2 ± 1.92	0.02*
Russula	1.8 ± 2.48	2.8 ± 3.03	0.25	7.6 ± 4.16	5.4 ± 4.10	0.42
Alnicola	–	–	–	0.8 ± 0.84	2.6 ± 0.89	0.01*
Amanita	0 ± 0	0.2 ± 0.45	0.19	–	–	–
Amphinema	–	–	–	0 ± 0	0.2 ± 0.45	0.19
Boletus	0.6 ± 0.55	0 ± 0	0.04*	–	–	–
Ceratobasidium	1.6 ± 0.55	1.6 ± 0.89	0.50	0 ± 0	0.2 ± 0.45	0.19
Clavicorona	–	–	–	0.4 ± 0.55	0 ± 0	0.09
Clavulina	0.6 ± 1.34	0.4 ± 0.55	0.38	0.6 ± 0.89	0.4 ± 0.55	0.34
Hebeloma	1.6 ± 2.61	0.4 ± 0.89	0.19	2.8 ± 0.84	2.6 ± 1.14	0.38
Hymenogaster	–	–	–	0.6 ± 0.55	0 ± 0	0.04*
Laccaria	–	–	–	1.4 ± 0.89	2.8 ± 0.84	0.02*
Lactarius	0.2 ± 0.44	0.8 ± 0.84	0.10	4.4 ± 3.36	1.8 ± 0.84	0.08
Leccinum	0.6 ± 0.55	0.8 ± 0.45	0.27	2.4 ± 1.34	1.6 ± 1.34	0.19
Membranomyces	0.4 ± 0.55	0 ± 0	0.09	–	–	–
Piloderma	0.2 ± 0.45	0 ± 0	0.19	–	–	–
Pseudotomentella	0.8 ± 1.30	0.4 ± 0.55	0.28	–	–	–
Sebacina	0.8 ± 1.79	0.4 ± 0.55	0.33	2.6 ± 1.34	1.4 ± 0.55	0.06
Sistotrema	1.4 ± 1.34	0.6 ± 1.34	0.19	3 ± 4.47	0.6 ± 0.89	0.15
Tomentellopsis	0.2 ± 0.45	0 ± 0	0.19	0 ± 0	0.4 ± 0.55	0.09
Tulasnella	0.8 ± 0.84	0.2 ± 0.45	0.10	–	–	–
C/ S/ MDS	42.2 ± 19.82	15.6 ± 3.78	0.02*	50 ± 17.13	33.6 ± 15.08	0.07
MDF/ L	11.8 ± 4.82	11 ± 6.63	0.42	22 ± 5	19.4 ± 16.59	0.38
Hi	34.6 ± 20.73	12 ± 4.69	0.04*	37.4 ± 11.68	25.4 ± 13.41	0.09
Ho	16.4 ± 8.02	11.6 ± 7.37	0.18	28.2 ± 8.93	22.6 ± 17.47	0.27
All ECM OTUs	54 ± 23.29	26.6 ± 8.02	0.03*	72.4 ± 22.02	53 ± 31.01	0.15

DC, dry tundra with ambient snow; DS, dry tundra with increased snow depth; MC, moist tundra with ambient snow; MS, moist tundra with increased snow depth; MDS, medium-distance smooth ET; MDF, medium-distance fringe ET; C, contact ET; S, short-distance ET; L, long-distance ET; Hi, hydrophilic; Ho, hydrophobic.
*Significant treatment effect ($\alpha = 0.05$).

$r^2 = 0.183$, total $r^2 = 0.849$ and orthogonality = 77.8%. The NMDS ordination plot was orthogonally rotated by the treatment to visualize correlations between snow depth and fungal community composition in general, and the taxonomic groups and EMM characteristics in particular (Fig. 3c). The MRPP analysis indicated a clear distinction between control and deep snow ECM community composition ($P = 0.0039$, $A = 0.04940$). The NMDS and MRPP analysis of the presence–absence matrix results yielded similar conclusions (Data S4c). The groups with the strongest negative correlation (Pearson's correlations) with the increased snow depth were OTUs of the contact, short-distance and medium-distance smooth hyphal ET ($r = -0.950$), *Tomentella* ($r = -0.937$), OTUs with hydrophilic hyphae ($r = -0.935$), *Inocybe* ($r = -0.935$), sequence counts ($r = -0.912$), total OTU richness ($r = -0.896$), *Sebacina* ($r = -0.682$), *Tulasnella* ($r = -0.582$), *Sistotrema* ($r = -0.533$). None of the groups showed a strong positive correlation ($r > 0.5$) with the increased snow depth. The control plots had on average 54 ± 23.29 OTUs per plot, while the treatment plots had 26.6 ± 8.02 OTUs per plot. This difference was statistically significant ($P = 0.03$). The comparison of sequence abundance also showed a significant decrease, in the same direction as the OTU richness patterns (Data S6).

Regarding taxonomic groups, *Tomentella* had the highest OTU richness in the control plots with 21 ± 13.55 OTUs per plot, followed by *Inocybe* with 10.4 ± 5.3. Interestingly, across the deep snow plots, both genera showed a significant decrease on the average OTU richness per plot ($P = 0.022$ and 0.047 respectively). On the other hand, OTU richness in *Cortinarius* was nearly unaffected and this genus had the highest

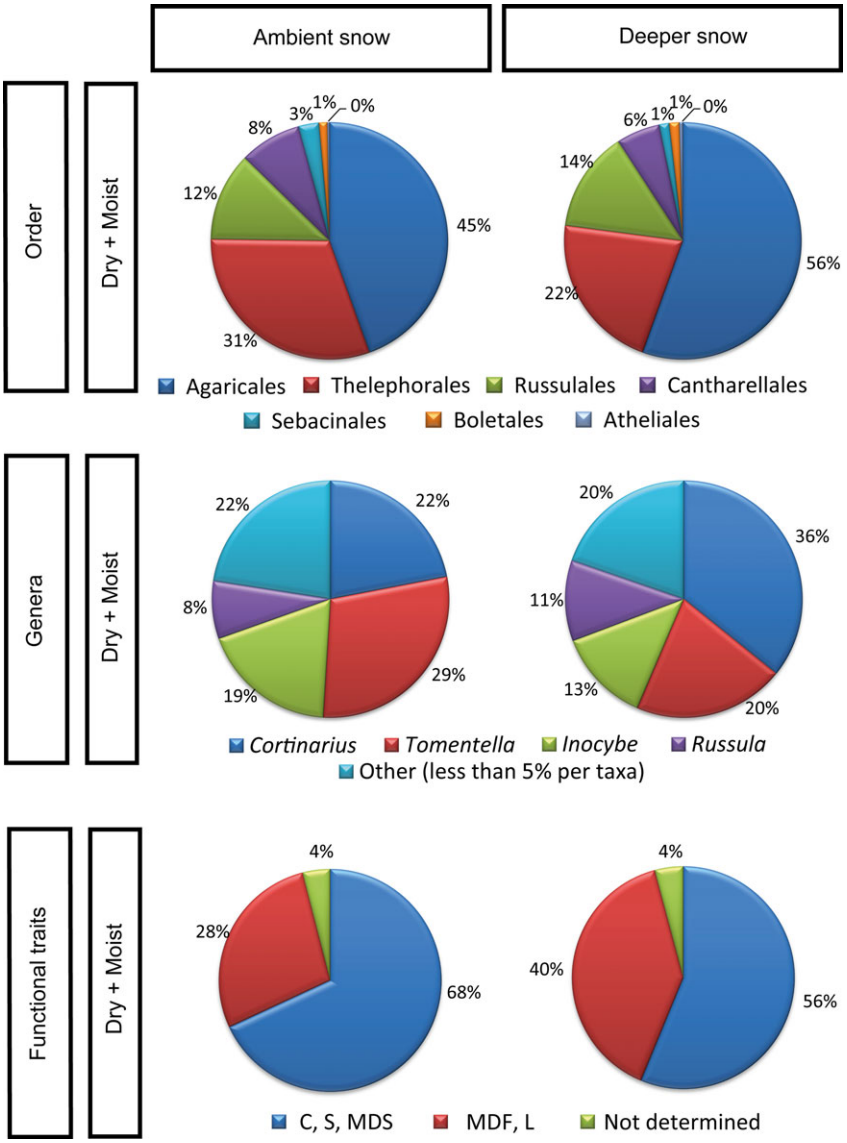

Fig. 1 Ectomycorrhizal basidiomycetes OTU richness, classified by taxonomic and functional traits in ambient snow and increased snow depth plots. The legend for each pair of graphics is organized by colors and in a clock-wise disposition. C, contact; S, short-distance; MDS, medium-distance smooth; MDF, medium-distance fringe; L, long-distance.

mean richness in the increased snow depth plots (Table 1). At the order level, there was an abrupt decrease in the proportion of Thelephorales OTUs, from 36% in the control plots to 17% in the deep snow plots, while most other orders had an increase in proportion between the ambient and increased snow depth plots, with Agaricales having 55% of all OTUs (across the deep snow plots) (Fig. 1).

In the ambient snow conditions, the functional group with contact, short-distance and medium-distance smooth ETs had the highest OTU richness with an average of 42.2 ± 19.82 OTUs per plot; while the group with long-distance and medium-distance fringed ETs solely had on average 11.8 ± 4.82 OTUs per plot. Interestingly, in the deeper snow plots the first group had a significant decrease ($P = 0.02$) in OTU richness, while the latter group maintained similar OTU richness ($P = 0.42$). Regarding relative sequence abundance, the same patterns were depicted for the above-mentioned functional groups, although the difference between control and increased snow depth plots for the contact, short-distance and medium-distance smooth ETs was only marginally significant ($P = 0.055$).The vast majority of OTUs were only present in the control plots (60%), a smaller percentage was present in both the control and the increased snow depth plots (20%), and

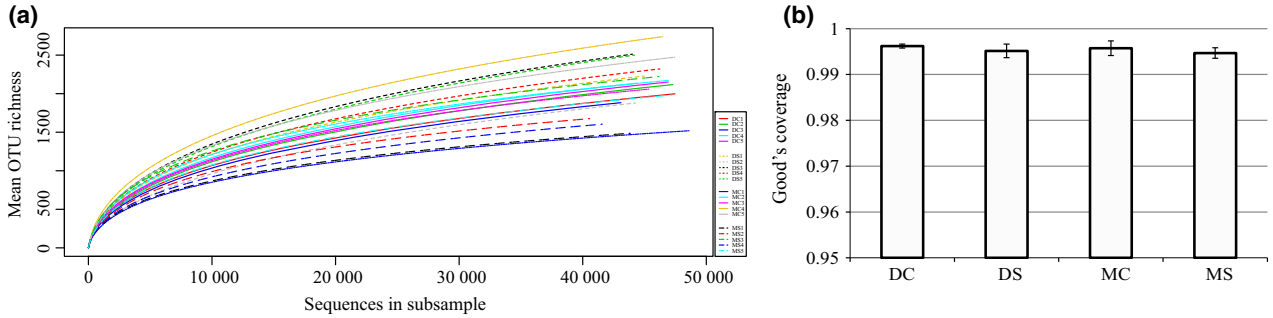

Fig. 2 (a) Observed number of OTUs *per* replicate sample; (b) Average Good's coverage *per* treatment and tundra type with standard deviation based on the five replicates. DC, dry tundra ambient snow; DS, dry tundra increased snow depth; MC, moist tundra ambient snow; MS, moist tundra increased snow depth.

only a minority (18%) was strictly present in the deeper snow plots (Fig. 4). The two genera with the highest OTU richness in the control plots, i.e. *Tomentella* and *Inocybe*, also follow this pattern. In comparison the percentage of OTUs that are solely present in the control plots in *Cortinarius* and *Russula* is considerably lower (Fig. 4). Indicator species analysis was significant ($\alpha = 0.05$) for five (undetermined) *Tomentella* OTUs in the ambient snow plots, while no OTU had significant values in the deeper snow plots (Table 3).

The correlation coefficient between the taxonomic groups and ETs revealed significant positive and negative correlations among specific groups (Data S7). Across the ambient snow depth plots, *Inocybe* OTU richness showed a significantly negative correlation with *Russula*, and a significantly positive correlation with *Tomentella*. Interestingly, across the increased snow depth plots, the negative correlations of OTU richness between the previously mention genera decreased sharply to nonsignificant values. The correlations for subsampled sequence abundance were all nonsignificant.

Moist tussock tundra

The NMDS analysis of the square-root sequence abundance matrix resulted in a 2-dimensional solution with a final stress and instability of 0.0759 and <0.00001 respectively. The results of the Monte Carlo test indicated that all two dimensional solutions using the real data were significantly better than occurrences by chance ($P < 0.01$). The coefficients of determination for the correlations between ordination distances and distances in the original *n*-dimensional space were axis 1: $r^2 = 0.607$; axis 2: $r^2 = 0.265$; total $r^2 = 0.872$; orthogonality = 93.1%. The NMDS ordination plot was orthogonally rotated by the treatment to visualize correlations between snow depth effect and fungal community composition in general, and the taxonomic groups in particular (Fig. 3b). The MRPP analysis indicated a clear distinction between ambient and deeper snow ECM community composition ($P = 0.0017$, $A = 0.0943$). The NMDS and MRPP analysis of the presence–absence matrix yielded similar results (Data S4b). *Inocybe* and the group of OTUs with short-distance exploration type had a strong negative correlation with the increased snow depth plots, $r = -0.757$ and -0.775, respectively, as well as the OTUs with hydrophilic hyphae ($r = -0.540$) and *Lactarius* ($r = -0.536$). On the other hand, *Alnicola* and *Laccaria* OTU richness had the strongest positive correlation with the deeper snow plots with $r = 0.696$ and $r = 0.510$, respectively. The control plots had on average 72.4 ± 22.02 OTUs per plot, while the deep snow plots had 53 ± 31.01 OTUs per plot. Despite the considerable decrease, the difference was not statistically significant ($P = 0.15$). Overall sequence abundance showed a similar pattern with a nonsignificant decreasing trend from the control to the increased snow depth plots.

The genus with the highest OTU richness in the ambient snow areas was *Tomentella* with 20.6 ± 7.4 OTUs per plot, followed by *Inocybe* with 10.4 ± 5.3. On the deeper snow areas, while *Tomentella* had only a marginally significant ($P = 0.07$) decrease on the average OTUs per plot to 12.6 ± 8.33, *Inocybe* had a significant ($P = 0.02$) decrease to 3.2 ± 1.92 OTUs per plot. On the other hand, *Cortinarius* was the genus with higher OTU richness in the deeper snow plots with 9.4 ± 6.8, and showed no significant changes ($P = 0.47$) on OTU richness compared with the ambient snow plots (Table 1). Interestingly, in the deeper snow plots, on the order ranking, Thelephorales increased the percentage of OTUs while all the remaining orders decreased (Fig. 1), mainly due to the decrease in *Inocybe*, *Russula*, and *Lactarius* OTU richness. Regarding EMM characteristics, in the control plots the contact, short-distance and medium-distance smooth had on average 50 ± 17.13 OTUs per plot, while the long-distance and medium-distance fringed had 22 ± 5 OTUs

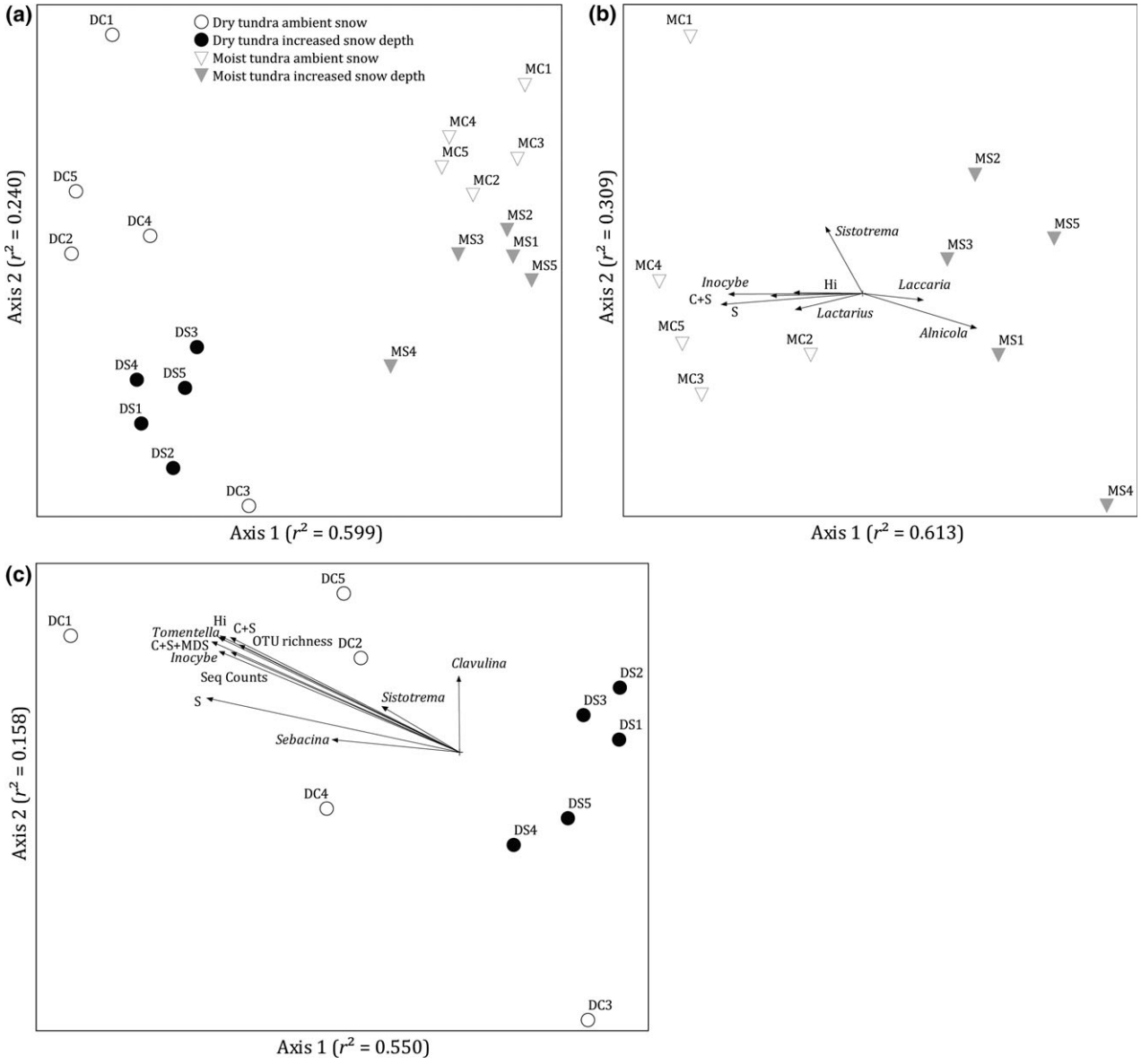

Fig. 3 Nonmetric multidimensional scaling (NMDS) ordination plots of ECM basidiomycetes communities from the ambient and increased snow depth plots based on OTU sequence square-root abundance in (a) the whole community (dry and moist tundra), (b) the moist tundra, (c) the dry tundra. Vectors with $|r|$ 0.5 are represented on the ordination plot. C, contact; S, short-distance; MDS, medium-distance smooth; MDF, medium-distance fringe; L, long-distance; Hi, hydrophilic; Seq counts, sequence reads.

per plot. In the deeper snow plots the first group had a marginally significant decrease (P = 0.07) to 33.6 ± 15.08 OTUs per plot, while the latter group did not change significantly (P = 0.38, 19.4 ± 16.59 OTUs per plot). Most OTUs, 45% were only present across the ambient snow plots, 25% were present in both the control and deeper snow plots and 30% were only present in the deeper snow plots (Fig. 4). *Tomentella* and *Russula* followed the overall OTU distribution pattern. Conversely, *Cortinarius* had a contrary pattern, with a higher percentage of OTUs solely recovered from the deep snow plots – 41%, 33% solely present on the control plots and 25% present in both the control and deeper snow areas. On the other hand, *Inocybe* had 63% of OTUs only recovered from the ambient snow plots, 23% recovered solely from the deeper snow areas and only 13% were found in both the ambient and deep snow plots (Fig. 4). OTUs from seven different genera had significant P-values (α = 0.05) in the indicator species analysis. Of these 13 OTUs were indicator of ambient snow plots and eight OTUs were indicator of deeper snow plots (Table 3).

Table 2 Beta diversity values using a Sørensen dissimilarity index, for all ECM basidiomycetes OTUs and the genera with the highest richness

	DC	DS	D	MC	MS	M
Cortinarius	0.985	0.984	0.984	0.788	0.806	0.871
Inocybe	0.974	0.979	0.976	0.738	0.833	0.883
Russula	0.957	0.961	0.958	0.650	0.682	0.821
Tomentella	0.981	0.988	0.982	0.638	0.739	0.830
All OTUs	0.995	0.996	0.995	0.701	0.719	0.836

DC, dry heath tundra with ambient snow; DS, dry heath tundra with increased snow depth; MC, moist acidic tussock tundra with ambient snow; MS, moist acidic tundra with increased snow depth; D, Dry control and increased snow depth; M, moist acidic tundra control and increased snow depth.

The correlation coefficient of OTU richness between the taxonomic groups and ETs revealed a significant positive correlation among two groups in the ambient snow plots: *Cortinarius* – *Russula*, and between the group of OTUs with contact, short-distance and medium-distance smooth ET, and the group of OTUs with medium-distance fringe and long-distance ET (Data S7). In the increased snow depth plots, the significant positive correlation, among these groups, was also observed, and further extended to the pairs *Tomentella* – *Cortinarius*, and (marginally significantly) for *Russula* – *Tomentella*. None of the groups tested had a negative correlation either at the control or the deeper snow plots. The correlation patterns for subsampled sequence abundance were similar in the increased snow depth plots, and all nonsignificant in the control plots.

Discussion

The results presented here clearly show that long-term increase in snow depth alters ECM fungal community composition in moist tussock and dry heath tundra, with a considerable portion of OTUs not being resistant to the resulting changes in environmental conditions. Such local extinction of many taxa is inferred from the significant decrease in average OTU richness with increased snow depth and from the high compositional turnover among the control and deep snow treatment in both tundra types. We find the strong decrease in ECM fungal richness surprising, because vegetation studies revealed a strong increase in shrub cover and size in the moist and to a lesser extent in the dry tundra that is presumably coupled with greater root biomass, i.e. more colonizable habitat for ECM fungi. Although our results suggest that only a subset of ECM fungi can withstand the altered environmental conditions caused

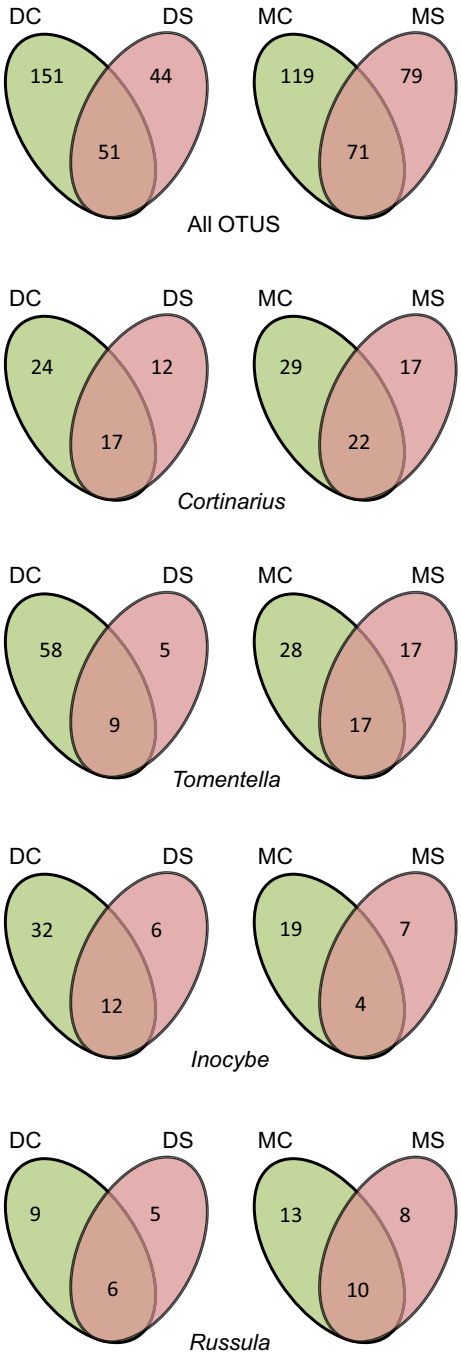

Fig. 4 Number of unique and shared OTUs *per* treatment (ambient snow and increased snow depth) in the dry and moist tundra types for all the community (All OTUs) and taxa with the highest richness. DC, dry tundra ambient snow; DS, dry tundra increased snow depth; MC, moist tundra ambient snow; MS, moist tundra increased snow depth.

by the increased snow depth, there were some species present in the deep snow treatment that were not detected in the control plots. It is plausible that some of the taxa characteristic of the deep snow plots may be

Table 3 Indicator OTUs (resultant from indicator species analysis, $\alpha = 0.05$) per treatment (correlated site), with classification, similarity and origin of the reference sequence

OTU	Correlated site	Kõljalg *et al.* (2013) and UNITE classification	Similarity (%)	Best match sequence origin
473	DC	*Tomentella* sp. (JX630707)	97.4	Happy Valley, AK
6290	DC	*Tomentella* sp. (UDB018363)	92.4	North India
6579	DC	*Tomentella* sp. (JX630707)	95.3	Happy Valley, AK
6686	DC	*Tomentella* sp. (FJ581421)	95.6	China: southwestern alpine meadow
8011	DC	SH108139.05FU *Tomentella* sp. (HQ211689)	93.8	Toolik Lake, AK
6073	MC	SH106684.05FU *Tomentella* sp. (EF218830)	96.5	British Columbia Interior Cedar Hemlock Forest, Canada
1991	MC	*Tomentella* sp. (JQ347212)	97.8	Subalpine medow, China
55	MC	*Tomentella* sp. (JX630431)	97.5	Thule, Greenland
12782	MC	*Tomentella* sp. 33E (FN687652)	95.2	Mid alpine environment, Sweden
1354	MC	*Tomentella* sp. (JX630589)	100	Prince Patrick Island, Canada
12656	MC	SH103086.05FU *Tomentella badia* (JQ711987)	93.6	BC, Canada
251	MC	SH108158.05FU *Tomentella* sp. (JF304372)	97.6	North America Arctic Transect
390	MC	SH166458.05FU *Cortinarius huronensis* (UDB015917)	100	Kilingi-Nõmme, Estonia
80	MC	SH105172.05FU *Cortinarius* cf. *flos-paludis* (FJ039560)	98.1	Canada
1030	MC	SH099601.05FU *Inocybe leiocephala* (AM882793)	99.2	Sweden
872	MC	SH111588.05FU *Inocybe nitidiuscula* (HQ604382)	95.4	BC, Canada?
301	MC	SH102330.05FU *Russula renidens* (UDB011117)	99.1	Kilpisjärvi, Finland
828	MC	SH164699.05FU *Lactarius torminosus* (UDB011509)	99.4	Estonia
10373	MS	*Laccaria* sp. (JX630414)	97.9	Ellef Ringnes Island, Canada
328	MS	*Inocybe* sp. (JX630878)	96.8	Baffin Island, Canada
3465	MS	SH105206.05FU *Cortinarius* sp. 17C (FN687635)	94.3	Sweden
11539	MS	SH101144.05FU *Russula* sp. (HQ212276)	96.9	Toolik Lake, AK
1165	MS	SH101328.05FU *Alnicola* sp. (FJ197860)	98.4	Primary successional glacier foreland soil, Austria
7259	MS	SH101328.05FU *Alnicola* sp. (FJ197860)	96	Primary successional glacier foreland soil, Austria
7802	MS	SH101328.05FU *Alnicola* sp. (FJ197860)	95.9	Primary successional glacier foreland soil, Austria
1058	MS	SH112490.05FU *Tomentella lapida* (JQ724049)	99.6	Natural/naturalized willow site, Sweden

DC, dry heath tundra with ambient snow; MC, moist acidic tussock tundra with ambient snow; MS, moist acidic tundra with increased snow depth.

snow bank specialists and may, therefore, be absent from the zonal (moist) and ridge-top (dry) control plots. Recently it has been shown that ECM fungi are also sensitive to summer warming with the communities of the moist tundra showing a more evident response to summer warming in terms of altered community composition, trait patterns and OTU richness levels (Geml *et al*., 2015; Morgado *et al*., 2015).

The strongest response to increased snow depth was observed in the dry heath tundra, where besides community composition, there was also an overall significant decrease in abundance (sequence reads) and richness of the ECM fungal community in response to increased snow depth. The richness and read abundance of the moist tundra also showed a decreasing trend but did not change in a significant manner. This was surprising, as we anticipated that ECM fungal community in the moist tussock tundra would have a stronger response to deeper snow, because the plant community and N dynamics had been reported to be more strongly affected by the increased snow depth in the moist tundra (Schimel *et al*., 2004; Wahren *et al*.,

2005; Mercado-Díaz, 2011). Together with our previous results (Morgado *et al*., 2015), we argue that the dry tundra ECM fungal community likely is more sensitive to changes in snow depth than to summer warming. The increased snow depth not only elevates winter soil temperature, but also increases soil moisture content as well (Wipf & Rixen, 2010), the effect of which likely is greater in the dry tundra. Because ambient snow in dry tundra areas is very shallow and provides little protection against cold and desiccating winds, the above compositional differences of fungal communities between ambient and deep snow may be partly caused by both temperature and moisture level differences. Our correlation analysis of OTU richness and sequence abundance with the different snow depths showed different patterns among the tundra types. For example, in the moist tundra OTU richness and sequence abundance between *Cortinarius* and *Tomentella* showed a significant positive correlation in the increased snow depth plots, while no significant correlation was observed in the control plots. On the other hand, in the dry tundra no correlation was observed between these two groups in either snow depths. This suggests that in our study the interaction between OTU richness and potential species abundance likely are habitat-specific, and are in agreement with studies that addressed species-species interactions (Kennedy *et al*., 2007; Simard *et al*., 2012; Fransson *et al*., 2013). Collectively, our findings reflect the complexity of arctic tundra responses to predicted changes in summer and winter climate and the need to undertake comparative studies that include multiple ecosystem types even at reduced spatial scales (Welker *et al*., 2000; Walker *et al*., 2008; Sullivan *et al*., 2008a,b; Rogers *et al*., 2011; Christensen *et al*., 2013; Leffler & Welker, 2013; Sharp *et al*., 2013; Lupascu *et al*., 2014). *Tomentella*, the genus with the highest richness in the control plots of both tundra types, showed a sharp negative response to increased snow depth, with a significant sixfold decrease in average OTU richness and a majority of the OTUs disappearing in the dry tundra, as well as an overall decrease in proportional sequence counts. In the moist tundra, *Tomentella* richness also showed a decreasing trend, but in a less striking manner than in the dry tundra. The higher beta diversity in the increased snow plots, especially in the moist tundra, reflects an increase in species turnover and intrageneric community dissimilarity indicating potential species-specific responses to the altered conditions and patchiness distribution of this group of species. Nevertheless, the elevated number of indicator OTUs associated with the control plots (five in the dry and seven in the moist tundra) point to the sensitivity of this group to altered conditions. Moreover, two of these OTUs were very closely related with an OTU (KJ792685) that was negatively affect by increased summer temperatures in the dry tundra (Morgado *et al*., 2015), further indicating that besides the general trends for the genus, at least one species of *Tomentella*, which is potentially widespread across the dry tundra, is very sensitive to summer and winter warming. Potential explanations to the observed patterns in our study may be linked with their functional traits and potential ecological roles. *Tomentella* and closely related genera (e.g. *Pseudotomentella* and *Tomentellopsis*) have melanized cell walls (Agerer, 1987–2002; Agerer, 2006), which is not a common feature in ECM basidiomycetes (Kõljalg *et al*., 2000). Melanins can be produced by fungi, plants, and animals, and are dark macromolecules composed of phenolic and indolic monomers, often coupled with protein and carbohydrates (Butler & Day, 1998). They usually constitute a considerable portion of total fungal cell weight and likely require a considerable energetic investment (Rast & Hollenstein, 1977; Butler & Day, 1998). This feature has been extensively argued and was recently shown in physiological experiments (Fernandez & Koide, 2013) to increase tolerance to several environmental stressors, such as freezing (Robinson, 2001) and hydric stress (Singaravelan *et al*., 2008; Fernandez & Koide, 2013). The increased snow depth not only elevates winter soil temperature, but also increases soil moisture content (Wipf & Rixen, 2010), the effect of which likely is greater in the dry tundra. The deep snow conditions with elevated soil temperature and moisture might reduce the competitive advantages of melanin-producing *Tomentella* adapted to the abovementioned stress factors (e.g., drought, and very low temperatures). Additionally, *Tomentella* has either contact, short or medium-distance smooth hyphal ETs, which have been argued to be adapted to labile N soil pools (Hobbie & Agerer, 2010). The plant community responded to increased snow depth with a significant increase in the shrubs and litter layer (Wahren *et al*., 2005; Mercado-Díaz, 2011), indicating a potential change in soil organic matter input and shifts in C:N ratio, that have been argued to be important regulators of arctic N dynamics (DeMarco *et al*., 2011). Therefore, it is possible that in the altered environmental conditions the combination of traits presented by *Tomentella* might not constitute an advantage in scavenging for soil nutrients, which might lead to a detrimental allocation of photosynthates by the ECM host and/or to competitive exclusion by other ECM fungi better suited to the altered conditions.

The decomposition and turnover of ECM fungal biomass likely has a significant role in C and other nutrient dynamics (Wallander *et al*., 2001; Clemmensen *et al*., 2013; Ekblad *et al*., 2013). Melanized hyphae have been argued to be relatively long lived, slow growing

(Robinson, 2001) and relatively resistant to decomposition (Coelho *et al*., 1997; Butler & Day, 1998; Butler *et al*., 2005), potentially representing a stable and recalcitrant component in the fungal biomass (Treseder & Lennon, 2015). In two laboratory experiments, Fernandez & Koide (2014) showed that the decomposition rate of ECM fungi was inversely correlated with the concentration of melanin and that the inhibition of melanin biosynthesis in an ECM fungi induced faster rates of decomposition. Moreover, Clemmensen *et al.* (2015), observed a correlation between the abundance of taxa with melanized hyphal content and higher carbon storage in the soil. If future climatic conditions lead to increased snow depth in the arctic tundra, the decreasing richness and relative abundance of *Tomentella* might contribute to soil C loss. Several other groups of root-associated fungi also have melanized hyphae, such as ericoid mycorrhizal fungi and dark septated endophytes. We therefore highlight the need to address responses of those root-associated fungi to increased snow depth to better understand if the above-mentioned warming-induced trend of decreasing richness is common in melanized fungi or is specific to certain phylogenetic lineages. Despite the uncertainties, our evidence and grounded speculations are in line with the results by Natali *et al.* (2014) that addressed winter warming effects on C cycle dynamics and indicated a net soil C loss due to winter warming.

We observed an abrupt decrease in mean OTU richness of *Inocybe* from the control to the increased snow depth plots in both tundra types. However, a considerable proportion of *Inocybe* OTUs that were found in the increased snow depth plots were also found in the control plots, particularly in the moist tundra. These results suggest that although arctic *Inocybe* spp. seem to be very sensitive to climate changes, a resistant subset of the species may be able to withstand changes in the climatic conditions. *Inocybe* spp. were previously argued to be sensitive to altered environmental conditions, such as soil compaction (Hartmann *et al*., 2014) and summer warming (Morgado *et al*., 2015). Additionally, there are evidences that in mature plant stands the rate of root-infection by *Inocybe* might decrease in sites with increased soil moisture (Fleming, 1984). It is possible that in the increased snow depth conditions, the lack of rizhomorphs and hydrophilic ectomycorrhizae of *Inocybe* (Agerer, 2006), a set of characteristics hypothesized to be adapted to labile N uptake (Hobbie & Agerer, 2010), might constitute detrimental traits in relation to other groups of ECM fungi. However, the increase in shrubs and litter layer might lead to potential patchiness of nutrient soil pools allowing for some species with hydrophilic hyphae to thrive in the increased snow depth.

The lack of significant changes in *Cortinarius* richness and the relative increase in overall sequence counts (a potential surrogate for relative abundance) between the control and increased snow depth plots indicate that this group might be more adapted to the altered conditions, and could become more dominant in the warming Arctic. A similar trend for this group was also observed in our previous work that reported ECM fungal responses to long-term summer warming (Morgado *et al*., 2015). However, in contrast, in the present study most OTUs are not shared between the two treatments, indicating that, although average OTU richness does not change, there seems to be a considerable turnover in species composition. This suggests that only a subset of the OTUs present in the control plots are resistant to increased snow depth and that there is substantial functional variation within the genus that allows for the exploitation of new niches created in the altered environment by incoming species. Species of *Cortinarius* form a dense mycelium with medium-distance fringe ET and hydrophobic rhizomorphs (differentiated mycelial cords) (Agerer, 2001). This foraging strategy is adapted for efficient N absorption and nutrient translocation (Hobbie & Agerer, 2010). Additionally, at least some *Cortinarius* spp. were reported to have the capability to assimilate organically bounded N (Hobbie & Agerer, 2010), and to transcribe Mn-peroxidase genes (which are involved in the production of exoenzymes) in field conditions; these were further linked through co-localization of DNA abundance with exoenzyme activity that interacts in complex organic matter breakdown (Bödeker *et al*., 2014). Another feature that may play a role is their physiological response to the altered conditions. Although, only a few studies focused on ECM fungal physiological responses to extreme cold, Ma *et al*. (2011) compared ECM fungi growth responses to very low temperatures (between −40 and +4 °C) and freeze-thaw events of four ECM species from distinct lineages. Their results indicated that *Cortinarius* had the lowest tolerance to freeze-thaw events and the fastest growth when temperatures reach near 0 °C. Because reduced temperatures, hydric stress, and freeze-thaw events inhibit the rate of chemical and microbial activity (Robinson, 2001), and given the characteristics and potential ecological role of *Cortinarius* spp., it seems feasible to argue that long-term increased winter soil temperatures, summer moisture, and reduced fluctuations in soil temperatures might convey advantages to this group over other ECM fungal groups.

The increasing dominance of fast growing ECM species with high EMM production and fast N mobilization might lead to increased C storage in the soil pool; however, this will be determined by biomass turnover. In an interesting work relating fungal traits, community

structure and nutrient soil pools, Clemmensen *et al.* (2015) found a link between abundance of species with similar ETs to that of *Cortinarius* and low accumulation of root-derived soil C. Briefly, they hypothesized that the exploratory hyphae of already explored soil patches could be recycled in an autolytic process, leaving behind just the long-living rhizomorphs connecting the exploratory forefront of the EMM and the ectomycorrhizae. This strategy would enhance their nutrient acquisition and maintain or reduce their biomass, potentially reducing also energetic costs. Due to the potentially high turnover of this mycelium biomass, this theory also implies a reduction in stable soil pools, and therefore the long-term C and N sequestration.

Russula did not show any significant change in richness with increased snow depth, and a considerable portion of the OTUs were found both in the control and the treatment plots, indicating that many OTUs were resistant to altered conditions. While most *Russula* spp. lineages have an hydrophilic ectomycorrhizae and contact or short-distance ETs that lack rhizomorphs, other lineages in *Russula* have a medium-distance smooth ET and hydrophobic hyphae (Agerer, 2006). In our dataset, the *Russula* OTUs with short or contact ET did not show change in richness with increased snow depth. However, one OTU with contact or short-distance ET was indicator of the moist control plots while another was indicator of increased snow depth, suggesting species-specific responses to altered conditions. On the other hand, in the increased snow depth plots in the moist tundra, we observed a significant decrease in richness of OTUs that matched *Russula* species with hydrophobic and medium-distance smooth ET. These results indicate that even within a closely related group of species the functional diversity can differ. While some *Russula* species seem to have a considerable fast growth rate at low temperatures (Ma *et al.*, 2011), others are considered to have a slow growth rate (Nygren *et al.*, 2008). Moreover, there is evidence of intrageneric variability in N usage as well (Avis, 2012). It is possible that in some species the hydrophilic ectomycorrhizae allows for the rapid intake of labile N forms by the plants, without metabolizing, via diffusion through the mantle of the ectomycorrhizae directly to the plant-host root via the apoplast. This process would avoid energetic costs and the necessity of C allocation to the ECM fungi (Nygren *et al.*, 2008). This may influence the competitive interactions between species with hydrophilic and hydrophobic mycelia.

In conclusion, our data provide first insights into the taxon-specific effects of increased snow depth on the ECM fungal community of Arctic tundra in Northern Alaska. We detected major shifts in ECM fungal community composition and its potential functional traits by coupling changes on fine scale taxonomic groups with their extramatrical mycelial characteristics. We postulate that ECM fungal community shifts induced by long-term increased snow depth likely stimulate C and N mobilization. However, the final balance induced by arctic ECM basidiomycete community in these nutrient pools will likely depend on the changes in the biomass of specific groups, particularly *Tomentella* and *Cortinarius*. Our results also highlight how the fundamental differences in tundra ecosystems control the nature of the existing fungal communities and their responses to deeper snow.

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Conflict of interests

The authors declare no conflict of interests.

References

Aanderud ZT, Jones SE, Schoolmaster DR, Fierer N, Lennon JT (2013) Sensitivity of soil respiration and microbial communities to altered snowfall. *Soil Biology and Biochemistry*, **57**, 217–227.

Agerer R (1987–2002) *Colour Atlas of Ectomycorrhizae*. Einhorn-Verlag, Schwäbish Gmünd, d-72525, Germany.

Agerer R (2001) Exploration types of ectomycorrhizae. A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycological Progress*, **11**, 107–114.

Agerer R (2006) Fungal relationships and structural identity of their ectomycorrhizae. *Mycological Progress*, **5**, 67–107.

Amend AS, Seifert KA, Bruns TD (2010) Quantifying microbial communities with 454 pyrosequencing: does read abundance count? *Molecular Ecology*, **19**, 5555–5565.

Averill C, Turner BL, Finzi AC (2014) Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature*, **505**, 543–545.

Avis PG (2012) Ectomycorrhizal iconoclasts: the ITS rDNA diversity and nitrophilic tendencies of fetid *Russula*. *Mycologia*, **104**, 998–1007.

Baselga A (2010) Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, **19**, 134–143.

Baselga A, Orme CDL (2012) Betapart: an R package for the study of beta diversity. *Methods in Ecology and Evolution*, **3**, 808–812.

Bintanja R, Selten FM (2014) Future increases in Arctic precipitation linked to local evaporation and sea-ice retreat. *Nature*, **509**, 479–482.

Bjorbækmo MFM, Carlsen T, Brysting A *et al.* (2010) High diversity of root associated fungi in both alpine and arctic *Dryas octopetala*. *BMC Plant Biology*, **10**, 244.

Blaalid R, Kumar S, Nilsson RH, Abarenkov K, Kirk PM, Kauserud H (2013) ITS1 versus ITS2 as DNA metabarcodes for fungi. *Molecular Ecology Resources*, **13**, 218–224.

Blok D, Weijers S, Welker JM, Cooper E, Michelsen A, Elberling B (2015) Deepened winter snow increases stem growth and alters stem? ^{13}C and ^{15}N in evergreen dwarf shrub *Cassiope tetragona* in high-arctic Svalbard tundra. *Ecological Research Letters*, **10**, 044008.

Bödeker ITM, Clemmensen KE, de Boer W, Martin F, Olson A, Lindahl BD (2014) Ectomycorrhizal *Cortinarius* species participate in enzymatic oxidation of humus in northern forest ecosystems. *New Phytologist*, **203**, 245–256.

Borner AP, Kielland K, Walker MD (2008) Effects of simulated climate change on plant phenology and nitrogen mineralization in Alaskan arctic tundra. *Arctic, Antarctic, and Alpine Research*, **40**, 27–38.

Boyle H, Zimdars B, Renker C, Buscot F (2006) A molecular phylogeny of *Hebeloma* species from Europe. *Mycological Research*, **110**, 369–380.

Brown SP, Callaham MA, Oliver AK, Jumpponen A (2013) Deep Ion Torrent sequencing identifies soil fungal community shifts after frequent prescribed fires in a southeastern US forest ecosystem. *FEMS Microbiology Ecology*, **86**, 557–566.

Buckeridge KM, Grogan P (2008) Deepened snow alters soil microbial nutrient limitations in arctic birch hummock tundra. *Applied Soil Ecology*, **39**, 210–222.

Butler MJ, Day AW (1998) Fungal melanins: a review. *Canadian Journal of Microbiology*, **44**, 1115–1136.

Butler MJ, Gardiner RB, Day AW (2005) Degradation of melanin or inhibition of its synthesis: Are these a significant approach as a biological control of phytopathogenic fungi? *Biological Control*, **32**, 326–336.

Callaghan TV, Björn LO, Chernov Y *et al.* (2005) Tundra and polar desert ecosystems. In: *ACIA. Arctic climate impacts assessment* (eds Symon C, Arris L, Heal B), pp. 243–345. Cambridge University Press, Cambridge, UK.

Christensen JH, Kumar KK, Aldrian E *et al.* (2013) Climate phenomena and their relevance for future regional climate change. In *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Stocker TF, Plattner QD, Tignor G-K *et al.*), pp. 1217–1311. Cambridge University Press, Cambridge, UK.

Clemmensen KE, Bahr A, Ovaskainen O *et al.* (2013) Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science*, **339**, 1615–1618.

Clemmensen KE, Finlay RD, Dahlberg A, Stenlid J, Wardle DA, Lindahl BD (2015) Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. *New Phytologist*, **205**, 1525–1536.

Coelho RRR, Sacramento DR, Linhares LF (1997) Amino sugars in fungal melanins and soil humic acids. *European Journal of Soil Science*, **48**, 425–429.

Collins M, Knutti R, Arblaster J *et al.* (2013) Long-term climate change: Projections, commitments and irreversibility. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Doschung J *et al.*), pp. 1029–1136. Cambridge University Press, Cambridge, UK.

Comiso JC, Hall DK (2014) Climate trends in the Arctic as observed from space. *Wiley Interdisciplinary Reviews Climate Change*, **3**, 389–409.

Davey M, Blaalid R, Vik U, Carlsen T, Kauserud H, Eidesen PB (2015) Primary succession of *Bistorta vivipara* (L.) Delabre (Polygonaceae) root associated fungi mirrors plant succession in two glacial chronosequences. *Environmental Microbiology*, **17**, 2777–2790.

DeMarco J, Mack MC, Bret-Harte MS (2011) The effects of snow, soil microenvironment, and soil organic matter quality on N availability in three Alaskan arctic plant communities. *Ecosystems*, **14**, 804–817.

Deslippe JR, Hartmann M, Mohn WW, Simard SW (2011) Long-term experimental manipulation of climate alters the ectomycorrhizal community of *Betula nana* in Arctic tundra. *Global Change Biology*, **17**, 1625–1636.

Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible assymetrical approach. *Ecological Monographs*, **67**, 345–366.

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, **26**, 2460–2461.

Ekblad A, Wallander H, Godbold DL *et al.* (2013) The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: role in carbon cycling. *Plant and Soil*, **366**, 1–27.

Elmendorf S, Henry GHR, Hollister RD *et al.* (2012) Plot-scale evidence of tundra vegetation change to recent summer climate warming. *Nature Climate Change*, **2**, 453–457.

Fernandez CW, Koide RT (2013) The function of melanin in the ectomycorrhizal fungus *Cenococcum geophilum* under water stress. *Fungal Ecology*, **6**, 479–486.

Fernandez CW, Koide RT (2014) Initial melanin and nitrogen concentrations control the decomposition of ectomycorrhizal fungal litter. *Soil Biology and Biochemistry*, **77**, 150–157.

Fleming LV (1984) Effects of soil trenching and coring on the formation of ectomycorrhizas on birch seedlings grown around mature trees. *New Phytologist*, **98**, 143–153.

Fransson PMA, Toljander YK, Baum C, Weih M (2013) Host plant — ectomycorrhizal fungus combination drives resource allocation in willow: evidence for complex species interaction from a simple experiment. *Ecoscience*, **20**, 112–121.

Gardes M, Dahlberg A (1996) Mycorrhizal diversity in arctic and alpine tundra: an open question. *New Phytologist*, **133**, 147–157.

Geml J, Laursen GA, Taylor DL (2008) Molecular diversity assessment of arctic and boreal *Agaricus* taxa. *Mycologia*, **100**, 577–589.

Geml J, Laursen GA, Timling I *et al.* (2009) Molecular phylogenetic biodiversity assessment of arctic and boreal ectomycorrhizal Lactarius Pers. (Russulales; Basidiomycota) in Alaska, based on soil and sporocarp DNA. *Molecular Ecology*, **18**, 2213–2227.

Geml J, Timling I, Robinson CH *et al.* (2012) An arctic community of symbiotic fungi assembled by long-distance dispersers: phylogenetic diversity of ectomycorrhizal basidiomycetes in Svalbard based on soil and sporocarp DNA. *Journal of Biogeography*, **39**, 74–88.

Geml J, Gravendeel B, Neilen M, Lammers Y, Raes N, Semenova TA, Noordeloos ME (2014a) DNA metabarcoding reveals high fungal diversity and pH-correlated habitat partitioning in protected coastal *Salix repens* communities in the Netherlands. *PLoS One*, **9**, e99852.

Geml J, Pastor N,, Fernandez L *et al.* (2014b) Large-scale fungal diversity assessment in the Andean Yungas forests reveals strong community turnover among forest types along an altitudinal gradient. *Molecular Ecology*, **23**, 2452–2472.

Geml J, Morgado LN, Semenova TA, Welker JM, Walker MD, Smets E (2015) Long-term warming alters richness and composition of taxonomic and functional groups of arctic fungi. *FEMS Microbiology Ecology*, **9**, 1–13.

Gihring TM, Green SJ, Schadt CW (2012) Massively parallel rRNA gene sequencing exacerbates the potential for biased community diversity comparisons due to variable library sizes. *Environ Microbiology*, **14**, 285–290.

Glen M, Tommerup IC, Bougher NL, O'Brien PA (2002) Are Sebacinaceae common and widespread ectomycorrhizal associates of *Eucalyptus* species in Australian forests? *Mycorrhiza*, **12**, 243–247.

Harley JL (1971) Fungi in ecosystems. *Journal of Applied Ecology*, **8**, 627–642.

Hartmann M, Niklaus PA, Zimmermann S *et al.* (2014) Resistance and resilience of the forest soil microbiome to logging-associated compaction. *The ISME Journal*, **8**, 226–244.

Henry GHR, Molau U (1997) Tundra plants and climate change: the International Tundra Experiment (ITEX). *Global Change Biology*, **3**, 1–9.

Higgins KL, Arnold AE, Miadlikowska J, Sarvate SD, Lutzoni F (2007) Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic endophytes from three major plant lineages. *Molecular Phylogentics and Evolution*, **42**, 543–555.

Hobbie EA (2006) Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. *Ecology*, **87**, 563–569.

Hobbie EA, Agerer R (2010) Nitrogen isotopes in ectomycorrhizal sporocarps correspond to belowground exploration types. *Plant and Soil*, **327**, 71–83.

Hobbie JE, Hobbie EA (2006) ^{15}N in symbiotic fungi and plants estimates nitrogen and carbon flux rates in Arctic tundra. *Journal of Ecology*, **87**, 816–822.

Hughes KW, Petersen RH, Lickey EB (2009) Using heterozygosity to estimate a percentage DNA sequence similarity for environmental species' delimitation across basidiomycete fungi. *New Phytologist*, **182**, 795–798.

Ihrmark K, Bodeker ITM, Cruz-Martinez K *et al.* (2012) New primers to amplify the fungal ITS2 region–evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology*, **82**, 666–677.

Jones DL, Kielland K (2012) Amino acid, peptide and protein mineralization dynamics in a taiga forest soil. *Soil Biology and Biochemistry*, **55**, 60–69.

Jones MH, Fahnestock JT, Walker DA, Walker MD, Welker JM (1998) Carbon dioxide fluxes in moist and dry arctic tundra during the snow-free season: responses to increases in summer temperature and winter snow accumulation. *Arctic and Alpine Research*, **30**, 373–380.

Kade A, Walker DA, Raynolds MK (2005) Plant communities and soils in cryoturbated tundra along a bioclimate gradient in the Low Arctic, Alaska. *Phytocoenologia*, **35**, 761–820.

Kattsov VM, Walsh JE (2000) Twentieth-century trends of Arctic precipitation from observational data and a climate model simulation. *Journal of Climate*, **13**, 1362–1370.

Kauserud H, Kumar S, Brysting AK, Nordén J, Carlsen T (2012) High consistency between replicate 454 pyrosequencing analyses of ectomycorrhizal plant root samples. *Mycorrhiza*, **22**, 309–315.

Kennedy PG, Hortal S, Bergemann SE, Bruns TD (2007) Competitive interactions among three ectomycorrhizal fungi and their relation to host plant performance. *Journal of Ecology*, **95**, 1338–1345.

Koide RT, Fernandez C, Malcolm G (2014) Determining place and process: functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. *New Phytologist*, **201**, 433–439.

Kõljalg U, Dahlberg A, Taylor AF *et al*. (2000) Diversity and abundance of resupinate thelephoroid fungi as ectomycorrhizal symbionts in Swedish boreal forests. *Molecular ecology*, **9**, 1985–1996.

Kõljalg U, Nilsson RH, Taylor AFS *et al*. (2013) Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology*, **22**, 5271–5277.

Leffler J, Welker JM (2013) Long-term increases in snow elevate leaf N and photosynthesis in *Salix arctica*: response to a snow fence experiment in NW Greenland. *Environmental Research Letters*, **8**, 025023.

Lilleskov EA, Hobbie EA, Horton TR (2011) Conservation of ectomycorrhizal fungi: exploring the linkages between functional and taxonomic responses to anthropogenic N deposition. *Fungal Ecology*, **4**, 174–183.

Lindahl BD, Nilsson RH, Tedersoo L *et al*. (2013) Fungal community analysis by high-throughput sequencing of amplified markers–a user's guide. *New Phytologist*, **199**, 288–299.

Liston GE, Mcfadden JP, Sturm M, Pielke RA (2002) Modelled changes in arctic tundra snow, energy and moisture fluxes due to increased shrubs. *Global Change Biology*, **8**, 17–32.

Lupascu M, Welker JM, Xu X, Czimczik CI (2014) Rates and radiocarbon content of summer ecosystem respiration in response to long-term deeper snow in the High Arctic of NW Greenland. *JGR Biogeoscience*, **119**, 1180–1194.

Ma D, Yang G, Mu L, Li C (2011) Tolerance of ectomycorrhizal fungus mycelium to low temperature and freezing-thawing. *Canadian Journal of Microbiology*, **57**, 328–332.

McCune B, Grace JB (2002) *Analysis of Ecological Communities*. MjM Software, Gleneden Beach, OR, USA.

Mercado-Díaz J (2011) Plant community responses of the Alaskan Arctic tundra to environmental and experimental changes in climate. MSc. Thesis. University of Puerto Rico, Rio Piedras Campus, PR.

Miller SL, Buyck B (2002) Molecular phylogeny of the genus *Russula* in Europe with a comparison of modern infrageneric classifications. *Mycological Research*, **106**, 259–276.

Morgado LN, Semenova TA, Welker JM, Walker MD, Smets E, Geml J (2015) Summer temperature increase has distinct effects on the ectomycorrhizal fungal communities of moist tussock and dry tundra in Arctic Alaska. *Global Change Biology*, **21**, 959–972.

Natali SM, Schuur EAG, Rubin RL (2012) Increased plant productivity in Alaskan tundra as a result of experimental warming of soil and permafrost. *Journal of Ecology*, **100**, 488–498.

Natali SM, Schuur EAG, Webb EE, Pries CEH, Crummer KG (2014) Permafrost degradation stimulates carbon loss from experimentally warmed tundra. *Ecology*, **95**, 602–608.

Nilsson RH, Abarenkov K, Larsson K-H, Kõljalg U (2011) Molecular identification of fungi: rationale, philosophical concerns, and the UNITE database. *The Open Applied Informatics Journal*, **5**, 81–86.

Nygren CMR, Eberhardt U, Karlsson M, Parrent JL, Lindahl BD, Taylor AFS (2008) Growth on nitrate and occurrence of nitrate reductase-encoding genes in a phylogenetically diverse range of ectomycorrhizal fungi. *New Phytologist*, **180**, 875–889.

O'Brien H, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R (2005) Fungal community analysis by large-scale sequencing of environmental samples. *Applied and Environmental Microbiology*, **71**, 5544–5550.

Oksanen J, Blanchet FG, Kindt R *et al*. (2012) *Vegan: Community Ecology Package*. R package version 2.0-3. Available at: http://CRAN.R-project.org/package=vegan (accessed 4 November 2014).

Oliveros JC (2007) VENNY. An interactive tool for comparing lists with venn diagrams. Available at: http://bioinfogp.cnb.csic.es/tools/venny/index.html (accessed 27 November 2014).

Pattison RR, Welker JM (2014) Differential ecophysiological response of deciduous shrubs and a graminoid to long-term experimental snow reduction and addition in moist tundra, Northern Alaska. *Oecologia*, **174**, 339–350.

Rast DM, Hollenstein GO (1977) Architecture of the *Agaricus bisporus* spore wall. *Canadian Journal of Botany*, **55**, 2251–2262.

Read DJ, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? *New Phytologist*, **157**, 475–492.

Read DJ, Leake JR, Perez-Moreno J (2004) Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Canadian Journal of Botany*, **83**, 1243–1263.

Robinson CH (2001) Cold adaptation in Arctic and Antarctic fungi. *New Phytologist*, **151**, 341–353.

Rogers MC, Sullivan PF, Welker JM (2011) Evidence of nonlinearity in the response of net ecosystem CO2 exchange to increasing levels of winter snow depth in the High Arctic of Northwest Greenland. *Arctic, Antarctic and Alpine Research*, **43**, 95–106.

Ryberg M, Larsson E, Molau U (2009) Ectomycorrhizal diversity on *Dryas octopetala* and *Salix reticulata* in an alpine cliff ecosystem. *Arctic, Antarctic and Alpine Research*, **41**, 506–514.

Schimel JP, Bennet J (2004) Nitrogen mineralization: challenges of a changing paradigm. *Journal of Ecology*, **85**, 591–602.

Schimel JP, Bilbrough C, Welker JM (2004) Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. *Soil Biology and Biochemistry*, **36**, 217–227.

Schloss PD, Westcott SL, Ryabin T *et al*. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied Environmental Microbiology*, **75**, 7537–7541.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 6241–6246.

Screen JA, Simmonds I (2012) Declining summer snowfall in the Arctic: causes, impacts and feedbacks. *Climate Dynamics*, **38**, 2243–2256.

Semenova TA, Morgado LN, Welker JM, Walker MD, Smets E, Geml J (2015) Long-term experimental warming alters community composition of ascomycetes in Alaskan moist and dry arctic tundra. *Molecular Ecology*, **24**, 424–437.

Sharp E, Sullivan P, Steltzer H, Csank A, Welker JM (2013) Complex carbon cycling responses to multi-level warming and supplemental summer rain in a High Arctic ecosystem. *Global Change Biology*, **19**, 1780–1792.

Simard SW, Beiler KJ, Bingham MA, Deslippe JR, Philip LJ, Teste FP (2012) Mycorrhizal networks: mechanisms, ecology and modelling. *Fungal Biology Reviews*, **26**, 39–60.

Singaravelan N, Grishkan I, Beharav A, Wakamatsu K, Ito S, Nevo E (2008) Adaptive melanin response of the soil fungus *Aspergillus niger* to UV radiation stress at "Evolution Canyon", Mount Carmel, Israel. *PLoS One*, **3**, e2993.

Stroeve JC, Markus T, Boisvert L, Miller J, Barrett A (2014) Changes in Arctic melt season and implications for sea ice loss. *Geophysical Research Letters*, **41**, 1216–1225.

Sturm M, Schimel J, Michaelson G *et al*. (2005) Winter biological processes could help convert arctic tundra to Shrubland. *BioScience*, **55**, 17–26.

Sullivan PF, Arens SJT, Chimner RA, Welker JM (2008a) Temperature and microtopography interact to control carbon cycling in a high arctic fen. *Ecosystems*, **11**, 61–76.

Sullivan PF, Welker JM, Steltzer H, Sletten R, Hagedorn B, Arens SJT, Horwath JL (2008b) Energy and water additions give rise to simple responses in plant canopy and soil microclimates of a high arctic ecosystem. *Journal of Geophysical Research*, **113**, G03S08.

Talbot JM, Treseder KK (2010) Controls over mycorrhizal uptake of organic nitrogen. *Pedobiologia*, **53**, 169–179.

Talbot JM, Bruns TD, Smith DP *et al*. (2013) Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. *Soil Biology and Biochemistry*, **57**, 282–291.

Tape KD, Hallinger M, Welker JM, Ruess RW (2012) Landscape heterogeneity of shrub expansion in Arctic Alaska. *Ecosystems*, **15**, 711–724.

Tedersoo L, Smith ME (2013) Lineages of ectomycorrhizal fungi revisited: foraging strategies and novel lineages revealed by sequences from belowground. *Fungal Biology Reviews*, **27**, 83–99.

Tedersoo L, May TW, Smith ME (2010) Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. *Mycorrhiza*, **20**, 217–263.

Tedersoo L, Naadel T, Bahram M *et al*. (2012) Enzymatic activities and stable isotope patterns of ectomycorrhizal fungi in relation to phylogeny and exploration types in an afrotropical rain forest. *New Phytologist*, **195**, 832–843.

Timling I, Taylor DL (2012) Peeking through a frosty window: molecular insights into the ecology of Arctic soil fungi. *Fungal Ecology*, **5**, 419–429.

Timling I, Dahlberg A, Walker DA, Gardes M, Charcosset J-Y, Welker JM, Taylor DL (2012) Distribution and drivers of ectomycorrhizal fungal communities across the North American Arctic. *Ecosphere*, **3**, 111.

Treseder KK, Lennon JT (2015) Fungal traits that drive ecosystem dynamics on land. *Microbiology and Molecular Biology Reviews*, **79**, 1–15.

Urban A, Weib M, Bauer R (2003) Ectomycorrhizas involving sebacinoid mycobionts. *Mycological Research*, **107**, 3–14.

Wahren C-HA, Walker MD, Bret-Harte MS (2005) Vegetation responses in Alaskan arctic tundra after 8 years of a summer warming and winter snow manipulation experiment. *Global Change Biology*, **11**, 537–552.

Walker MD, Walker DA, Welker JM *et al.* (1999) Long-term experimental manipulation of winter snow regime and summer temperature in arctic and alpine tundra. *Hydrological Processes*, **13**, 2315–2330.

Walker DA, Raynolds MK, Daniëls FJA *et al.* (2005) The Circumpolar Arctic vegetation map. *Journal of Vegetation Science*, **16**, 267–282.

Walker DA, Epstein HE, Welker JM (2008) Introduction to the special section: biocomplexity in Arctic terrestrial environments. *Journal of Geophysical Research*, **113**, G03S14.

Wallander H, Nilsson LO, Hagerberg D, Bååth E (2001) Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. *New Phytologist*, **151**, 753–760.

Welker JM, Molau U, Parsons AN, Robinson CH, Wookey PA (1997) Response of Dryas *octopetala* to ITEX manipulations: a synthesis with circumpolar comparisons. *Global Change Biology*, **3**, 61–73.

Welker JM, Fahnestock JT, Jones MH (2000) Annual CO2 flux from dry and moist arctic tundra: field responses to increases in summer temperature and winter snow depth. *Climatic Change*, **44**, 139–150.

Welker JM, Fahnestock JT, Sullivan PF, Chimner RA (2005) Leaf mineral nutrition of arctic plants in response to long-term warming and deeper snow in northern Alaska. *Oikos*, **109**, 167–177.

White TM, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications* (eds Innis MA, Gelfand DH, Sninsky JJ, White TJ), pp. 315–321. Academic Press, San Diego, USA.

Wild B, Schnecker J, Bárta J, Čapek P, Guggenberger G, Hofhansel F (2013) Nitrogen dynamics in turbic cryosols from Siberia and Greenland. *Soil Biology and Biochemistry*, **67**, 85–93.

Wipf S, Rixen C (2010) A review of snow manipulation experiments in Arctic and alpine tundra ecosystems. *Polar Research*, **29**, 95–109.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Fasta file with the OTU sequences used in the paper.
Data S2. OTU classification based on the curated database published by Kõljalg *et al.* (2013) with read abundance *per* plot.
Data S3. Total ECM fungal sequence counts, classified by taxonomic and functional traits, comparing ambient snow with increased snow depth plots.
Data S4. Nonmetric multidimensional scaling (NMDS) ordination plots of ECM basidiomycetes communities based on OTU presence–absence data matrix.
Data S5. Average OTU richness per hyphal exploration groups in dry and moist tundra types of the communities with ambient and increased snow depth and Student's *t*-test comparison.
Data S6. Average sequence abundance per treatment and tundra type combination with standard deviation and Student's *t*-test comparison.
Data S7. Paired correlation of OTU richness and sequence abundance (for 5 replicates) between the genera with higher richness, and between grouped hyphal exploration types (ET).