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Compositional and functional shifts in arctic fungal communities in response to experimentally increased snow depth

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ABSTRACT

Climate warming leads to more intensive evaporation from the Arctic sea resulting in increased precipitation in the low Arctic, e.g., higher snowfall during winter. Deeper snow keeps the arctic soils warmer and alters soil attributes and vegetation, e.g., increase in nitrogen availability, expansion of shrubs and decline in shade-intolerant lichens and bryophytes. Changes in soil properties and vegetation are expected to influence on saprotrophic and plant-symbiotic fungi, but how increased snow depth affects their community composition remain unknown. In the present work, we used DNA metabarcoding to study the effects of long-term experimental manipulations of snow depth on soil fungal communities in dry heath and moist tussock tundra in Arctic Alaska. We report strong changes in fungal community compositions in the two tundra types, with pronounced declines observed in the majority of fungal functional guilds, including ectomycorrhizal, lichenized, plant pathogenic, saprotrophic and bryophyte-associated species. The observed changes in lichenized and bryophyte-associated fungi are in agreement with previously published above-ground changes, i.e. decrease of lichen and bryophyte cover and diversity. However, the majority of observed trends, including the decline of ectomycorrhizal fungi (that were anticipated to benefit from the expansion of their host plants), suggest that changes in fungal communities do not entirely correspond to and are not primarily driven by shifts in vegetation. Instead, arctic fungal communities appear to exhibit faster turnover that may be influenced by dynamic interactions with numerous biotic and abiotic factors, e.g., soil nutrient cycling and community dynamics in other groups of soil microorganisms. We highlight the importance of "below-ground studies" in assessing ecosystem responses to climatic changes, because faster turnover of microbial communities may be applicable for monitoring early-stage alterations caused by climatic changes.

1. Introduction

Arctic ecosystems are among the biomes with the greatest rate of climate warming (IPCC, 2007; Kaufman et al., 2009; Serreze and Barry, 2011; Bintaja and Selten, 2014). Annual temperature increases reported over the last three decades for the arctic region approached 0.1 °C (Anisimov et al., 2007), which is considerably higher than the the global average value of 0.017 °C (Comiso and Hall, 2014). Rising temperatures intensify local surface evaporation of the Arctic sea, as well as enhance moisture inflow from lower latitudes (Bintaja and Selten, 2014), leading to increased precipitation in the Arctic region (Kattsov and Walsh, 2000; Stocker et al., 2013; Bintaja and Selten, 2014). According to models, arctic precipitation may increase more than 30 to 50 percent by the end of twenty-first century (Stocker et al., 2013; Bintaja and Selten, 2014). In the arctic tundra, where winter can be up to 9 months long and winter temperatures are well below the freezing point, most of the precipitation falls as snow, resulting in deeper snow cover in winter (Derksen et al., 2015). Increased snow depth is expected based on forecasted precipitation increases (Bintaja and Selten, 2014) and

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due to expansion of shrubs that increase snow depth by 10–25% due to increased wind shelter, i.e. snow trapping effect (Larsen et al., 2007; Sturm et al., 2001).

Deeper snow, in turn, insulates soils and prevents it from becoming excessively cold, which has consequences for soil processes and vegetation during winter and the subsequent summers. (Jones et al., 1998; Schimel et al., 2004; Screen and Simmonds, 2012; Collins et al., 2013). These consequences include alterations in microbial N and C mineralization (Schimel et al., 2004; Sturm et al., 2005; Welker et al., 2005), increases in plant leaf N (Welker et al., 2005), greater C fixation (Pattison and Welker, 2014), shifts in nutrient cycling and changes in plant community composition (Sweet et al., 2014). For instance, in the moist tussock tundra of Arctic Alaska, experimental snow addition (ca 1–1.2 m) resulted in significant increases in 1) soil temperatures from December to April and greater monthly thaw depth (Natali et al., 2011), 2) soil moisture likely resulting from the surface subsidence (Natali et al., 2012), 3) ecosystem respiration during the growing season and a 2-fold increase in annual CO_2 loss to the atmosphere, 4) NH_4^+ levels in soils through the fall and winter (Schimel et al., 2004), 5) foliar and litter N mass (Natali et al., 2012), 6) mean N availability (Pattison and Welker, 2014), 7) overall plant growth and canopy height, 8) cover of deciduous shrubs *Betula nana* and *Salix pulchra* 9) cover of a graminoid *Eriophorum vaginatum*, and in 10) accumulation of litter (Mercado-Diaz, 2011). Simultaneously, the cover of shade-intolerant lichens and bryophytes has decreased (Wahren et al., 2005; Wipf and Rixen, 2010; Mercado-Diaz, 2011; Loranty and Goetz, 2012; Pattison and Welker, 2014). In the adjoining dry heath tundra, deeper snow resulted in 1) a nearly 4-fold increase in winter NH_4^+ levels, 2) increased cover of shrubs *Dryas octopetala*, *Arctostaphylus alpina*, *Vaccinium vitis-idea*, *Loiseleuria procumbens*, 3) decline of lichens, and in 4) increased accumulation of litter (Schimel et al., 2004; Fahnestock et al., 2000; Wahren et al., 2005; Mercado-Diaz, 2011).

Alterations in the arctic plant communities are generally expected to be coupled with the shifts in soil fungal communities (Dahlberg and Bültman, 2013), especially, in mycorrhizal and root-associated fungi, due to tight associations between fungi and plants in nutrient-poor tundra soils (Hobbie and Hobbie, 2006; Buckeridge and Grogan, 2008; Hobbie et al., 2009). Such a correspondence between changes in vegetation coverage, soil temperature and fungal community composition has been observed in summer warming experiments in the Arctic (Deslippe et al., 2011; Geml et al., 2015; Morgado et al., 2015; Semenova et al., 2015). In Alaska, an 18-year summer warming experiment (+2 °C, ca 2 months per year) resulted in reorganization of soil fungal communities, particularly in the moist tussock tundra: ectomycorrhizal basidiomycetes declined in richness and their community composition shifted according to their functional traits (e.g., mycelial characteristics) (Morgado et al., 2015), while the richness of ascomycetes did not change, and their communities shifted in accordance with the availability of hosts/-substrates for different ecological groups (Semenova et al., 2015).

Besides preventing soils from cooling fast (i.e. keeping them warm), deeper snow protects the soils and vegetation from the wind and frost disturbances (Blok et al., 2015), alters soil moisture, and shortens the vegetation season due to the delayed snowmelt (Wahren et al., 2005; Blok et al., 2015). The collective effects of deeper snow on arctic soil fungal communities are unknown, although there is some evidence that deeper snow increases the potential of pathogenic fungi to cause disease outbreaks in arctic and alpine ecosystems (Olofson et al., 2011; Natali and Mack, 2011; Barbeito et al., 2013).

In this study we investigated the responses of soil fungi to increased snow depth in arctic tundra of Northern Alaska. We compared fungal community compositions across the plots with ambient and experimentally increased snow depth in two main tundra types found throughout the region: dry heath and moist tussock tundra. By analyzing these four experimental treatments, we aimed to answer 1) how richness and community composition of fungi change in response to long-term increase in snow depth; 2) whether these responses are similar in dry and moist tundra; 3) how taxonomic and ecological groups of fungi alter in response to long-term winter warming.

2. Materials and methods

2.1. Study sites

The experimental sites were located at Toolik Lake area, situated at the northern foothills of the Brooks Range, Alaska (68°38′N, 149°34′W). Two main vegetation types are found throughout the region: dry heath and moist tussock tundra. The dry site is represented by the dwarf-shrub and fruticose-lichen tundra, and is characterized by *Dryas octopetala*, *Salix polaris* and *Vaccinium* spp. The moist site vegetation is tussock-sedge dwarf-shrub tundra, characterized by *Betula nana*, *Salix pulchra* and *Eriophorum vaginatum* (Walker et al., 1999). The detailed description of the both sites could be found at *arcticatlas.org* and in Walker et al. (1999) and Kade et al. (2005). The annual precipitation in the region ranges from 200 mm to 400 mm, and ca. 50% of the precipitation falls as snow. The average snow depth approaches ca. 50 cm (DeMarco et al., 2011). As a part of the International Tundra Experiment (ITEX) program initiated in 1994 (Henry and Molau, 1997; Welker et al., 1997, 2000; Jones et al., 1998), the snow fence experiments were established in both dry and moist tundra. Snow fences are wooden fences, 2.8 m tall and 60 m long, and they are set on east-west axes to accumulate snow (Fig. 1a) brought over by the predominant winds and storms blown from the Brooks Range to the south (Walker et al., 1999). Fences create a leeward drift of approximately 60 m long, with three zones: a deep zone with the snow depth of 2–3 m, moderate zone (0.5–2 m snow depth) and a shallow zone (<0.5 m snow depth) (Fig. 1a) (Walker et al., 1999; Pattison and Welker, 2014). Snow accumulation behind the fence causes more consistent and largely higher winter soil temperatures; for instance, at the 2 cm depth, the average soil temperatures in snow fence treatment approached −2.9 °C versus −4.7 °C in the control soils (Pattison and Welker, 2014). The lowest soil temperatures reported for the snow fence treatment were ca −7 °C versus ca. −35 °C observed across the control plots (Walker et al., 1999; Schimel et al., 2004). A suite of physical alterations in the ecosystem caused by the snow fence treatment led to significant shifts in the plant communities, described in details in Walker et al. (1999), Wahren et al. (2005), Welker et al. (2005), Mercado-Diaz (2011) and Pattison and Welker (2014). Because these studies indicated the strongest responses of the plant communities in the zone where the snow was ca 1–1.5 m deep, we sampled soils from that particular zone (Fig. 1a).

2.2. Soil sampling

In July 2012, we collected soils from moist and dry tundra, from the zones where the snow depth was ambient (control, i.e. < 0.5 m) and experimentally increased to ca 1–1.5 m (snow fence treatment). Five experimental replicates were taken from each of the four experimental treatments, i.e. dry and moist tundra types, ambient and increased snow depth (Fig. 1b). Each of the experimental replicates was a composite sample of five soil cores taken from the area of ca 1 m^2 by the soil corer of ca 2 cm in diameter and ca. 20 cm deep (Fig. 1b). In total, we sampled 100 soil cores and

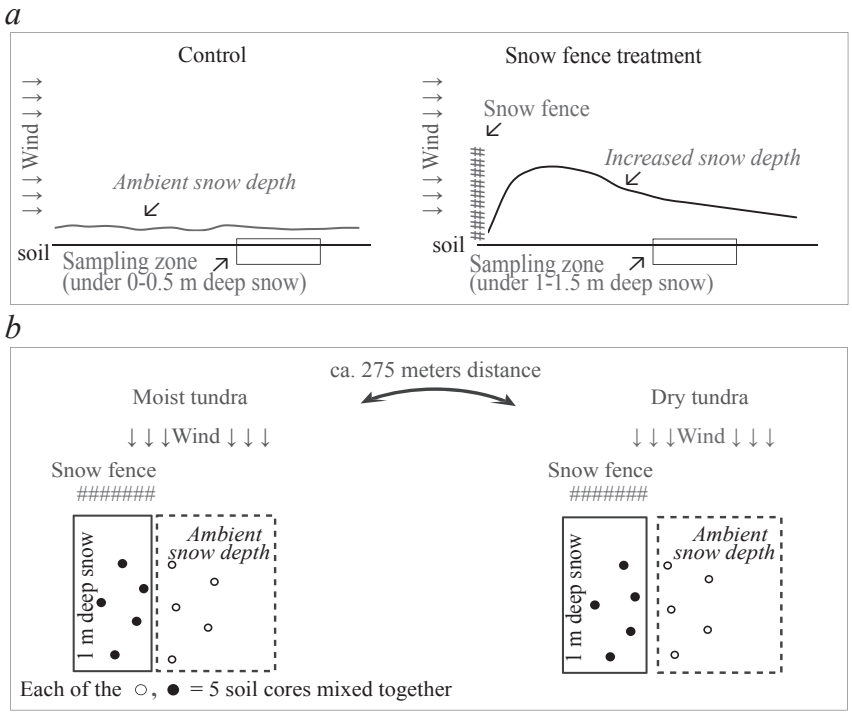

Fig. 1. Experimental setup. **(a)** A methodological set up to experimentally increase the snow depth by a snow fence. In ambient (control) conditions (left part of the figure) snow depth does not exceed 0.5 m. The snow fence (right part of the figure) leads to snow accumulation behind the fence by creating a leeward drift with a snow depth of ca. 2 m (deep zone), 1.5–1 m (medium zone) and 0.5 m (shallow zone). In dry and moist tundra types, soils were sampled from 1) control plots and 2) medium zone snow fence plots, where the snow was ca 1–1.5 m deep. **(b)** Location of the sampling plots in dry and moist tundra types. In each of the tundra types we sampled 5 plots in control conditions (shown as ○) and 5 plots in experimental treatment (1–1.5 m deep snow, shown with ●). Each of the 20 plots was a composite sample of five soil cores mixed together.

combined them in 20 samples. Coarse particles (litter, aboveground parts of the plants etc.) were removed from the samples, although some fine particles (e.g. plant roots) could still be present in the sample. Samples were kept frozen until lyophilisation was done, within 24 h of soil sample collection.

2.3. Molecular work

Prior to downstream applications the lyophilized soil samples were thoroughly mixed. DNA extraction was carried out using Macherey-Nagel NucleoSpin Soil kit (Macherey-Nagel Gmbh and Co., Düren, Germany), using SL2 lysis buffer. The volume of elution buffer was set to 30 μl. For each of the twenty samples the DNA extraction was carried out twice resulting in ca. 0.4–1 g of the soil used for DNA extraction per sample. (ca. 0.2–0.5 g of the soil could be processed in one extraction).

The forward primer fITS7-trP1 (Ihrmark et al., 2012) and reverse sample-specific primer ITS4 (White et al., 1990) were used to amplify the ITS2 r DNA region of ca. 250 bp. The ITS4 primer was labelled with sample-specific Multiplex Identification DNA-tags. For each of the 20 samples, the following PCR protocol was used for three positive and a one negative reactions: one cycle of 95 °C for 5 min, then 25 cycles of 95 °C for 20 s, 56 °C for 30 s, and 72 °C for 1.5 min, ending with one cycle of 72 °C for 7 min. Negative PCR reactions were made for each primer pair and contained elution buffer instead of DNA. PCR products were checked for DNA concentrations using QIAxcel Advanced System (QIAGEN). Emulsion PCR and Ion Torrent sequencing was carried out at the Naturalis Biodiversity Center. We used the sequencing Ion 318™ Chip to allow for highest possible sequencing coverage. Ion Torrent sequencing resulted in 3 960 925 reads with the median sequence length of 268 bp.

2.4. Bioinformatic analyses

The initial clean-up of the raw sequence data was carried out using the online platform Galaxy (https://main.g2.bx.psu.edu/root), in which the sequences were sorted according to samples, and adapters (identification tags) were removed. The poor-quality ends were trimmed off based on 0.02 error probability limit in Geneious Pro 5.6.1 (BioMatters, New Zealand). Subsequently, sequences were filtered using USEARCH v.8.0 (Edgar, 2010) based on the following settings: all sequences were truncated to 200 bp and sequences with expected error > 1 were discarded. The resulting 1,971 748 high-quality sequences were grouped into 5169 operational taxonomic units (OTUs) by UPARSE algorithm in USEARCH at 97% sequence similarity, following other fungal metabarcoding studies (e.g., Bjorbækmo et al., 2010; Geml et al., 2010; Bellemain et al., 2013; Tedersoo et al., 2014), while simultaneously excluding 14 067 putative chimeric sequences. We assigned sequences to taxonomic groups based on pairwise similarity searches against the curated UNITE fungal ITS sequence database containing identified fungal sequences with assignments to Species Hypothesis groups (Nilsson et al., 2011; Kõljalg et al., 2013). After discarding global singletons and OTUs that did not have at least 80% similarity to any fungal sequence in UNITE, the final dataset contained 3550 OTUs.

The ecological functions for the OTUs were determined using FUNGuild software (Nguyen et al., 2015) and the dataset of Tedersoo et al. (2014), resulting in 1174 OTUs with identified functional guilds. For the same OTUs we independently assigned ecological functions based on the isolation source for the closest reference sequences in UNITE. The two approaches resulted in largely similar (ca. 81%) datasets. The inconsistence was observed in fungal genera that have been generally considered saprotrophic but were isolated from surface-sterilized or ectomycorrhizal roots.

For example, species of *Penicillium* – *Penicillium swiecickii* SH279517.07FU (Min et al., 2014), *P spinulosum* SH207148.07FU (Summerbell, 1989), *P neocrassum* SH107619.07FU, *P soppii* SH199403.07FU (Summerbell, 2005), *P angulare* SH182512.07FU, *P thomii* SH407691.07FU (Ghen et al., 2007) were assigned to root-associated or endophytic fungi. Root-associated lifestyle was assigned to *Mycena metata* SH220724.07FU (Toju et al., 2013) and *M. cinerella* SH220744.07FU. A guild of dark septated endophytes involved *Meliniomyces variabilis* SH181078.07FU, *Cadophora finlandica* SH214265.07FU, *Leptodontidium* sp SH205736.07FU, *Phialocephala fortinii* SH204986.07FU and *Acephala* sp SH020838.07FU (Addy et al., 2005; Newsham, 2011). The ecological assignment based on the isolation source for the reference sequence (Species Hypothesis in UNITE) was included in the analysis, to further distinguish saprotrophic and root-associated fungal types based on their ecological function.

Raw sequencing data was deposited to DRYAD, http://dx.doi.org/10.5061/dryad.cq2rb. Fungal OTUs were deposited in Genbank, accession numbers KX401620–KX404870.

2.5. Statistical analyses

Depth of sequencing coverage was quantified by rarefaction curve and coverage estimators. Rarefaction analysis, Good's coverage, Shannon's (H) and Simpson's diversity indexes, OTU richness (S) and evenness (H/ln S) was carried out/calculated using "rarefy" function in Vegan package (Oksanen et al., 2012) in R software for statistical computing (R Core team, 2013). The rarefaction curves reached plateau suggesting that we sequenced almost all fungal species in the sampled plots (Fig S1a). High Good's coverage estimators (98.9 ± 0.2%) indicated equally deep OTU recovery across the treatments (Fig S1b). There was no significant difference in richness, evenness, and diversity estimators across the treatments (Fig S1b).

The effect of increased snow depth on fungal community composition was estimated using PC-ORD v. 5.32 (McCune and Grace, 2002) separately for dry and moist tundra, in order to eliminate all the factors unrelated to the treatment but contributing to the variation in fungal community compositions. The presence-absence data (for abundance-based data see Supplementary material for the paper, Fig S2) was used to estimate shifts in richness of fungal taxa and ecological groups by non-metric multidimensional scaling (NMDS) in PC-ORD. Following recommendations of Lindahl et al. (2013), presence was set as >4 sequences on a per sample basis. The primary matrix contained of experimental plots by OTUs (i.e. fungal community composition). The secondary matrix contained of plots by richness of fungal taxa (i.e. number of OTUs belonging to specific taxa) and richness in ecological groups containing at least 8 OTUs per group in interest. For the moist tundra, this final dataset contained of 1363 fungal OTUs, and for dry tundra – of 1328 OTUs. The dataset was subjected to 500 iterations per run using the Sørensen similarity (Bray-Curtis index) and a random starting number. The resulting NMDS solution with the lowest final stress was rotated to maximize the correlation between the snow fence treatment and the axis 1 (i.e. left part of the axis indicated ambient snow depth and right part – increased snow depth). Although, Pearson's correlation coefficient $r^2 > 0.2$ could be used as indication of correlation (as in McCune and Grace (2002), we present the results for $|r| > 0.5$ that are indicated by PC-ORD as strong correlations and that are important for characterizing shifts in fungal communities (Rogers et al., 2011). To test whether fungal community compositions in ambient and deep snow zones plots were statistically different, we used a multi-response permutation procedure (MRPP) and permutation-based nonparametric MANOVA (Anderson, 2001), also in PC-ORD. In addition, this software was used to examine if specific fungal OTUs were characteristic for any of the experimental treatments using indicator species analysis (Dufrêne and Legendre, 1997).

To test for statistical difference in read counts between the control and treatment, we carried out a two-group analysis in Comprehensive Meta-Analysis software (Smith, 2014) on a per-OTU basis. Using sequence read abundance as proxy for fungal biomass is constrained due to interspecific differences in copy numbers and length of ITS regions as well as other species-specific biological factors. However, for a particular OTU, changes in sequence counts in different samples can be considered indicative of relative changes and trends with respect to abundance (or biomass) (Amend et al., 2010). The input matrix contained the mean read abundance, standard deviation and sample size (5 replicates) across the control and deep snow plots. For each OTU we compared the mean read counts across the ambient and deep snow plots to calculate size effect and 95% confidence range. The analyses were run for 10 functional groups containing 10 OTUs at very least, including animal pathogenic, bryophyte-associated, dark septated endophytes, endolichenic, lichenized, mycoparasitic, root-associated, ectomycorrhizal, plant pathogenic and saprotrophic fungi. In addition, we run an analysis for the overall fungal community. This approach allowed us to depict the variation in responses of individual OTUs to increased snow depth as well as to evaluate the overall response of the functional groups.

3. Results

3.1. Increased snow depth alters fungal community composition

Tests of community differences revealed that fungal assemblages changed significantly under the deeper snow in both tundra types. For the moist tundra, NMDS analysis resulted in a two-dimensional solution with a final stress of 6.53 and final instability <0.00001 (Fig. 2). Correlation coefficients for ordination axes were axis 1: $r^2 = 0.489$ and axis 2: $r^2 = 0.399$. Strong effect of deep snow on fungal community compositions was revealed by both MRPP ($P = 0.0027$; $A = 0.076$) and MANOVA ($P = 0.0074$; $F = 2.56$) analyses. According to the MANOVA, snow fence treatment explained 23.7% of the variation in fungal community compositions. The effect of increased snow depth on richness in fungal taxa and ecological groups is presented in Tables 1 and 2. We observed a decline in seventeen fungal taxa and functional groups including bryophyte-associated, dark septated endophytic, ectomycorrhizal, lichenized, plant pathogenic and saprotrophic fungi, although no taxonomic or ecological groups increased in richness under deep snow.

In the dry tundra, NMDS resulted in a two-dimensional solution with a final stress of 5.78 and final instability <0.00001. Correlation coefficients for ordination axes were: axes 1: $r^2 = 0.294$, axis 2: $r^2 = 0.628$, with orthogonality 81.8%. Significant changes in fungal community compositions were shown by both MRPP ($P = 0.015$; $A = 0.052$) and MANOVA ($P = 0.022$; $F = 1.90$) analyses. Snow fence treatment explained 15.2% of the variation in fungal community composition, as revealed by MANOVA statistics. Shifts in richness of fungal taxa and ecological groups are presented in Tables 1 and 2. Increased snow depth resulted in declines in four fungal orders (Capnodiales, Lecanorales, Peltigerales and Thelephorales), although three taxa increased in richness across the treatment plots (Archaeorrhizomycetales, Venturiales and *Clavaria*). Among the ecological groups, ectomycorrhizal and lichenized species declined, and root-associated increased in richness (Table 2).

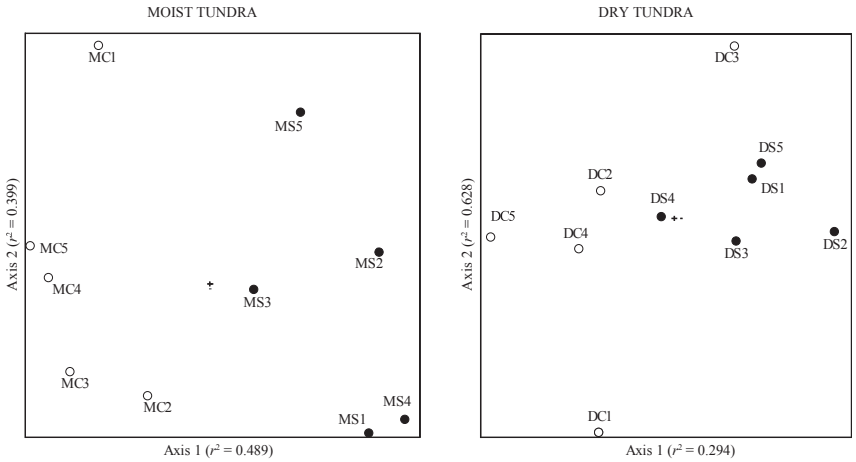

Fig. 2. The effect of increased snow depth on fungal community compositions in moist and dry tundra. D – dry tundra, M – moist tundra, S – snow fence treatment, C – ambient snow depth. The communities of the plots with ambient and increased snow depth are shown with ○ and ●, respectively.

Table 1
Effect of increased snow depth on richness of fungal taxonomic groups in dry and moist tundra, as revealed by PC-ORD. Correlation values are shown for fungal taxonomic groups that correlated with ordination axes at $|r| > 0.5$. Negative and positive values indicate higher richness of the group in interest across the control and treatment plots, respectively.

Taxonomic group	No of OTUs	Correlation
Moist Tundra		
Agaricales	132	$r = -0.758$
Chaetothyriales	104	$r = -0.755$
Helotiales	241	$r = -0.781$
Hypocreales	26	$r = -0.644$
Lecanorales	14	$r = -0.667$
Pezizales	15	$r = -0.824$
Pleosporales	28	$r = -0.704$
Russulales	17	$r = -0.504$
Sebacinales	86	$r = -0.558$
Sporidiobolales	14	$r = -0.712$
Thelephorales	32	$r = -0.704$
Trechisporales	9	$r = -0.535$
Tremellales	39	$r = -0.732$
Clavaria	8	$r = -0.844$
Inocybe	10	$r = -0.710$
Cryptococcus	25	$r = -0.631$
Mycena	10	$r = -0.575$
Dry Tundra		
Capnodiales	48	$r = -0.492$
Lecanorales	54	$r = -0.724$
Peltigerales	11	$r = -0.642$
Thelephorales	28	$r = -0.887$
Archaeorhizomycetales	22	$r = 0.716$
Clavaria	15	$r = 0.581$
Venturiales	8	$r = 0.654$

3.2. Indicator species analysis

In moist tundra, we observed 30 OTUs that were indicators (all $P < 0.05$) for either control or snow fence treatment. The complete list of indicator OTUs, their distribution across the sampling plots, taxonomic affinities, putative ecological functions and *P*-values is presented in Table S1. Among the 21 OTUs that were indicators of the control habitats, we observed 11 ascomycete and 7 basidiomycete species, with ectomycorrhizal, root-associated or saprotrophic lifestyles. For example, we observed the root-associated Sebaciales (SH201961.07FU and SH083523.07FU), dark septate endophytic *Phialocephala* sp (SH218122.07FU) and ectomycorrhizal *Inocybe leiocephala* (SH219800.07FU), as indicators of control conditions. Increased snow depth was favourable for 9 OTUs only. Among those that were identified to genus, we observed the saprotrophic *Capronia* (SH180465.07FU) and the ectomycorrhizal *Tomentella lapida* (SH189354.07FU).

In dry tundra, the indicator species analysis revealed an opposite trend: there were many more OTUs characteristic for the snow fence treatment (16 OTUs) compared to the control conditions (5 OTUs). The observed tendency was possibly due to increased moisture in the snow fence treatment plots, as moisture limits the growth of many fungal species in dry tundra (Jones et al., 1998). There was no clear pattern for any of the ecological groups, although we anticipated more lichenized fungi to be indicators of the control conditions. For example, the lichenized *Pertusaria* sp (SH206383.07FU) and the plant pathogenic *Ilyonectria mors-panacis* (SH202967.07FU) were characteristic of the control plots, while the saprotrophic *Guehomyces pullulans* (SH212824.07FU) and *Phaeomoniella* sp (SH015552.07FU) and the root-associated *Archaeorhizomyces* (SH004487.07FU) were indicators of the deep snow plots.

3.3. Snow fence treatment alters abundance of fungal ecological groups

Comprehensive meta-analysis revealed significant changes in abundance of several fungal ecological groups (Table 2, Fig. 3). In moist tundra, we observed a decline in bryophyte-associated ($P = 0.006$), ectomycorrhizal ($P < 0.001$), lichenized ($P < 0.001$) and saprotrophic ($P = 0.002$) species, as well as a decline in total fungal abundance ($P < 0.001$) under the deeper snow. These results were largely concordant with the trends observed for the richness: both analyses showed a decline in bryophyte-associated, ectomycorrhizal and lichenized fungi. We did not observe conflicting patterns in richness and abundance in any of the groups tested.

In dry tundra, snow fence treatment resulted in increase of ectomycorrhizal ($P < 0.001$) fungi and a decline in lichenized ($P < 0.001$) species (Table 2, Fig. 3). For lichenized species, this aligned with the decline in species richness revealed previously by NMDS. For ectomycorrhizal fungi an increase in abundance coupled with a decline in species richness across the treatment plots suggested that a relatively small subset of taxa could benefit from deeper snow, while the majority of ectomycorrhizal species petered out under the increased snow depth. Total fungal abundance in dry tundra was not affected significantly ($P = 0.67$).

Table 2
Effect ofin increased snow depth on richness and abundance of fungal ecological groups in dry and moist tundra. Shifts in fungal abundance were analysed by Comprehensive Meta-analysis: positive size effect values correspond to an increase in abundance, and negative size effect values show a decline in abundance of ecological group under the snow fence treatment. ** correspond to $P < 0.01$, and *** – to $P < 0.001$. The effect on richness was analysed by non-metric multidimensional scaling in PC-Ord. Correlations $|r| > 0.5$ are shown in bold. For convenience, significant changes are shown with ⬇ for decrease and ⬆ for increase in richness/abundance of the group in interest.

Functional group	Moist tundra, effect on:					Dry tundra, effect on:				
	No of OTUs	Abundance		Richness		*No of OTUs*	Abundance		Richness	
		Size effect	*P value*	*r*	Effect		Size effect	*P value*	*r*	Effect
animal pathogens	*6*					*14*	−0.09	0.599	−0.242	
bryophyte-associated	*10*	−0.57 ⬇	**0.006** **	**−0.596**	⬇	*6*				
dse	*23*	−0.12	0.379	**−0.617**	⬇	*32*	0.07	0.535	0.143	
ectomycorrhizal	*93*	−0.24 ⬇	**<0.001** ***	**−0.675**	⬇	*95*	0.269 ⬆	**<0.001*** **	**−0.749**	⬇
endolichenic	*7*					*16*	−0.234	0.145	−0.321	
endophytic	*30*	−0.07	0.577	0.214		*25*	0.043	0.736	−0.08	
lichenized	*30*	−0.503 ⬇	**<0.001** ***	**−0.797**	⬇	*107*	−0.318 ⬇	**<0.001*** **	**−0.866**	⬇
mycoparasitic	*18*	−0.235	0.122	−0.292		*21*	−0.004	0.98	−0.004	
plant pathogenic	*52*	−0.169	0.059	**−0.643**	⬇	*46*	0.137	0.152	0.215	
root-associated	*42*	0.076	0.807	−0.026		*49*	−0.014	0.88	**0.662**	⬆
saprotrophic	*365*	−0.106	**0.002** **	**−0.653**	⬇	*322*	0.018	0.62	−0.091	
Total Fungi		−0.15 ⬇	**<0.001*** **	**−0.876**	⬇		0.046	0.67	−0.382	

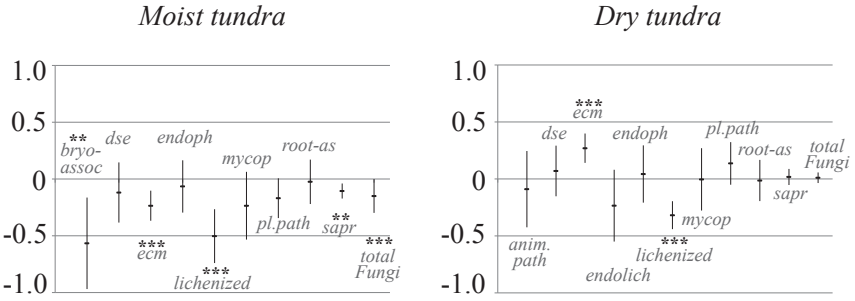

Fig. 3. Responses of fungal functional groups to deeper snow, an abundance-based comprehensive meta-analysis. The graph shows the size effect with a 95% confidence interval calculated for various functional groups of fungi. Positive and negative values indicate increase and decrease in abundance in the deep snow plots, respectively. Significant correlations are shown with ** for $P < 0.01$ and *** – for $P < 0.001$. *anim. path* - animal pathogenic fungi, *dse* – dark septated endophytes, *bryo-assoc* – bryophyte-associated, *ecm* – ectomycorrhizal, *endolich* – endolichenic, *root-as* – root-associated, *sapr* – saprotrophic, *mycop* – mycoparaistic, *pl.path.* – plant pathogenic species of fungi.

4. Discussion

4.1. Changes in fungal community composition

In both moist tussock and dry heath tundra, community composition of arctic soil fungi in tundra sites subjected to 18 years of increased snow depth differed significantly from communities in the control sites. The changes in both tundra communities were different from those observed in response to experimental summer warming, where only fungal communities in moist tundra exhibited significant compositional changes (Geml et al., 2015; Morgado et al., 2015; Semenova et al., 2015). The more pronounced fungal community response to deep snow compared to summer warming in dry heath tundra likely relates to the fact that higher snowpack not only maintains higher soil temperature in winter, but also enhances the soil moisture regime in summer due to additional snow melt water (Pattison and Welker, 2014), considering that low soil moisture is often the main limitation for fungal growth (Griffin, 1963). The duration of snow pack may also contribute to the strong fungal community response; since winters are longer than summers in the Arctic, the fungal communities are exposed longer to experimentally increased snow depth than to summer warming (Jones et al., 1998; Welker et al., 2000).

Our data suggest marked changes in fungal community compositions associated with this snow depth, although it was difficult to compare our results to any similar previous studies. For instance, Penton et al. (2013) found almost no effect of 1 year of snow fence treatment (1.2 m deep snow) on fungal richness and community composition in boreal Alaskan discontinuous permafrost. Their analysis of OTU abundance revealed a decline in only one ascomycete family – Helotiaceae, and increases in basidiomycete genera *Russula*, *Lactarius* and *Cortinarius*, which did not coincide with our results. Within the Canadian low Arctic, Buckeridge and Grogan (2008) found no compositional changes in fungal biomass and hyphal length in birch hummock tundra after 3 years of deeper snow (ca. 1 m in the treatment vs ca. 0.3 m in the control), and no information was provided on the taxonomic composition of that community. We believe that it may take several years for the effects of snow pack to affect the composition of soil fungi, either directly or by altering the vegetation and soil attributes, so our findings may not be comparable to those from other ecosystems. (Wahren et al., 2005; Mercado-Diaz, 2011). In the same area at Toolik Lake, in both

dry and moist tundra, we observed a decline in richness of ectomycorrhizal fungi under deep snow, which coincides with the trends observed for ectomycorrhizal basidiomycetes in long-term summer warming (Morgado et al., 2015) and snow addition experiments (Morgado et al., 2016).

4.2. Changes in taxonomic and functional groups

Increased snow depth resulted in declines in richness in many fungal taxonomic and functional groups. In dry tundra, the total species richness was not affected, likely because the loss of species in one fungal lineage was coupled with increased richness in another fungal taxa. In moist tundra, total fungal richness decreased in the deep snow treatment, as suggested by the NMDS Pearson correlation value, although the difference was not significant in the *t*-test, likely because of the high standard deviation values possibly caused by the large spatial heterogeneity, i.e. "patchiness" of fungal species (Blaalid et al., 2012). All taxonomic and functional groups with strong correlation values had lower richness and/or abundance in the deep snow plots. It is possible that trend would be significant if more samples were collected per plot. Unfortunately, given the destructive nature of soil sampling, we could not take more than 5 soil cores per plot that are also used for a variety of long-term research projects. In general, loss of species richness implies greater fluctuations in ecosystem functioning (e.g., productivity, rates of decomposition or nutrient cycling), as well as reduced robustness towards fluctuations in abiotic factors, e.g., extreme temperatures or water regimes (Naeem et al., 1999). However, the negative consequences for the moist tundra ecosystems caused by the decline in fungal species richness are difficult to predict due to functional redundancy of the soil microbial community and numerous ways in which this decline may be compensated by other groups of fungi and bacteria (Coleman and Whitman, 2005 and references therein).

Patterns of richness and read abundance in our study revealed partly concordant results with previously reported changes in plant communities (Wahren et al., 2005; Wipf and Rixen, 2010; Mercado-Diaz, 2011; Loranty and Goetz, 2012; Pattison and Welker, 2014) in response to higher snowpack levels in arctic tundra. In all analyses in both the dry and the moist tundra, we observed a significant decline of lichenized fungi, implying, therefore, strong decrease in richness as well as abundance. The decline in richness and abundance of bryophyte-associated species observed in the moist tundra was in agreement with the previously reported losses of bryophytes caused by increased snow depth (Wahren et al., 2005; Mercado Diaz, 2011). A similar pattern was observed in response to long-term experimental summer warming (Geml et al., 2015; Semenova et al., 2015).

Somewhat unexpectedly, ericoid-, ectomycorrhizal and endophytic fungi either decreased or showed no significant change in richness and abundance under increased snow depth, despite higher shrub density and biomass reported in the deep snow areas in both tundra types (Mercado-Diaz, 2011; Pattison and Welker, 2014). Greater aboveground biomass and shrub density were assumed to reflect increased root biomass (Sullivan et al., 2007), which, in turn, would broaden niches for mentioned above fungal species. For instance, such increase in ectomycorrhizal fungi associated with *Betula nana* was reported in summer warming greenhouse experiments (Deslippe et al., 2011). However, our data clearly demonstrate a strong decline in ectomycorrhizal species in both richness and abundance in dry and moist tundra, while an increase was only observed for richness of root-associated fungi in dry tundra. We assume that in the moist tundra this decline could be due to decreased aeration caused by excess moisture following snow melt in the spring, as in general these fungi tend to avoid overly flooded habitats (van der Wal et al., 2013). In the dry tundra, ectomycorrhizal fungi may be influenced by factors other than moisture, e.g., rising NH_4^+ concentrations under the deep snow (Boxman et al., 1986) or acidification of rhizosphere that follows higher uptake of NH_4^+ by the plants (Zhang and Bai, 2003).

It is currently unclear whether the decline in sensitive ectomycorrhizal fungi is a direct consequence of altered environmental conditions or a result of changes in competition dynamics affected by the increased snow depth. Morgado et al. (2015) and Geml et al. (2015) report a strong decline in ectomycorrhizal basidiomycetes in arctic tundra under summer warming, including the genera *Inocybe* and *Sebacina*, that strongly correlated with the control sites. Therefore, declines in ectomycorrhizal fungi observed in our study may not be related to altered moisture or NH_4^+ concentrations but instead may be caused by the effects of temperature increase on fungal metabolism (i.e. production of extracellular enzymes) and fungus-plant and fungus-fungus interactions (Morgado et al., 2015).

Similarly, we anticipated an increase in saprotrophic fungi across the deep snow plots, that was not supported by our data. An increase in richness of saprotrophic ascomycetes was reported in our summer warming experiments, likely as a consequence of accumulation of leaf litter across the warmed plots (Semenova et al., 2015). Because litter accumulation occurs also in the deep snow plots (Mercado-Diaz, 2011), we anticipated higher richness of saprotrophs in the snow fence experiments as well. However, no significant response was observed in dry tundra and strong declines in richness and abundance of saprotrophic fungi was observed in moist tundra. Because of their key roles in decomposition processes, saprotrophs are essential for nutrient turnover and soil C storage. In microcosm experiments (Hunt et al., 1987) deletion of saprotrophic fungi or bacteria led to extinction of other groups of organisms, although the system was still functioning when mycorrhizal fungi were removed. On the other hand, possible consequences of ca. 10 percent loss in saprotroph abundance observed in moist tundra are difficult to predict given high functional redundancy of microbial communities (Coleman and Whitman, 2005).

We did not observe any changes in abundance of plant pathogenic fungi, while richness in this guild declined in moist tundra. More intensive growth of shrubs and higher winter temperatures in deeper snow areas were anticipated to favour plant pathogens, similar to what was known from former studies (Olofson et al., 2011; Natali et al., 2012). For example, a six year snow fence experiment (increased snow depth by 0.6–0.8 m) in northern Sweden resulted in outbreaks of the plant pathogenic ascomycete *Arwidssonia empetri* that caused shoot mortality and reduced the coverage of the dominant dwarf-shrub *Empetrum hermaphoditum* by 70% (Olofson et al., 2011; Natali et al., 2012). Possibly, an observed decline in richness of plant pathogenic fungi in moist tundra was related to increased plant fitness due to lesser frost cleft and higher nitrogen availability under deep snow, or other factors that overweighed the benefits of winter warming for plant parasitic fungi. In addition, *Arwidssonia empetri* (or syn. *Heterosphaeria* spp.) was not among the species detected in our dataset, despite the fact that *Empetrum* is present in both the dry and the moist plots. Therefore, it remains unknown if disease outbreaks similar to the ones reported from the Swedish snow fence experiment may occur in the arctic tundra.

4.3. Plant-versus fungal community responses to deep snow

Lichenized and bryophyte-associated fungi in our dataset responded to deeper snow in agreement with formerly reported shifts in plant communities across the same sampling plots,

however shifts in many fungal lineages, i.e. ectomycorrhizal, dark septated endophytes, plant pathogenic and saprotrophic species appear counterintuitive in light of observed vegetation change and temperature records. A correspondence between plant and fungal responses is generally anticipated due to known tight associations between fungi and plants in nutrient-poor soils of arctic tundra (Hobbie et al., 2009), suggesting that fungal communities would change in their assemblages following the trends known for their hosts (Dahlberg and Bültmann, 2013). On the other hand, plant and fungal responses to climate change involve a variety of strategies, including shifts in population ranges, symbiotic partners or timing of phenological events that provide a high potential for mismatches between interacting plants and their symbiotic microbes (Classen et al., 2015). Belowground communities in general have a much faster turnover compared to aboveground ones, and both may be strongly structured by different direct and indirect environmental drivers (Fierer and Jackson, 2006; Kardol et al., 2014; Classen et al., 2015). For example, bacteria-to-fungal ratio may increase (DeAngelis et al., 2015) or decrease (Deslippe et al., 2012) in warmer soils, with a short-term impact on fungal assemblages but a retarded effect on climax arctic plant communities. Because of faster turnover, the state of present microbial communities may correspond to the ongoing climatic changes that will be reflected in plant community composition in future years. We, therefore, highlight the importance of studies in soil biology because monitoring the below-ground communities may be suitable for predicting and managing ecosystem disturbances on earlier stages.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.06.001.

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