

RESEARCH ARTICLE

Long-term warming alters richness and composition of taxonomic and functional groups of arctic fungi

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One sentence summary: Long-term experimental warming alters diversity and community composition of taxonomic and functional groups of arctic fungi in moist and dry tundra types.

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ABSTRACT

Fungi, including symbionts, pathogens and decomposers, play crucial roles in community dynamics and nutrient cycling in terrestrial ecosystems. Despite their ecological importance, the response of most arctic fungi to climate warming is unknown, so are their potential roles in driving the observed and predicted changes in tundra communities. We carried out deep DNA sequencing of soil samples to study the long-term effects of experimental warming on fungal communities in dry heath and moist tussock tundra in Arctic Alaska. The data presented here indicate that fungal community composition responds strongly to warming in the moist tundra, but not in the dry tundra. While total fungal richness was not significantly affected by warming, there were clear correlations among operational taxonomic unit richness of various ecological and taxonomic groups and long-term warming. Richness of ectomycorrhizal, ericoid mycorrhizal and lichenized fungi generally decreased with warming, while richness of saprotrophic, plant and animal pathogenic, and root endophytic fungi tended to increase in the warmed plots. More importantly, various taxa within these functional guilds followed opposing trends that highlight the importance of species-specific responses to warming. We recommend that species-level ecological differences be taken into account in climate change and nutrient cycling studies that involve arctic fungi.

Keywords: Alaska; climate change; ITEX; ITS; metabarcoding; Toolik Lake

INTRODUCTION

The arctic tundra occupies an area of 8 million km² and is on the threshold of significant structural and functional changes (Tape et al. 2012). Because arctic soils store a great portion of the Earth's reactive carbon (C), nutrient cycling in the Arctic has major consequences for global change (Welker, Fahnestock and Jones 2000;

Callaghan et al. 2004; Welker et al. 2004; Welker, Rayback and Henry 2005; Oechel et al. 2014). Regional rates of warming in several arctic areas, e.g. in the region that is the focus of this paper, are among the highest globally, reaching a mean temperature increase of 0.1°C per year over the past 35 years and further increases in temperature are predicted (Anisimov et al. 2007).

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Marked changes have already been observed in terrestrial arctic ecosystems, including increased microbial activity leading to increased plant nitrogen (N) availability (Chapin 1983; Nadelhoffer et al. 1992; Aerts 2006), faster C turnover in soils (Hobbie and Chapin 1998; Shaver et al. 2006) and shifts in land surface vegetation (Bret-Harte, Shaver and Chapin 2002; Stow et al. 2004; Chapin et al. 2005) in response to warming. For example, increases in the abundance and extent of arctic shrubs have been reported (Sturm et al. 2001, 2005; Stow et al. 2004; Tape, Sturm and Racine 2006; Tape et al. 2012). In addition, vegetation studies carried out in plots of long-term experimental warming featured in this study indicated significant increases in the cover and height of shrubs (e.g. *Betula nana* and *Salix pulchra*) (Walker et al. 2006; Pattison and Welker 2014). Conversely, the bryophytes and lichens decreased significantly (Mercado-Díaz 2011), most likely due to competitive exclusion by shrubs (Cornelissen et al. 2001; Jägerbrand et al. 2009).

Fungi are central to the functioning of terrestrial arctic ecosystems due to their roles as symbionts (e.g. mycorrhizae, endophytes, lichens) and decomposers. Almost all arctic plants are highly dependent on mutualistic relationships with mycorrhizal fungi for survival in these nutrient-poor environments (Gardes and Dahlberg 1996; Hobbie et al. 2009; Bjorbaekmo et al. 2010). Such associations include ectomycorrhizal (ECM), arbuscular mycorrhizal (AM) and ericoid mycorrhizal (ERM) fungi (Väre, Vestberg and Eurola 1992; Michaelson et al. 2008; Newsham, Upson and Read 2009). It has been estimated that 61–86% of N in arctic tundra plants is obtained through mycorrhizal fungi (Hobbie and Hobbie 2006). In addition, endophytic fungi appear to be ubiquitous in the roots and above-ground parts of arctic–alpine plants (Väre, Vestberg and Eurola 1992; Higgins et al. 2007; Newsham, Upson and Read 2009), but little is known about their diversity, identity and ecological role in the Arctic. Given their intimate relationships with plants in a wide range of symbioses and importance in nutrient cycling, fungi are expected to play an important role in arctic vegetation change.

Currently, our ability to predict the response of fungal and plant communities to climate change factors is hampered both by the few detailed descriptions of the members of these communities and our limited understanding of the ecological role of many fungal species. Globally, approximately 100 000 species of fungi have been described, but their true diversity may be as high as 5 million species (Blackwell 2011). In recent years, an increasing number of molecular studies have been devoted to studying arctic fungi. The vast majority of these focused on root-associated, particularly ECM fungi, amassing valuable information on their diversity and biogeographic patterns (Bjorbaekmo et al. 2010; Blaaid et al. 2012; Geml et al. 2012; Timling et al. 2012) and their responses to experimental warming (Clemmensen et al. 2006; Deslippe et al. 2011; Morgado et al. 2015). While ECM species are among the most ecologically important taxa, they represent a small fraction of the taxonomic and functional diversity of fungi. Yet, most other groups of arctic fungi have received little attention with the exceptions of the work of Semenova et al. (2015) on arctic ascomycetes and that of Timling et al. (2014) on soil fungal communities in zonal tundra vegetation types along a latitudinal transect spanning the low and high arctic bioclimatic subzones of North America.

Despite these important advances in our knowledge, the effect of long-term experimental warming on the taxonomic and functional diversity of a broad range of arctic soil fungal communities remains largely unknown. This knowledge is important to understand what roles fungi may have in the observed and predicted changes in tundra communities. In this study, we use

deep DNA sequencing of soil samples to study the long-term effects of experimental warming on total fungal community in dry heath and moist tussock tundra in Northern Alaska. Specifically, we aimed to examine (1) how total fungal richness changes after 18 years of summer warming, (2) which ecological and taxonomic groups are favoured or hindered by warming, and (3) how the observed changes in fungal community composition relate to formerly reported vegetation trends.

MATERIALS AND METHODS

Experimental design and soil sampling

The study was undertaken at the International Tundra Experiment (ITEX) long-term research site in the Toolik Lake region in the northern foothills of the Brooks Range, Alaska, USA (68°37' N, 149°32' W; 760 m above sea level) (Walker et al. 1999; Welker, Fahnestock and Jones 2000; Pattison and Welker 2014). The region lies within the bioclimatic subzone E that is the warmest subzone of the arctic tundra with mean July temperatures ranging from 9 to 12°C (Walker et al. 2005). The two main vegetation types of the region are dry acidic heath tundra, characterized by *Dryas octopetala*, *Salix polaris*, *Vaccinium* species and fruticose lichens, and moist acidic tussock tundra, dominated by *Betula nana*, *Salix pulchra* species, the sedge *Eriophorum vaginatum* and several peat moss species (*Sphagnum* spp.). Detailed descriptions of the plant communities can be found in Walker et al. (1999) and Kade, Walker and Reynolds (2005).

We sampled soil from 20 plots representing the dry and the moist tussock tundra in the last week of July 2012. In each tundra type, we sampled five plots that were subjected to passively increased summer air and upper soil temperature by hexagonal open top chambers (OTCs), subsequently referred to as 'treatment', and five adjacent areas with unaltered conditions ('control'). The OTCs have a 1 m² surface area, are 0.4 m high and are made of translucent fibreglass (Marion et al. 1997; Walker et al. 1999); they increase the summer air and upper soil temperature by a mean daily average of 1.5–2 °C measured at 15 cm height and 5 cm depth, respectively, within the OTCs (Jones et al. 1998; Walker et al. 1999). Every year since 1994, the OTCs were set up as soon as 50% of the ground area of a given plot was snow-free (late May or early June) and were removed at the end of August or early September, following the ITEX protocol (Welker et al. 1997). It has been repeatedly shown that OTCs provide a reasonable approximation to the predicted climatic changes in the Arctic as they alter daytime temperature significantly and minimize unwanted ecological effects, such as changes in soil moisture, the influence of wind speed on air temperature, etc. (Marion et al. 1997; Bokhorst et al. 2013 and references therein). Therefore, OTCs have been recommended to study the response of high-latitude ecosystems to warming (Marion et al. 1997). The soil sampling was performed with a soil core of 2 cm × 20 cm (diameter × depth). In each of the 20 plots, five randomly chosen soil cores were taken, thoroughly mixed and kept frozen until lyophilization.

Molecular work

Genomic DNA was extracted from 1 ml (0.4–1 g) of lyophilized soil from each of the 20 samples using NucleoSpin[®] soil kit (Macherey-Nagel GmbH & Co., Düren, Germany), according to manufacturer's protocol. For each sample, two independent DNA extractions were carried out and pooled in order to optimize the homogenization of the extraction. PCR amplification

and Ion Torrent sequencing of the ITS2 region (ca. 250 bp) of the nuclear ribosomal rDNA repeat were carried out as described in detail by Geml et al. (2014b) using primers fITS7 (Ihrmark et al. 2012) and ITS4 (White et al. 1990). The ITS4 primer was labelled with sample-specific multiplex identification DNA tags. The amplicon library was sequenced at the Naturalis Biodiversity Center using an Ion 318™ Chip and an Ion Torrent Personal Genome Machine (Life Technologies, Guilford, CT, USA).

Sequence data analysis

The raw sequence data contained 4046 811 reads with an average length of 212 ± 111 bp (SD). 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 sequence regions of primers and adapters (identification tags) were removed. We used a parallel version of MOTHUR v. 1.32.1 (Schloss et al. 2009) for subsequent sequence analyses following the protocol described in detail in Geml et al. (2014a,b). A total of 2068 216 sequences remained after quality filtering and trimming with an average read length of 255 ± 56 (mean \pm SD). The quality-filtered sequences were normalized following Gihring, Green and Schadt (2012) by rarefying to the lowest number of sequences obtained for a sample (56 483 reads). The resulting 1129 660 sequences were clustered into operational taxonomic units (OTUs) using OTUPIPE (Edgar 2010) with the simultaneous removal of putatively chimeric sequences with both *de novo* and reference-based filtering using curated dataset of fungal ITS sequences with representative of known taxonomic groups across the kingdom of fungi (Nilsson et al. 2011) as reference. We used a 97% sequence similarity clustering threshold as has been routinely done in fungal ecology studies (e.g. O'Brien et al. 2005; Geml, Laursen and Taylor 2008; Geml et al. 2009, 2010; Kausserud et al. 2012; Brown et al. 2013). Global singletons were discarded from further analysis. Because of the very high number of sequences generated per sample and because most singletons in next-generation sequencing datasets tend to be artefactual and can overestimate the diversity of 'rare taxa' (e.g. see Kunin et al. 2010; Tedersoo et al. 2010), we opted to be conservative and excluded all global singletons (OTUs that were found only once across all samples despite the deep sequencing effort) from further analyses. 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 also excluded from the final analysis due to unreliable classification. The raw sequence data for all samples have been deposited to Dryad (doi:10.5061/dryad.2fc32), while the OTU distribution matrices for the moist and dry tundra types and the representative sequences of OTUs are provided in Tables S1–3 (Supporting Information).

Statistical analysis

We calculated Good's coverage (complement of the ratio between local singletons and the total sequence count) for each sample to estimate the exhaustiveness of our deep sequencing efforts. The effect of tundra and treatment type on observed richness of OTUs (S), Good's coverage estimators, Simpson's diversity ($D = 1 - \sum [p_i^2]$, where p_i is the importance probability in element i) and Shannon's diversity ($H = \sum [p_i \times \ln p_i]$) were tested across all sites using analysis of variance in R (Faraway 2002). In addition, we calculated the species–area curve of all fungal OTUs vs. the number of sampled sites with first-

and second-order jackknife to estimate the total fungal diversity in the moist and dry tundra types. Beta diversity was calculated for each tundra type and treatment combination following Whitaker (1972), i.e. $\beta = S_c / S - 1$, where S_c is the total number of OTUs in all plots in a certain tundra and treatment type and S is the average number of OTUs per plot.

Because preliminary analyses using one-way clustering based on an OTU vs. plot matrix revealed substantial differences in the community composition of the dry and moist tundra plots (Fig. S1, Supporting Information), we carried out the ordinations separately for the two tundra types in order to eliminate the influence of habitat and to focus only on the effects of warming. We used PC-Ord v. 5.32 (McCune and Grace 2002) to run non-metric multidimensional scaling (NMDS) on a primary matrix of experimental plots by OTUs and a secondary matrix of plots by OTU richness in various taxonomic and ecological groups. Because of demonstrated uncertainties regarding the reliability of read abundance as indicator of taxon abundance or biomass in the samples (Amend, Seifert and Bruns 2010; Baldrian et al. 2013), we carried out the ordination analyses based on presence-absence values as well as with square-root transformed read abundance to moderate the influence of OTUs with high sequence counts, while maintaining some approximation of template abundance. Given the very high sequencing coverage we achieved, 'presence' was defined as ≥ 5 sequences on a per sample basis following the recommendations 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). The dataset was subjected to 500 iterations per run using the Sørensen similarity (Bray–Curtis index) and a random starting number. Orthogonal rotation of the resulting NMDS solution with the lowest stress was used to maximize correlation between the warming treatment and the major axes. Pearson's correlation coefficient (r) values between relative OTU richness, OTU diversity per taxon, and axes 1 and 2 were calculated. OTU richness values were calculated for fungal taxonomic groups based on the current classification in Index Fungorum as implemented in UNITE, while we assigned OTUs to ecological groups based on the identities of the most similar sequences coupled with the source information of the reference sequence, when available, or based on published ecological information of the taxon in question (Kirk et al. 2008; Tedersoo, May and Smith 2010; Grünig, Queloz and Sieber 2011; Tedersoo and Smith 2013). For the latter, only OTUs with $\geq 95\%$ similarity to a reference sequence identified to genus were used. We tested whether fungal communities were statistically different across the replicates using two different methods: multiresponse permutation procedure (MRPP) and permutation-based nonparametric MANOVA (Anderson 2001). We determined any preferences of individual OTUs for either control or treatment plots in moist tussock and dry tundra using indicator species analyses (Dufrêne and Legendre 1997) as implemented in PC-Ord v. 5.32. Finally, we compared the OTU richness values of functional groups among the control and warmed plots using Student's t -test.

RESULTS

Fungal diversity

Out of the 4046 811 original sequences, 2068 216 passed the series of quality-filtering steps. After normalizing the library size across all samples by rarefying, 1129 660 sequences were assembled into 6887 non-singleton OTUs, while 1249 singletons and 3148 putatively chimeric clusters were removed. After excluding

OTUs with <80% similarity or <150 bp pairwise alignment length to a fungal sequence as performed by USEARCH, 5438 OTUs were retained for further analyses. The observed number of OTUs did not differ significantly among the tundra and treatment types ($F = 1.22$, $p = 0.33$) (Fig. 1a). Good's coverage estimators (0.993 ± 0.001 , mean \pm SD across all sites) indicate that the deep sequencing allowed for a very high OTU coverage. The coverage estimators did not differ among tundra or treatment types ($F = 1.02$, $p = 0.41$), suggesting that our sequencing effort were similarly deep across all plots (Fig. 1b). Similarly, there was no significant difference among Shannon's diversity index ($F = 0.42$, $p = 0.74$) and Simpson's diversity index ($F = 0.04$, $p = 0.99$) values among the tundra and treatment types (Fig. 1c and d). The species-area curves generated based on the accumulating number of OTUs with increased number of sites indicated that the majority of the soil fungi that occur in the moist tussock and dry heath tundra types in the Toolik Lake region likely have been sampled in our study. In the moist tundra, there were 3534 observed OTUs (Sobs), with first- and second-order jackknife estimates of 4429 and 4725, respectively (Fig. 1e), while the values were somewhat higher in the dry tundra, with 3543 observed OTUs and first- and second-order jackknife estimates of 4503 and 4894, respectively (Fig. 1f). Beta diversity values were relatively low within the tundra and treatment types, although in both the dry and the moist tundra, warmed plots had slightly decreased beta diversity values among the replicates (dry control: 1.389, dry warmed: 1.147, moist control: 1.143, moist warmed: 1.123).

Of the 5438 fungal OTUs, Ascomycota was by far the most OTU-rich phylum and accounted for 2189 OTUs (40.25%), followed by Basidiomycota with 1206 (22.18%). Glomeromycota was represented by 9 OTUs (0.17%), while basal lineages formerly classified in Zygomycota accounted for 145 OTUs (2.66%) and Chytridiomycota for 5 OTUs (0.09%). In addition, there were 1884 (34.65%) unidentified fungal OTUs with most similar sequences to other environmental sequences without assignment to a phylum. In Ascomycota, there were several taxonomic orders with a high number of OTUs, such as Helotiales, Chaetothyriales and Lecanorales, while in Basidiomycota, the order Agaricales was the most diverse, followed by Sebaciales and Thelephorales. The proportional distribution of OTUs representing the taxonomic phyla and orders is shown in Fig. S2 (Supporting Information).

The effect of warming on fungal community composition

For the moist tussock and dry heath tundra types, NMDS analyses resulted in 2D solutions with final stress values of 0.049 52 (square-root abundance) and 0.051 31 (presence-absence) in the moist and 0.069 31 (square-root abundance) and 0.046 41 (presence-absence) in the dry tundra, with final instability values <0.000 01 (Figs 2 and S3, Supporting Information). The Monte Carlo test results indicated that all 2D solutions using the real data were significantly better than chance occurrences ($p < 0.01$). In the moist tundra, coefficients of determination for the correlations between ordination distances and distances in the original n -dimensional space were axis 1: $r^2 = 0.537$, axis 2: $r^2 = 0.323$, total $r^2 = 0.861$, with orthogonality = 84.9% (square-root abundance), axis 1: $r^2 = 0.550$, axis 2: $r^2 = 0.377$, total $r^2 = 0.927$, with orthogonality = 88.1% (presence-absence). For dry tundra, these values were axis 1: $r^2 = 0.538$, axis 2: $r^2 = 0.357$, total $r^2 = 0.895$, with orthogonality = 99.9% (square-root abundance), axis 1: $r^2 = 0.618$, axis 2: $r^2 = 0.334$, total $r^2 = 0.952$, with orthogonality = 100% (presence-

absence). The NMDS ordination plots were orthogonally rotated by treatment. Because the ordination plots based on square-root abundance and presence-absence were almost identical (Figs 2 and S3, Supporting Information, respectively), only the square-root abundance NMDS plots were used to calculate the Pearson correlation coefficient (r) values between all environmental and fungal community variables and the ordination axes. Following Rogers, Moore and Ryel (2009), variables with $|r| \geq 0.5$ values for either axis were considered important for characterizing changes in fungal community structure and were superimposed on the ordination plot as direction and strength vectors to best illustrate differences among ecological groups (Fig. 2a and b), ascomycete (Fig. 2c and d) and basidiomycete (Fig. 2e and f) genera.

The NMDS plot revealed a strong structuring of fungal communities according to the warming treatment in the moist tundra, while in the dry tundra the compositional difference between the warmed and the control plots was less pronounced (Fig. 2). MRPP confirmed that the total fungal community composition was significantly altered by long-term warming in the moist tundra (effect size $A = 0.132$ 93, probability $p = 0.003$ 79), while the changes were not significant in the dry tundra ($A = 0.015$ 46, $p = 0.125$ 76). Similarly, permutation-based MANOVA indicated that fungal community structure in the warmed and control plots differed significantly in the moist tundra ($p = 0.0064$), where the treatment explained 36.26% of the variation. In contrast, the warming treatment did not significantly alter fungal community composition in the dry tundra ($p = 0.1224$) and accounted for only 5.54% of the variation.

With respect to functional groups, ECM basidiomycetes represented the most OTU-rich ecological guild in both tundra types, followed by lichenized fungi in the dry tundra and saprotrophic zygomycetes in the moist tundra. In the moist tussock tundra, OTU richness in the following groups was negatively correlated with axis 1 (warming treatment): lichens, ERM basidiomycetes (in Sebaciales), endophytes of herbs, dung/litter fungi, moss endophytes and ECM basidiomycetes. In contrast, some groups were more diverse in the moist warmed plots, such as predominantly saprotrophic (and opportunistically pathogenic) soil asco- and zygomycetes, DSE fungi, ERM ascomycetes, animal pathogens and wood-rotting ascomycetes (Fig. 2a). The patterns were different in the dry heath tundra, where only ERM ascomycetes showed strong negative correlation with warming, while ECM asco- and basidiomycetes, plant pathogens, wood-rotting basidiomycetes, herb endophytes, moss endophytes, lichens, saprotrophic soil basidiomycetes and AM fungi were represented by more OTUs in the warmed plots (Fig. 2b). These patterns were confirmed by statistical comparisons of the ecological groups using t -tests that the majority of the above differences between control and warmed plots were significant ($p < 0.05$) or marginally significant ($p < 0.1$) (Fig. 3).

In the moist tundra, the following genera had higher OTU richness in the control plots: *Alatospora*, *Cladonia*, *Phialea* and *Sclerotinia* in Ascomycota, and *Clavaria*, *Galerina*, *Hebeloma*, *Hemimycena*, *Inocybe*, *Lactarius*, *Ramariopsis*, *Rhodotorula*, *Sebacia* and *Tomentella* in Basidiomycota. Genera with strong positive correlation with warming included *Cladosporium*, *Cryptosporiopsis*, *Hypocrea*, *Lachnum*, *Mollisia*, *Penicillium*, *Phialocephala*, *Pseudogymnoascus* and *Tropoporella* in Ascomycota and *Exobasidium* and *Omphalina* in Basidiomycota (Fig. 2c and e). In the dry tundra, OTU richness in *Archaeorhizomyces*, *Cladonia*, *Hypodiscus*, *Mycoblastus*, *Rhizoscyphus* and *Sphaerophorus* (Ascomycota) and that in *Clavaria* and *Malassezia* (Basidiomycota) were

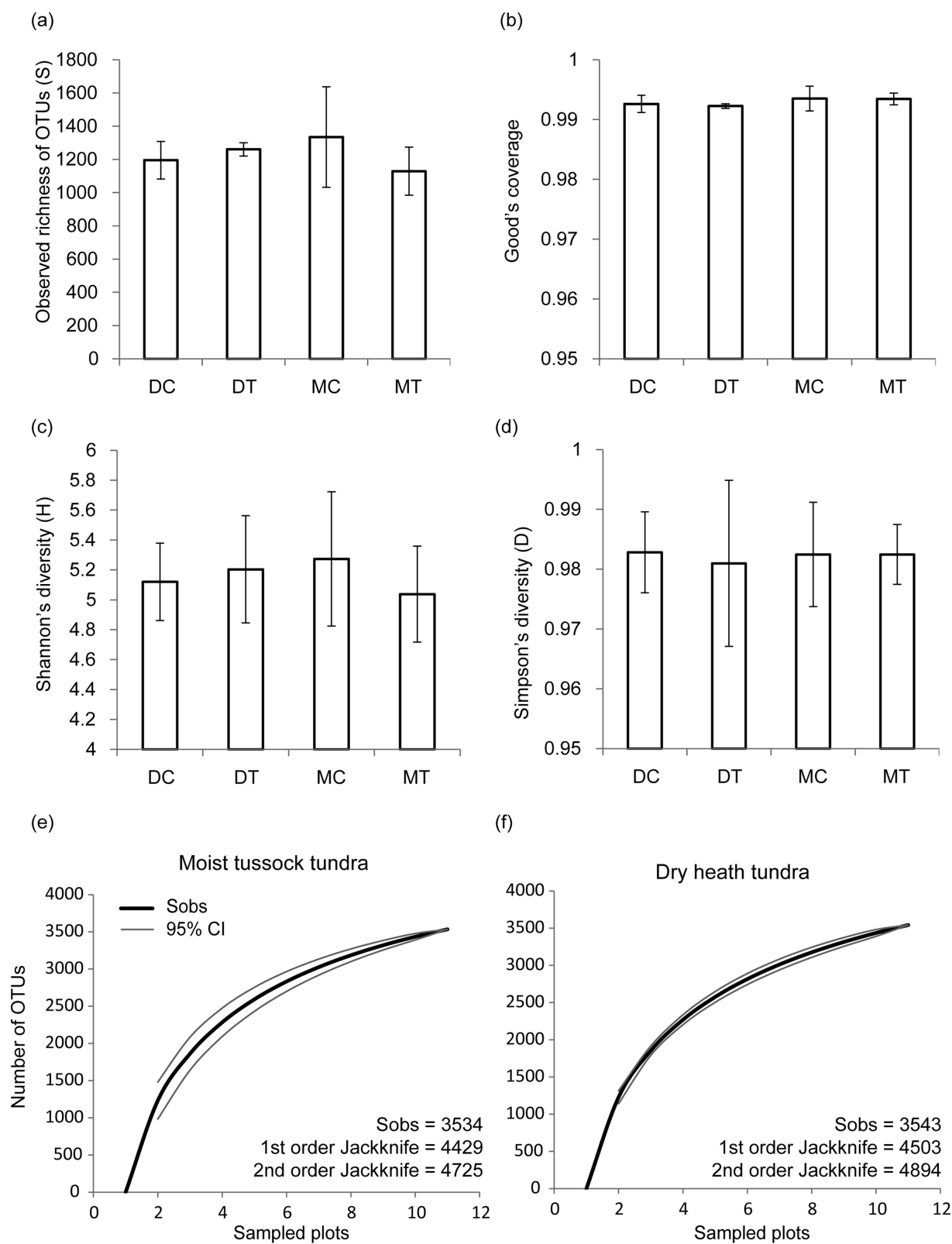


Figure 1. Community richness and coverage estimators in the warmed and control plots in the dry and moist tundra types with standard deviations: (a) observed number of OTUs (S), (b) Good's coverage, (c) Shannon's diversity index (H), (d) Simpson's diversity index (D), and (e) and (f) rarefaction curves of the total number of fungal OTUs with 95% confidence interval and with first- and second-order jackknife estimates of OTU richness in the dry and moist tundra types. Abbreviations are M = moist tundra, D = dry tundra, C = control, T = warming.

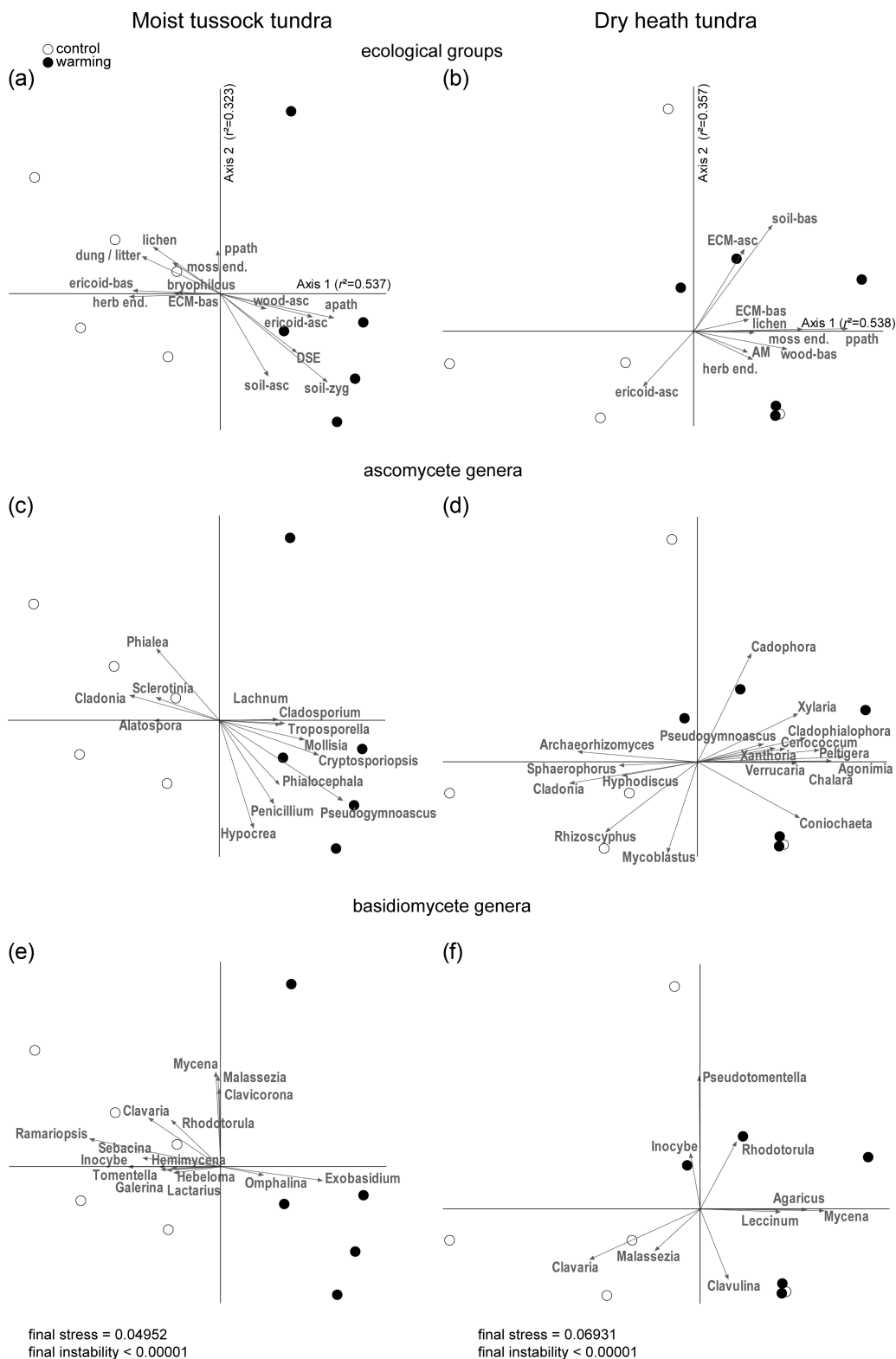


Figure 2. NMDS ordination plots for fungal communities from the warmed and control plots in the dry and moist tundra types based square-root abundance. Because of the high number of variables tested, vectors of variables representing OTU richness in ecological groups and in genera of Ascomycota and Basidiomycota are distributed over six identical ordination plots. Only variables that correlated with any ordination axis at $|r| \geq 0.5$ are displayed. Abbreviations are apath = animal pathogen, bryophilous = fungi living on mosses, DSE = dark-septate endophyte, dung/litter = secondary decomposer fungi that live on litter and/or dung, ECM-asc = ectomycorrhizal ascomycete, ECM-bas = ectomycorrhizal basidiomycete, ericoid-asc. = ericoid mycorrhizal ascomycete, ericoid-bas. = ericoid mycorrhizal basidiomycete, herb end. = endophyte of herbs, lichen = lichenized fungi, moss end. = endophyte of mosses, ppath = plant pathogen, soil-asc = soil saprotrophic ascomycete, soil-bas = soil saprotrophic basidiomycete, soil-zyg = soil saprotrophic zygomycete, wood-asc = wood-rotting ascomycete, wood-bas = wood-rotting basidiomycete.

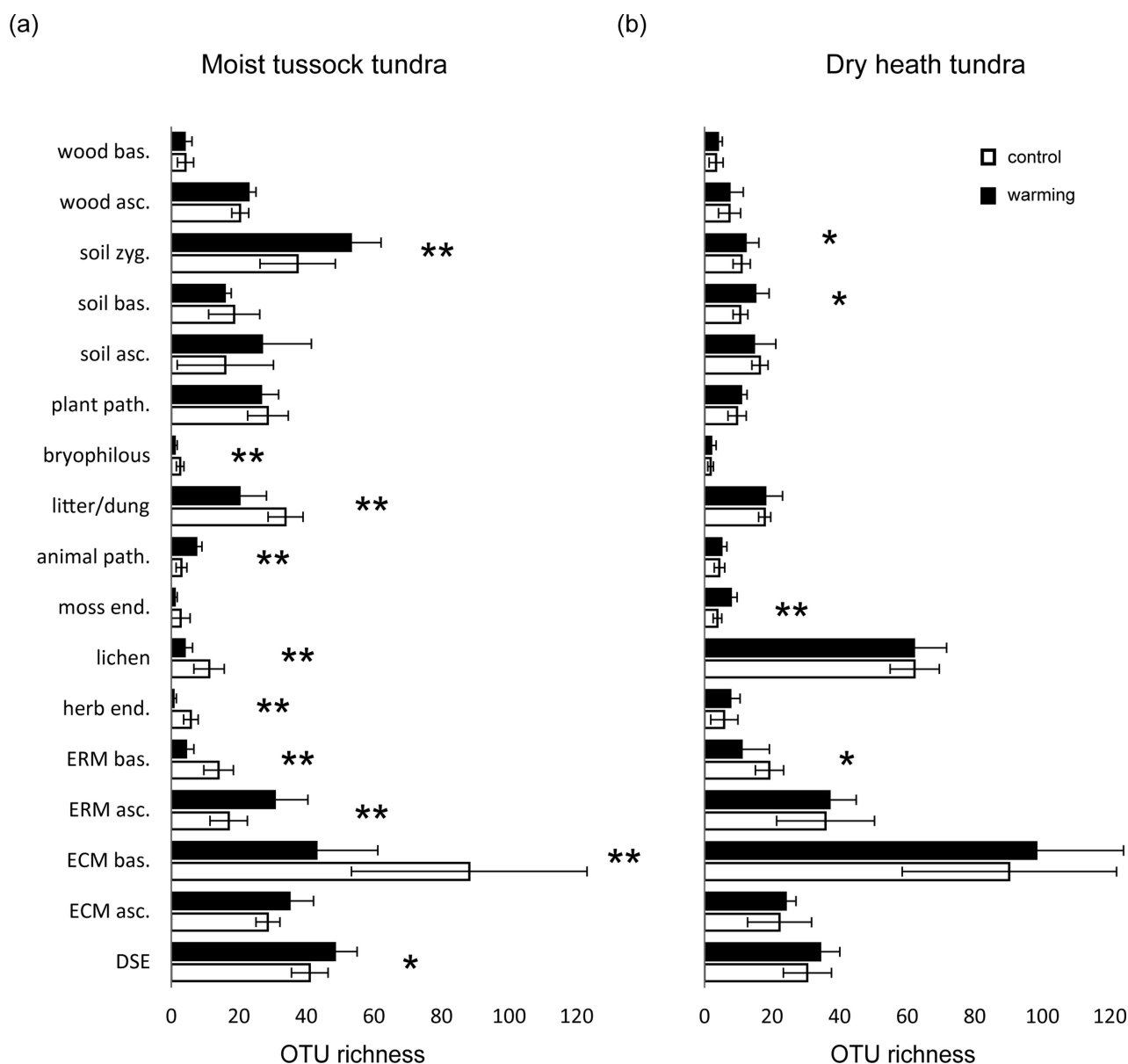


Figure 3. OTU richness of ecological groups of fungi represented by at least five OTUs with standard deviations. Significance values of Student's t-tests are indicated as follows: * = marginally significant ($P < 0.1$), ** = significant ($P < 0.05$). Abbreviations are as explained in Table 1.

strongly negatively correlated with warming. In contrast, OTU richness in the following genera was higher in the warmed plots: *Agonomia*, *Cadophora*, *Cenococcum*, *Chalara*, *Cladophialophora*, *Coniochaeta*, *Peltigera*, *Pseudogymnoascus*, *Verrucaria*, *Xanthoria* and *Xylaria* in Ascomycota and *Agaricus*, *Mycena* and *Leccinum* in Basidiomycota (Fig. 2d and f). Pearson correlation values of all ecological groups and genera are displayed in Table 1.

Indicator species

In the moist tundra, 365 OTUs had significant (<0.05) p -values in the indicator species analyses. Of these, 212 were indicators for the control and 153 for the warming treatment. In contrast, there were only 83 OTUs in the dry tundra with significant indicator values, of which 25 were indicators of the control and 58 of the warmed plots. We conservatively identified 130 indicator OTUs to species or species complexes

based on $\geq 97\%$ sequence similarity to a reference sequence (Table S4, Supporting Information). Among plant-associated fungi in the moist tundra, ECM basidiomycetes were generally negatively affected by the warming, and all indicator species representing this ecological group were associated with the control treatment. In the moist tundra, ECM basidiomycetes negatively affected by warming included *Hebeloma radicosum* SH039561.06FU, *Russula renidens* SH025103.06FU, *Sebacina* sp. SH231852.06FU and *Tomentella* spp. SH220174.06FU, SH202711.06FU, SH202533.06FU and SH220001.06FU. In the dry tundra, *Tomentella* sp. SH112690.05FU and the unidentified ECM agaric fungus SH220993.06FU were indicators of the control treatment (Table S4, Supporting Information).

Similarly, ERM and root endophytic fungi belonging to the Sebaciniales appeared to be negatively affected by warming. In the moist tundra, the unidentified sebacinoid ERM species SH113701.05FU, SH101765.05FU and SH265789.06FU, and root

Table 1. Pearson's correlation values (r) for variables in the NMDS ordination performed with the OTU vs. site matrix. Variables with $|r| \geq 0.5$ values are shown in bold and are displayed in the NMDS ordinations in Fig. 2, where axis 1 represents the warming treatment. Pearson's correlation values were not calculated for taxonomic orders with less than three OTUs in any site. Abbreviations are apath = animal pathogen, bryophilous = fungi living on mosses, DSE = dark-septate endophyte, dung/litter = secondary decomposer fungi that live on litter and/or dung, ECM-asc = ectomycorrhizal ascomycete, ECM-bas = ectomycorrhizal basidiomycete, ericoid-asc. = ericoid mycorrhizal ascomycete, ericoid-bas. = ericoid mycorrhizal basidiomycete, herb end = endophyte of herbs, lichen = lichenized fungi, moss end = endophyte of mosses, ppath = plant pathogen, soil-asc = soil saprotrophic ascomycete, soil-bas = soil saprotrophic basidiomycete, soil-zyg = soil saprotrophic zygomycete, wood-asc = wood-rotting ascomycete, wood-bas = wood-rotting basidiomycete.

	Moist Axes		Dry Axes			Moist Axes		Dry Axes			Moist Axes		Dry Axes	
	1	2	1	2		1	2	1	2		1	2	1	2
AM			0.500	-0.312	<i>Agaricus</i>			0.705	-0.050	<i>Acremonium</i>			0.452	0.108
apath	0.829	-0.382	-0.107	0.075	<i>Alnicola</i>	0.002	-0.338			<i>Agonimia</i>			0.826	0.152
bryophilous	-0.513	0.089	0.264	0.056	<i>Arrhenia</i>			0.049	0.422	<i>Alatospora</i>	-0.628	0.024		
DSE	0.680	-0.598	0.420	0.095	<i>Clavaria</i>	-0.662	0.545	-0.720	-0.486	<i>Archaeorhizomyces</i>	-0.070	-0.278	-0.750	0.223
dung/litter	-0.690	0.474	0.390	-0.427	<i>Clavicornia</i>	-0.106	0.687			<i>Cadophora</i>	-0.237	-0.234	0.500	0.713
ECM-asc	0.445	-0.098	0.486	0.620	<i>Clavulina</i>	0.017	-0.145	0.360	-0.574	<i>Capronia</i>	0.230	0.492	0.405	0.164
ECM-bas	-0.532	0.067	0.505	0.239	<i>Corticium</i>			0.153	-0.423	<i>Cenococcum</i>	-0.343	0.154	0.638	0.246
ericoid-asc	0.747	-0.376	-0.486	-0.506	<i>Cortinarius</i>	-0.287	0.346	0.358	-0.108	<i>Chalara</i>	-0.739	0.390	0.791	0.076
ericoid-bas	-0.730	0.143	-0.304	-0.101	<i>Cryptococcus</i>	-0.106	-0.134	0.378	0.257	<i>Cladonia</i>	-0.075	0.185	-0.775	-0.316
herb end	-0.744	-0.125	0.524	-0.362	<i>Dioszegia</i>	-0.392	0.204			<i>Cladophialophora</i>	0.630	-0.132	0.712	0.336
lichen	-0.640	0.535	0.531	-0.067	<i>Ertoloma</i>	0.027	0.129	-0.369	0.156	<i>Clathrosphaerina</i>	-0.190	0.291		
moss end	-0.539	0.435	0.713	0.102	<i>Exobasidium</i>	0.785	-0.286			<i>Coniochaeta</i>			0.691	-0.512
ppath	-0.126	0.513	0.849	0.114	<i>Fellomyces</i>	0.278	-0.122			<i>Cryptosporiopsis</i>	0.775	-0.458		
soil-asc	0.538	-0.706	-0.083	0.380	<i>Galerina</i>	-0.570	-0.144			<i>Hyaloscypha</i>	-0.063	-0.239		
soil-bas	-0.096	0.150	0.606	0.706	<i>Hebeloma</i>	-0.544	-0.071	0.140	0.225	<i>Hymenoscyphus</i>	-0.197	0.272		
soil-zyg	0.805	-0.730	0.247	0.232	<i>Hemimycena</i>	-0.611	-0.006			<i>Hypodiscus</i>	-0.326	0.286	-0.596	-0.254
wood-asc	0.524	-0.297	0.215	0.199	<i>Inocybe</i>	-0.748	-0.014	-0.216	0.511	<i>Hypocrea</i>	0.455	-0.810		
wood-bas	0.072	0.271	0.660	-0.288	<i>Kockovaella</i>	0.079	0.042			<i>Hypogymnia</i>			-0.261	-0.185
					<i>Lactarius</i>	-0.535	-0.191			<i>Lachnum</i>	0.595	0.073	-0.009	-0.076
					<i>Leccinum</i>	-0.238	-0.285	0.612	-0.115	<i>Lecanora</i>	-0.165	0.186		
					<i>Malassezia</i>	-0.124	0.743	-0.460	-0.441	<i>Lecythophora</i>	0.055	-0.100	0.566	0.177
					<i>Mrakia</i>	0.390	0.350			<i>Meliniomyces</i>	0.462	-0.111	0.339	-0.222
					<i>Mycena</i>	-0.171	0.758	0.761	-0.081	<i>Mollisia</i>	0.718	-0.339		
					<i>Omphalina</i>	0.511	-0.225			<i>Mycoblastus</i>			-0.374	-0.653
					<i>Psathyrella</i>	-0.444	-0.023			<i>Neobulgaria</i>	-0.057	0.361		
					<i>Pseudotomentella</i>			-0.074	0.789	<i>Parmelia</i>			-0.060	-0.308
					<i>Psilocybe</i>	-0.041	-0.164			<i>Peltigera</i>			0.755	0.239
					<i>Ramariopsis</i>	-0.893	0.410			<i>Penicillium</i>	0.572	-0.711	0.352	-0.089
					<i>Rhodocybe</i>			0.412	0.173	<i>Pertusaria</i>			0.172	0.466
					<i>Rhodotorula</i>	-0.546	0.531	0.411	0.562	<i>Phaeoconiella</i>			-0.276	-0.248
					<i>Russula</i>	-0.488	0.143	0.021	0.272	<i>Phialea</i>	-0.619	0.659		
					<i>Sebacina</i>	-0.686	0.234	-0.021	0.381	<i>Phialocephala</i>	0.601	-0.624		
					<i>Sistotrema</i>			-0.278	-0.113	<i>Phoma</i>			0.343	0.453
					<i>Sporobolomyces</i>	-0.109	0.343			<i>Pseudogymnoascus</i>	0.864	-0.697	0.555	0.289
					<i>Tomentella</i>	-0.600	-0.124	0.291	0.029	<i>Ramalina</i>			0.554	0.205
					<i>Trechispora</i>			0.232	-0.263	<i>Rhizoscyphus</i>	0.093	0.344	-0.656	-0.573
					<i>Tremella</i>	-0.198	0.409			<i>Sclerotinia</i>	-0.620	0.372		
										<i>Sphaerophorus</i>			-0.606	-0.128
										<i>Trichocladium</i>			0.363	0.242
										<i>Tropospora</i>	0.607	-0.148		
										<i>Venturia</i>	0.029	0.322		
										<i>Verrucaria</i>			0.682	-0.076
										<i>Xanthoria</i>			0.602	0.256
										<i>Xylaria</i>			0.685	0.476

endophytic SH265824.06FU, SH167198.06FU and SH285151.06FU, as well as other members of the Sebacinales previously sequenced from soils (SH214727.06FU and SH265810.06FU) were all indicators of the control plots.

In contrast, trends in ascomycetes capable of forming both ECM and DSE symbioses were more complex and appeared to be taxon-specific. ECM/DSE ascomycete indica-

tors of the moist control treatment were *Cladophialophora* sp. SH228315.06FU, *Meliniomyces bicolor* SH161266.06FU, *Meliniomyces* sp. SH207177.06FU, and unidentified Helotiales SH209187.06FU and SH208255.06FU. ECM/DSE ascomycetes that were indicators of the moist warmed plots included *Cadophora melinii* SH267533.06FU, *Cladophialophora* sp. SH228303.06FU, *Lachnum* spp. SH189825.06FU and SH189775.06FU, *Meliniomyces*

bicolor SH207165.06FU, *Phialocephala fortinii* SH113844.06FU, and unidentified ECM fungi in Chaetothyriales and Helotiales, SH220001.06FU and SH207207.06FU, respectively. In the dry tundra, the unidentified ECM Chaetothyriales SH305874.06FU was indicator of the control plots, while the unidentified ECM Helotiales SH209187.06FU, SH108616.06FU and SH209187.06FU, *Meliniomyces bicolor* SH207295.06FU and *Meliniomyces* sp. SH207172.06FU were significant indicators of warming treatment.

DISCUSSION

Our results show that arctic tundra fungal communities respond strongly to long-term experimental summer warming, particularly in the moist tussock tundra. We found that while total fungal diversity and richness are not significantly affected by warming and are comparable across moist and dry tundra sites, there are clear patterns of correlations among OTU richness of various ecological and taxonomic groups and long-term warming. In general, the changes are more pronounced in the moist tundra, where the effect of warming on the total fungal community was strongly significant and there were more ecological and taxonomic groups with significant differences in OTU richness between the control and the warmed plots. The greater responsiveness of fungi in the moist tussock tundra may partly be explained by the fact that ambient moist tundra soils, being generally cool throughout the summer, tend to experience less fluctuations in temperature than dry tundra soils that are regularly exposed to higher temperatures as well as pronounced water stress in the upper layers (Jones et al. 1998). Sedge-dominated moist tundra sites were also shown to be more responsive to warming than dry tundra sites in a study focusing on the richness and community composition of nitrogen-cycling bacterial communities in the Canadian High Arctic (Walker, Egger KN, Henry 2008). These findings and the accumulating evidence from alpine and arctic dry tundra plant communities (Welker et al. 1993, 1997; Lupascu et al. 2013, 2014; Sharp et al. 2013) suggest that temperature responses of microbial and plant communities likely are predicated on soil water conditions and resulting differences in productivity among tundra types.

The warming-induced changes we report here are particularly notable when taking into account that warming-induced vegetation shifts at the same experimental site were caused by changes in the relative abundance of various plant functional groups rather than changes in richness or species identities (Wahren, Walker and Bret-Harte 2005). In fungi, most of the differences in community composition among the control and warmed plots were caused by the presence of many OTUs in a particular treatment type and absence in the other, as shown by the ordinations and the indicator species analyses. While the currently prevailing view is that altered plant community composition drives fungal community change in the Arctic (Dahlberg et al. 2013), we conclude that fungal community composition may change independently and that fungi may be particularly well suited to monitor early responses to environmental changes.

One finding in our paper is that OTU richness of ERM and ECM basidiomycetes was negatively affected by warming in the moist tundra. ECM basidiomycetes represent the most OTU-rich functional guild, and the strong decrease in richness in the moist warmed plots in almost all ECM basidiomycete genera may have functional implications. For example, in the moist tundra, warming appeared to favour taxa with medium-distance

fringe mycelial exploration types (e.g. *Cortinarius*), which showed non-significant decrease in richness as opposed to the significant decrease seen in all other exploration types, potentially affecting the mobilization of different nutrient pools in the soil (Morgado et al. 2015). In the dry tundra, although total ECM basidiomycete richness tended to increase slightly in response to the warming treatment in dry tundra, the difference was not significant. Similarly, Morgado et al. (2015) did not detect significant differences among the control and treatment plots in various genera of ECM basidiomycetes, although *Cortinarius*, *Hebeloma*, *Leccinum* and *Tomentella* tended to be more diverse in the warmed plots, in agreement with our study. In ERM basidiomycetes (Sebacinales), the warming-induced decrease in OTU richness was evident in both tundra types, albeit with only marginally significant *p*-values in the dry tundra. The decrease in the number of observed OTUs of bryophilous and moss endophytic fungi (many of them sebacinoid) in the moist tundra is in line with the previously reported decline of moss cover in the warmed plots (Wahren, Walker and Bret-Harte 2005; Walker et al. 2006). However, moss endophytes were significantly more OTU rich in the warmed dry tundra plots. A similar pattern was shared by endophytes of herbs that had significantly less OTUs in the warmed moist plots, but showed positive correlation with warming in the dry tundra.

The responses of root-associated ascomycetes to warming appear less clear, as different species within various groups of root-associated fungi respond differently to warming. For example, OTU richness of DSE and ECM ascomycetes (e.g. *Cadophora*, *Cenococcum*, *Cladophialophora*, *Cryptosporiopsis*, *Phialocephala*) tended to be higher in the warmed plots in both tundra types, although the difference was only significant for DSE fungi in the moist tundra. Because these fungi are able to mineralize organic nitrogen in the rhizosphere (Newsham, Upson and Read 2009), they may contribute significantly to the growth of their hosts, possibly including increased shrub growth. However, it is worth noting that although several OTUs of DSE and ECM ascomycetes were indicators for the warmed plots, there were also some that showed significant association with the controls as shown in the section 'Results'. Similarly, we observed differential responses in ascomycete genera capable of forming ERM symbioses, e.g. *Pseudogymnoascus* and *Rhizoscyphus*. OTU richness in *Rhizoscyphus* declined in the warmed dry plots, but showed no difference in the moist plots, while *Pseudogymnoascus* clearly benefited from the warming in both tundra types. Because several *Pseudogymnoascus* species are also known to live as psychrotolerant saprotrophs (Rice and Currah 2006), the increase in available nutrients resulting from the warming-induced thawing of permafrost (Schoor et al. 2008) may contribute to the increase in their richness. This indicates that, to some extent, response to warming may be species-specific within these broadly defined ecological groups. In addition, it is likely that many binomials in our species list refer to morphological species and which, in reality, may harbour multiple phylogenetic species. For example, the DSE/ECM *Meliniomyces bicolor* was among the indicators of both the moist control and warmed plots and the dry warmed plots. However, the matching sequences and the phylogeny-based species hypothesis numbers differed among the indicators of the distinct treatments, suggesting that *Meliniomyces bicolor* may in fact include multiple evolutionary lineages, each with a specific niche and distinct response to warming. These results further emphasize the need for ecological studies at fine taxonomic scales.

The significant decrease of OTU richness of litter/dung fungi (e.g. *Clavaria*, *Coprinus*, *Lycoperdon*, *Preussia* spp.) in the moist

warmed plots is surprising, given the increased litter accumulation observed in the warmed plots (Walker et al. 2006). OTU richness in diverse taxonomic groups of soil saprotrophic (e.g. *Cladosporium*, *Coniochaeta*, *Hypocrea*, *Mollisia*, *Penicillium*, *Troposporella* and *Xylaria* in Ascomycota, *Rhodotorula* in Basidiomycota, and *Mortierella* and *Umbelopsis* in Zygomycota) and animal pathogenic fungi (*Isaria* sp., *Lecanicillium* sp., *Pochonia bulbilosa*) increased or were indicators of the warmed plots, although less so in the dry tundra that may be explained by the general environmental conditions described above. These results are in agreement with previous findings on warming-induced increase in microbial decomposition of organic matter (Nadelhoffer et al. 1992; Sistla et al. 2013) and in insect abundance in the Arctic (Hasle 2013). Similar patterns were observed in plant pathogens as well, e.g. *Chalara*, *Davidiella* and *Exobasidium*. It is unclear whether these plant pathogens primarily benefit from the increased biomass of the deciduous and evergreen shrubs (Walker et al. 2006) or from higher temperatures (or both). However, similar to other broader groups, distinct species-specific responses to warming were revealed by the indicator species analyses in saprotrophic and pathogenic taxa as well. For example, while most plant pathogen or saprotrophic indicators were associated with the moist or dry warmed plots (*Bullera* sp., *Capronia* sp., *Davidiella tassiana*, *Exobasidium areolens*, *E. woronichinii*, *Leptodontidium* sp., various *Mortierella* spp., *Phacidium lacerum*, *Rhodotorula* sp., *Pseudocercospora* sp., *Trimmatostroma botulinum*, various *Umbelopsis* spp. and *Venturia alpina*), *Leptosphaeria dolioleum* and the unidentified *Archaeorhizomyces* sp. and *Dothierella* sp. were indicators of the control plots.

Lichens showed a mixed response to warming both in terms of tundra type and lichenized fungal genera. The negative effect of warming on lichen richness in the moist plots may be partly explained by the observed decrease in cover of shade-intolerant lichens and plants in the moist warmed plots (Walker et al. 2006), while the low abundance of erect shrubs results in little shading in the dry plots. However, strong taxon-specific responses were observed again, as OTU richness of *Cladonia* and *Shaerophorus* strongly negatively correlated with warming particularly in the dry plots, while *Agonimia*, *Peltigera*, *Verrucaria* and *Xanthoria* benefited from the increased temperatures in the dry tundra. The reindeer lichens (*Cladonia* spp.) are considered keystone components in arctic ecosystems and are the main winter food source for caribou (Dahlberg et al. 2013). *Cladonia* was one of the very few fungal genera that showed consistent and strong warming-induced decline in richness in both tundra types. Because the sampled tundra types represent communities with the greatest surface areas in Northern Alaska (Walker et al. 2005), the observed trend of declining lichen richness (Semenova et al. 2015 and this study) and cover (Walker et al. 2006; Joly, Jandt and Klein 2009) may have detrimental effects on Alaskan caribou populations, with potentially profound social implications for local people (Joly, Jandt and Klein 2009).

The low diversity of AM fungi (Glomeromycota) in our samples is somewhat surprising, given that, although AM fungi tend to be rare in high arctic regions (Kohn and Stasovski 1990; Väre, Vestberg and Euroala 1992), they have nevertheless been reported from numerous sites and plant species, particularly in the Low Arctic (Strelkova 1956; Miller and Laursen 1978; Olsson, Eriksson and Dahlberg 2004). It seems unlikely that the low diversity of AM is a methodological artefact, although this possibility cannot be ruled out. Glomeromycota was represented by 409 OTUs (ca. 3% of all fungal OTUs) from diverse taxonomic orders in a Neotropical study that used the same primers and methodology as in the present study (Geml et al. 2014b). Also, Stockinger,

Krüger and Schüßler (2010) showed that primers routinely used to amplify ITS2 rDNA (including primer ITS4 used in this study) show mismatches in only a small fraction of known glomeromycete sequence types and they considered the ITS2 fragment a good candidate for AM species identification by metabarcoding studies. It is possible that AM fungi are present in very low biomass in the sampled arctic soils and some species may have been missed by our sampling efforts. The positive correlation of AM fungi with warming is in agreement with their presumed preference for higher temperatures, although the low number of AM OTUs prevented their statistical comparison between the controls and the warming treatments. In addition, because AM fungi have been shown to prefer non-acidic soils in a wide range of ecosystems (Porter, Robson and Abbott 1987; Coughlan et al. 2000; Geml et al. 2014a,b), it is possible that their richness in the acidic tundra sites, such as those sampled in this study, is unusually low. Future studies should assess the diversity of AM fungi in non-acidic arctic tundra habitats as well.

CONCLUSIONS

The data presented in this study describe changes within arctic soil fungal communities induced by long-term experimental warming. Many OTUs in this dataset could not be confidently assigned to species or genera due to limited fungal reference sequences. Nevertheless, we were able to show how the richness and composition of fungal functional groups may respond to decades of summer temperature increases. We were also able to present evidence that compositional changes in fungal communities in response to warming are species-specific, and may be masked when communities are compared at higher taxonomic levels. Therefore, we recommend that studies of arctic fungal communities (for example, their roles in nutrient cycling) take into account species-level differences.

DATA ACCESSIBILITY

The raw sequence data have been submitted to Dryad (doi:10.5061/dryad.2fc32). The representative sequences of OTUs and the OTU distribution matrices with read abundance for the moist and dry tundra are shown in the Supplementary Material.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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Conflict of interest. None declared.

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