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# Ectomycorrhizal fungal response to warming is linked to poor host performance at the boreal-temperate ecotone

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## Abstract

Rising temperatures associated with climate change have been shown to negatively affect the photosynthetic rates of boreal forest tree saplings at their southern range limits. To quantify the responses of ectomycorrhizal (EM) fungal communities associated with poorly performing hosts, we sampled the roots of *Betula papyrifera* and *Abies balsamea* saplings growing in the B4Warmed (Boreal Forest Warming at an Ecotone in Danger) experiment. EM fungi on the root systems of both hosts were compared from ambient and +3.4 °C air and soil warmed plots at two sites in northern Minnesota. EM fungal communities were assessed with high-throughput sequencing along with measures of plant photosynthesis, soil temperature, moisture, and nitrogen. Warming selectively altered EM fungal community composition at both the phylum and genus levels, but had no significant effect on EM fungal operational taxonomic unit (OTU) diversity. Notably, warming strongly favored EM Ascomycetes and EM fungi with short-contact hyphal exploration types. Declining host photosynthetic rates were also significantly inversely correlated with EM Ascomycete and EM short-contact exploration type abundance, which may reflect a shift to less carbon demanding fungi due to lower photosynthetic capacity. Given the variation in EM host responses to warming, both within and between ecosystems, better understanding the link between host performance and EM fungal community structure will to clarify how climate change effects cascade belowground.

*Keywords:* Ascomycete, boreal forest, climate change, ectomycorrhiza, fungi, host photosynthesis

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## Introduction

Global climate change poses a major threat to the health and functioning of many forest ecosystems, particularly those in boreal regions where changes are occurring rapidly (Hansen *et al*., 1996; Bonan, 2008). These high-latitude systems hold a substantial proportion of biosphere carbon in both biotic and soil pools (Lal, 2004) and elevated temperatures are likely to convert these ecosystems from atmospheric carbon sinks to sources (Davidson & Janssens, 2006; Arneth *et al*., 2010; Bradshaw & Warkentin, 2015). Boreal tree species (or populations within species) that are adapted to colder climates often respond negatively when grown under higher temperatures, in terms of photosynthetic capacity, growth, and survival (Reich & Oleksyn, 2008; Fisichelli *et al*., 2012; Reich *et al*., 2015). This is particularly true for tree populations occurring at the warmer lower end of their latitudinal ranges, where boreal species have been shown to have significant negative responses to warming compared to co-occurring temperate species (Fisichelli *et al*., 2012; Reich *et al*., 2015). The observed changes in boreal tree productivity are likely to have cascading effects belowground (Carney *et al*., 2007; Drigo *et al*., 2010). Soil microbial communities are integral components of ecosystems that mediate both decomposition and production processes (Van der Heijden *et al*., 1998, 2008; Zak *et al*., 2003; Wardle *et al*., 2004) and shifts in soil microbial communities due to changes in aboveground carbon inputs can have significant consequences for soil fertility, carbon storage, and ecosystem productivity (Zogg *et al*., 1997; Hopkins *et al*., 2014).

Ectomycorrhizal (EM) fungi dominate soil microbial communities in boreal forests and are essential for host

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nutrition and productivity in these systems (Read & Perez-Moreno, 2003; Hobbie, 2006). Trees can allocate up to 22% of net primary productivity to sustaining symbioses with EM fungi (Hobbie, 2006; Ekblad *et al.*, 2013) and recent research has highlighted the importance of this fungal guild as a major contributor and mediator of stable soil organic matter pools in forest systems (Clemmensen *et al.*, 2013, 2015; Averill *et al.*, 2014; Fernandez & Kennedy, 2016; Fernandez *et al.*, 2016). It is increasingly well recognized that EM fungal communities are typically highly diverse and composed of fungi that vary widely in their functional traits and life history strategies (Lilleskov *et al.*, 2002; Parrent *et al.*, 2010; Rineau & Courty, 2011; Koide *et al.*, 2014). Because of this functional diversity, the structure of these communities is likely to have significant direct and indirect effects on host performance and ecosystem processes. With respect to global climate change, community shifts associated with elevated temperatures may have important downstream effects on ecosystem functioning (Koide *et al.*, 2014). To date, however, understanding of changes in EM fungal community structure in response to elevated temperatures has been largely relegated to high-latitude arctic systems (Clemmensen *et al.*, 2006; Deslippe *et al.*, 2011; Geml *et al.*, 2015; Morgado *et al.*, 2015). Because tree EM hosts in arctic systems respond positively to warming in terms of photosynthetic performance (Shaver *et al.*, 2000), experiments targeting other systems where EM hosts respond differently are needed to broaden our understanding of EM fungal community response to climate change.

Fungi are known to tolerate a wide range of temperatures (*ca.* −17.5–40 °C) (Pietikäinen *et al.*, 2005), so it is likely that EM fungal responses to climate warming will manifest primarily through altered host performance or changes in soil nutrient cycling (Mohan *et al.*, 2014). The rate at which plants fix carbon is, in part, dependent on temperature (Berry & Bjorkman, 1980) and any reductions in productivity in response to warmer temperatures (or associated reductions in soil moisture) will have direct consequences for plant carbon allocation strategies (Litton *et al.*, 2007). These changes can ultimately result in reduced or increased carbon allocation belowground to EM fungi, which may affect species richness, composition, and abundance (Pena *et al.*, 2010). In addition, increased nitrogen mineralization rates and inorganic nitrogen availability have been associated with elevated soil temperatures through the stimulation of heterotrophic activity (Rustad *et al.*, 2001; Melillo *et al.*, 2011). Increased nitrogen availability to host trees often results in lowered carbon allocation to EM fungi (Nilsson & Wallander, 2003) as well as altered EM community structure (Lilleskov *et al.*, 2002; Avis *et al.*, 2003; Cox *et al.*, 2010). Finally, warming-associated reductions in water availability also have the ability to alter both EM community composition and functioning (Bell & Adams, 2004; Allison & Treseder, 2008; Gordon & Gehring, 2011).

Along with variation at the community level, how specific lineages of EM fungi will respond to changing climatic conditions is not well understood (Mohan *et al.*, 2014). While some EM genera such as *Cortinarius* appear to respond positively to warming (Clemmensen *et al.*, 2006; Deslippe *et al.*, 2011), other genera, such as *Russula*, generally respond negatively (Deslippe *et al.*, 2011). These shifts may have important consequences for nutrient cycling and host performance, as EM fungi in the genus *Cortinarius* have more extensive extraradical mycelia and higher organic nitrogen-acquiring abilities than those in the Russulaceae (Agerer, 2001; Lilleskov *et al.*, 2002). At higher taxonomic levels, a number of studies have observed positive shifts in the abundances of Ascomycetes in plots passively warmed by open-topped chambers that both warm and dry soils (Allison & Treseder, 2008; Deslippe *et al.*, 2011; Geml *et al.*, 2015; Morgado *et al.*, 2015). Ascomycetes often represent a more dominant component of EM communities in xeric than in mesic environments (Gehring *et al.*, 1998; Smith *et al.*, 2007; Gordon & Gehring, 2011), suggesting this pattern may be driven by reductions in water availability. In general, however, the limited experimental-based work and the narrow range of systems examined has hampered our ability to make consistent predictions about the effects of climate warming on specific groups of EM fungi.

In this study, we sampled a multi-year field experiment in which both air and soil temperatures have been elevated to examine the effects on EM fungal communities colonizing *Abies balsamea* and *Betula papyrifera* saplings in two sites at the boreal-temperate ecotone in Minnesota, USA. We focused on these two tree species due to their boreal distributions and reduced photosynthetic responses to warming in previous work in this study system (Reich *et al.*, 2015). Our specific questions were: (i) Do warming-induced declines in host performance influence EM community diversity? (ii) Do EM fungal communities hosted by *B. papyrifera* and *A. balsamea* saplings respond similarly to warming treatments? (iii) Are particular EM taxonomic groups or EM functional traits (i.e. mycelium exploration type and hydrophobicity) strongly associated with warming? (iv) Is there a relationship between decreasing host performance and EM fungal community composition?

## Materials and methods

### *Experimental design*

The study was part of the B4Warmed experiment (Rich *et al*., 2015), which is located in northern Minnesota at the Cloquet Forestry Center (Cloquet, MN: 46°40′46″N, 92°31′12″W, 382 m a.s.l., 4.8 °C mean annual temperature, 783 mm mean annual precipitation) and the Hubachek Wilderness Research Center (Ely, MN: 47°56′46″N, 91°45′29″W, 415 m a.s.l., 2.6 °C mean annual temperature, 726 mm mean annual precipitation). The experimental set-up at each site consisted of 36 circular 3 m diameter plots exposed to different levels of plant and soil warming (ambient, +1.7 °C, +3.4 °C; $n = 12$ for each treatment). Warming was accomplished via infrared lamp heaters aboveground and soil heating cables belowground (Rich *et al*., 2015). Lamps and cables were turned on annually from early spring to late autumn in open air (that is, without chambers) via a feedback control that acted concurrently and independently at the plot scale to maintain a fixed temperature differential from ambient conditions above- and belowground (see Rich *et al*., 2015 for additional details).

Saplings of 11 tree species were planted into existing vegetation (40- to 60-year-old stands dominated by aspen, birch, and fir) in each plot in early spring 2008. The 11 species included six native angiosperm (*Acer rubrum*, *A. saccharum*, *B. papyrifera*, *Populus tremuloides*, *Quercus macrocarpa* and *Q. rubrum*), one naturalized angiosperm (*Rhamnus cathartica*), and four native gymnosperm (*Abies balsamea*, *Picea glauca*, *Pinus banksiana*, and *Pinus strobus*) species, all of which are present in the region. Local ecotypes of all native saplings, which were in their second year of growth, were obtained from the Minnesota Department of Natural Resources. Individual saplings were planted within a randomized matrix in each plot, with 11 replicate individuals/species. Dead individuals or those that grew beyond the height of the heating lamps were removed and replanted with new individuals of the same species.

### *Plant and soil sampling*

Photosynthetic rate ($A_{net}$) at light saturating conditions (800 μmol $m^{-2}$ $s^{-1}$ light level were measured *in situ* at both sites across all temperature treatments in all years (2008–2013) of the experiment using six Li-Cor 6400 portable photosynthesis systems (Li-Cor, Lincoln, NE, USA). Photosynthetic surveys were conducted on *a priori* randomly selected individuals with fully expanded, healthy leaves. Soil temperatures were measured every ten seconds for the duration of the experiment within each plot using two sealed thermocouples (type T) installed at the depth of 10 cm. Soil moisture was monitored hourly within each plot from 0 to 20 cm depth using Time Delay Reflectometer (TDR) probes manufactured by Campbell Scientific (Campbell Scientific, Logan, UT, USA) and expressed as volumetric water content. In 2013, soil inorganic nitrogen pools ($NH_4^+$ and $NO_3^-$) and net nitrogen mineralization rates were measured over four consecutive time periods (November 2012–April, 2013, May–June, 2013; July–August, 2013, and September–October, 2013). Two 2.5 cm-diameter soil cores were obtained from each plot (9 cm depth) at the beginning of each period, sieved (2 mm), homogenized, shaken with 2 M KCl for one hour, filtered, and the extracts analyzed for $NH_4^+$ and $NO_3^-$. One additional five cm diameter nine cm deep PVC core was installed in each plot, the tops capped, and left to incubate until the end of the period, when it was processed in the same way as the initial samples. Net nitrogen mineralization rates were calculated as the difference in the inorganic nitrogen pools at the end and the beginning of each incubation period, with rates summed over all incubations periods during the year to obtain an annual rate.

### *Ectomycorrhizal fungal sampling*

In early April 2014 (after six full seasons of growth in the experiment), saplings of both host species (i.e. *A. balsamea* and *B. papyrifera*) were harvested from all of the ambient and +3.4 °C (hereafter referred to as warmed) understory plots (i.e. plots with intact canopies of 40–60 year old *Populus*-dominated forest). The root systems of 2–3 saplings per species/plot were rinsed of adhering soil in the field, combined into a pooled sample and kept in plastic bags, and transferred on ice to the laboratory within 48 h. In the lab, each pooled root sample was carefully re-washed and dried at 40 °C for 48 h. Samples were then gently individually crushed inside of folded paper to separate the smaller EM colonized roots from the larger non-colonized coarse roots. Twenty mg of the fine root pool from each sample were placed in screw-cap tubes with glass beads and homogenized for one minute via bead beating at continuous variable shaking speeds of 2000–3450 strokes per minute (BioSpec Products, Bartlesville, OK, USA). Total genomic DNA from each of the root homogenate samples was extracted using the chloroform extraction method detailed in Kennedy *et al*. (2003).

### *Molecular identification and bioinformatics*

To identify the EM fungi present on *A. balsamea* and *B. papyrifera* sapling roots, the ITS1 rDNA subunit was PCR amplified using a barcoded fungal-specific ITS1F-ITS2 primer set and cycling conditions detailed in Smith and Peay (2014). Amplified products were magnetically cleaned using the Agencourt AMPure XP kit (Beckman Coulter, Brea, CA, USA) and quantified using a Qubit dsDNA HS Fluorometer (Life Technologies, Carlsbad, CA, USA). Each of the *A. balsamea* and *B. papyrifera* root samples were pooled into a single library and sequenced at the University of Minnesota Genomics Center using the 250 bp paired-end MiSeq Illumina platform. Raw sequences and associated metadata were deposited in the NCBI Short Read Archive (Accession #: SRP080680).

Using both the QIIME and MOTHUR packages (QIIME v 1.8 (Caporaso *et al*. 2010) and MOTHUR v 1.33.3 (Schloss *et al*. 2009), we demultiplexed and quality filtered the raw sequences (i.e. culled sequences with Phred scores <20, <75 bp long, with any ambiguous bases, or a homopolymer run of >8 bp). The reverse reads in this MiSeq run were found to be of relatively

poor quality, so we used only the forward reads for all analyses. After quality filtering, we employed a multi-step operational taxonomic unit (OTU) picking strategy, first clustering all sequences with USEARCH at a 95% sequence similarity followed by reclustering with UCLUST at 95%. We found that this strategy best recovered the mock community that we included as a positive control (see Nguyen *et al*., 2015 for details) and we therefore applied it to all the experimental samples. The UNITE database (v6, Kõljalg *et al*., 2013) was used for chimera checking, OTU clustering, and assigning taxonomy. Since we have previously found that OTUs with length/query length ≤0.845 often have ambiguous taxonomies (i.e. they may not be fungal) (Nguyen *et al*., 2015), we excluded any OTUs below that threshold. For any fungal OTUs present in the mock community sample but not part of its original composition (likely due to low-level tag switching Carlsen *et al.* 2012), we subtracted the number of sequence reads of that OTU in the mock sample from the number of sequence reads of that OTU in each of the experimental samples (as described in Nguyen *et al*., 2015). As an additional quality control step, we removed all sequence reads <10 per sample for all remaining OTUs, based on the combined recommendations of Lindahl *et al.* (2013) and Oliver *et al.* (2015).

EM fungal OTUs were separated from those belonging to other guilds using the online tool FUNGuild (Nguyen *et al*., 2016). For the final EM OTU × sample matrix, we included all OTUs that FUNGuild assigned as having a 'highly probable' and 'probable' likelihood of being an EM fungal taxon. For all OTUs that had a 'possible' EM designation, we checked the species-level matches for inclusion in the final dataset and removed two *Entoloma* OTUs, one *Ceratobasidium* OTU, and one *Lyophyllum* OTU that matched more closely to non-EM than EM fungal sequences. Among the OTUs that were unassigned in FUNGuild, we determined that some were likely EM despite not being assigned to that guild (due to missing family- and/or genus-level taxonomy). We therefore checked the individual UNITE database species hypotheses (SH in Kõljalg *et al*., 2013) for each unassigned OTU. Using the criteria: (i) >90% sequence match to top BLAST hist, (ii) belonged to a lineage designated EcM in UNITE, and (iii) matched another sequence identified as ectomycorrhizal from a tree host within 3% sequence similarity, we reassigned 72 of the 335 unassigned OTUs as EM fungi. The final non-transformed EM fungal OTU × sample matrix, including taxonomic identification for each OTU, is provided in Table S1.

### *Statistical analyses*

We examined the effects of treatment (ambient vs. warmed), site (Cloquet vs. Ely), and host (*A. balsamea* vs. *B. papyrifera*) on plant photosynthetic rate using a fully factorial three-way fixed-factor analysis of variance (ANOVA). We applied a similar approach for the five soil response variables (i.e. temperature, moisture content, ammonium, nitrate, net nitrogen mineralization) using two-way fully factorial fixed-factor ANOVAs, with site and treatment as the predictor variables. For all five response variables, we analyzed data collected in 2013. The photosynthetic rates were measured on multiple days in mid-August 2013. The soil temperature and moisture values were plot-level averages from all of the data collected between June 1 and August 31. The soil nitrogen pool sizes and mineralization rates were based on summer 2013 values (soil ammonium and nitrate from cores taken July 8, and mineralization from cores incubated July 8 to September 9). Although there is a temporal offset from when these plant and soil measures were taken and when the root harvesting was done, both the strong winter climate at our study sites (which severely limits biological activity) and sufficiently long average lifespan of EM roots (typically ranging from 3 to 6 months, although in some cases much longer (Fernandez *et al*., 2013), limit our concerns about a possibility of major shifts in EM community abundance or composition between datasets (see Appendix S1 for further justifications regarding use of the plant and soil data).

To assess the experimental effects on EM fungal OTU community diversity and composition, we used the observed rather than rarefied number of sequence reads per sample because there have been concerns raised about the application of rarefaction to next-generation sequencing community datasets (McMurdie & Holmes, 2014). Prior to running statistical analyses, however, we normalized the sequence data by number of reads per sample, so that comparisons were based on relative not absolute abundances. To test for changes in EM fungal OTU diversity (Shannon index) by treatment, site, and host, we used a three-way fixed-factor ANOVA. Prior to running the ANOVA, variances among groups were determined to be homogenous using Cochran's C test. To examine the effects of treatment, site, and host on EM fungal community composition, we used two different approaches. The first was a permutational multivariate analysis of variance (PERMANOVA) using the *adonis* function in the 'VEGAN' package in R (R Core Team, 2014). For that analysis, the normalized relative abundance data were first converted into a Bray-Curtis dissimilarity matrix and then run in a model including all main effects (i.e. treatment, site, and host) and two-way interactions, set at 999 permutations. The second approach was building a generalized linear model using the package 'MVABUND'. This analysis in this package, unlike distance-based metrics such as PERMANOVA, directly accounts for the strong mean–variance relationships present in most multivariate community abundance analyses (Wang *et al*., 2012). Using the *manyglm* function, we analyzed the effects of treatment, site, and host and all two-way interactions using a negative binomial model. To satisfy the data formatting parameters, the normalized relative abundance data was multiplied by 1000 and rounded up to the nearest integer (to be input as count data). Significance was calculated using a Wald statistic and *P* values assigned following 999 iterations (via PIT-trap resampling to account for correlation in testing) using the ANOVA function. To visualize variation in EM fungal community composition by treatment, site, and host, we also generated a series of non-metric multidimensional scaling plots using the *metaMDS* function in the 'VEGAN' package in R.

We also used the CLAssification Method program (CLAM) in the 'VEGAN' package in R to identify EM taxonomic clades (mostly genera but in some cases higher taxonomic level groupings) with significant preferences for ambient vs.

warmed plots (Chazdon *et al.*, 2011). This program uses a multinomial statistical approach to classify taxa as specialists or generalists based on relative abundance data in binary habitats, which in this case were the ambient and warmed plots. The algorithm assesses whether the relative abundance of a taxa is significantly greater in one habitat or the other by performing a one-sided statistical test, with the significance threshold adjusted for multiple comparisons. For these tests, we used the super majority specificity rule (2/3; $\alpha < 0.005$) to determine significance. As with the generalized linear model analysis, to satisfy the data format parameters, we multiplied the EM fungal OTU relative abundances by 1000 and rounded up to the next integer. We also calculated the individual EM fungal OTU responses by calculating the differences in relative abundance between treatments divided that by the relative abundance of the OTU in all samples. To further assess EM fungal community responses to elevated temperature, we compared mean relative abundances of EM fungal groupings: phylum (Ascomycota or Basidiomycota), extramatrical exploration type (Contact-Short, Contact-Medium, or Medium-Long), and mycelial hydrophobicity (Hydrophilic or Hydrophobic) with three-way fixed-factor ANOVAs. Assignments of exploration types and hydrophobicity followed Agerer (2001) and Lilleskov *et al.* (2011) (Table S11). Finally, based on the strong response of many EM Ascomycetes and Contact-Short exploration types to warming (see below), we examined the relationship between EM host photosynthetic rate and relative abundance of each of the groups mentioned above using simple linear regression analyses.

## Results

### *Plant and soil responses*

Photosynthetic rates in August 2013 for both *A. balsamea* and *B. papyrifera* saplings were significantly lower in the warmed plots (Treatment: $F_{1,16} = 31.39$, $P < 0.0001$), being 43% below those in the ambient plots (Table 1). The photosynthetic rates were significantly higher (23%) at Ely compared to Cloquet (Site: $F_{1,16} = 7.67$, $P = 0.0153$). Despite *B. papyrifera* saplings having photosynthetic rates that were 40% higher than *A. balsamea* saplings (Host: $F_{1,16} = 27.31$, $P < 0.0001$), there was no significant treatment × host interaction (Interaction: $F_{1,16} = 0.22$, $P = 0.6466$). All of the other higher order interactions were also not significant (Table S2). Experimental warming significantly elevated soil temperature (Treatment: $F_{1,20} = 916.11$, $P < 0.0001$), being, on average, 3.5 degrees higher in the warmed plots compared to the ambient plots (Table 1). Conversely, soil moisture content was significantly lower in the warmed plots (18% lower, Treatment: $F_{1,20} = 105.61$, $P < 0.0001$). Soil moisture was also significantly different by site (Site: $F_{1,20} = 163.3$, $P < 0.0001$); on average, Cloquet had both cooler and drier soils than Ely (Table 1). Soil ammonium, nitrate, and mineralization tended to be higher in the warmed plots and at Ely, but none of these three measures were significantly different by treatment or site (Table S3). There were also no significant site × treatment interactions for any of soil-related measures.

### *Ectomycorrhizal fungal responses*

Of the 2 174 374 sequence reads that passed quality control, 1 929 652 (89%) reads belonged to EM fungal taxa. Sequence abundance averaged 43 546 reads per sample (min: 13 744; max: 58 984) and there were a total of 217 EM fungal OTUs across the 44 samples. EM fungal OTU diversity did not differ significantly by treatment or host (Table S4), but was higher at Cloquet than Ely (Site: $F_{1,36} = 6.73$, $P = 0.014$; Fig. S1). For EM fungal community composition, the PERMANOVA model indicated a significant effect of site (Site: $F_{1,37} = 2.73$, $P < 0.001$) and host (Host: $F_{1,37} = 1.53$, $P = 0.049$), but not of treatment (Table S5). In contrast, the generalized linear model indicated that site (Site: $\text{Wald}_{1,41} = 17$, $P < 0.001$) and treatment (Treatment: $\text{Wald}_{1,42} = 15$, $P = 0.013$; Table S6) but not host (Table S6) significantly influenced EM fungal community composition. In addition, the effect of treatment in the generalized linear model was site and host dependent (Site × Treatment: $\text{Wald}_{1,39} = 9$, $P = 0.025$; Host × Treatment: $\text{Wald}_{1,38} = 10$, $P = 0.023$). NMDS visualization of EM community composition was consistent with interactive effects of site and treatment (Fig. 1). No other interaction terms were significant in either model (Tables S5 and S6).

The CLAM tests revealed that many EM fungal genera had significant differences in relative abundance between the warmed and ambient plots. *Cenococcum*, *Hebeloma*, *Hydnum*, *Laccaria*, *Peziza*, *Thelephora*, *Tomentellopsis*, and *Wilcoxina* all had significantly greater relative abundances in the warmed plots, while *Alnicola*, *Amphenima*, *Clavulina*, *Cortinarius*, *Lactarius*, *Pseudotomentella*, *Russula*, *Tomentella*, and *Tylospora*, all had significantly greater relative abundances in the ambient plots (Fig. 2).

When grouped at the phylum level, EM Ascomycete OTU relative abundance was significantly higher under elevated temperature treatment (ANOVA; $F_{1,43} = 4.91$, $P = 0.032$), while EM Basidiomycete OTU relative abundance was significantly lower in warmed plots (ANOVA; $F_{1,43} = 4.84$, $P = 0.033$) (Fig. 3a). Across treatments, sites, and hosts, the relative abundance of EM fungal Ascomycetes was also significantly negatively correlated with sapling photosynthetic rate at both the Cloquet ($P = 0.027$; $R^2 = 0.403$) and Ely ($P = 0.009$; $R^2 = 0.507$) sites (Fig. 3a; Table S8). When EM fungal OTUs were grouped by exploration type the 'Contact-Short' group had significantly higher relative

**Table 1** Plant host (A) and soil (B) measurements in ambient (0 °C) and warmed (+3.4 °C) plots at the B4Warmed study sites (Cloquet and Ely) in 2013. Values represent means (± one standard error). Units: Photosynthesis (umol $CO_2$ $m^{-2}$ $s^{-1}$), Temperature (°C), Volumetric Moisture ($cm^3$ $H_2O$ per $cm^3$ soil), Ammonium (mg N per g soil), Nitrate (mg N per g soil), Net Nitrogen Mineralization (mg N g $soil^{-1}$ $yr^{-1}$). See methods for details about the timing of measurements

| | Plant | | | |
|---|---|---|---|---|
| | *Abies* | | *Betula* | |
| (A) | Cloquet Photosynthesis | Ely Photosynthesis | Cloquet Photosynthesis | Ely Photosynthesis |
| Ambient | 3.78 (0.41) | 4.35 (0.66) | 5.64 (0.74) | 7.11 (0.53) |
| Warmed | 1.74 (0.48) | 2.23 (0.65) | 2.99 (0.56) | 4.83 (0.49) |

| | Soil Cloquet | | | | |
|---|---|---|---|---|---|
| (B) | Temperature | Moisture | Ammonium | Nitrate | Mineralization |
| Ambient | 15.6 (0.1) | 0.192 (0.003) | 0.0013 (0.0004) | 0.00012 (0.0002) | −0.0013 (0.0004) |
| Warmed | 19.1 (0.1) | 0.158 (0.003) | 0.0021 (0.0005) | 0.00021 (0.0006) | 0.0020 (0.0032) |
| | Ely | | | | |
| | Temperature | Moisture | Ammonium | Nitrate | Mineralization |
| Ambient | 15.7 (0.1) | 0.250 (0.006) | 0.0011 (0.0002) | 0.0014 (0.0003) | −0.0030 (0.0035) |
| Warmed | 19.2 (0.1) | 0.217 (0.006) | 0.00010 (0.0004) | 0.00011 (0.0001) | −0.0004 (0.0004) |

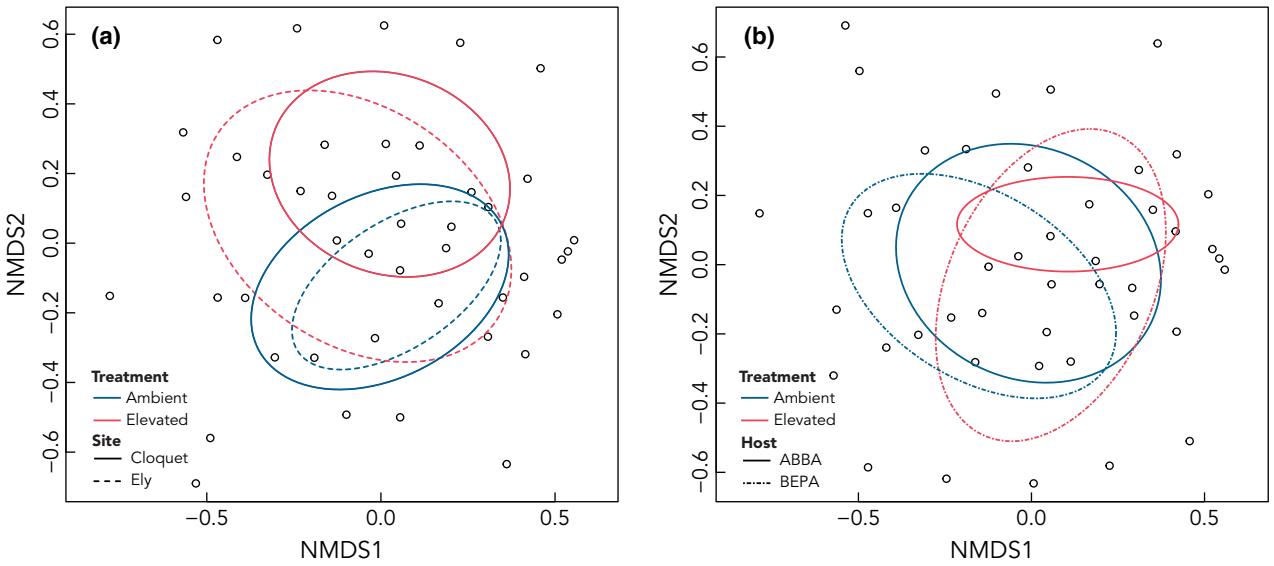


**Fig. 1** Non-metric multidimensional scaling (NMDS) plots of the ectomycorrhizal fungal communities on *Abies balsamea* and *Betula papyrifera* sapling roots at the two B4Warmed study sites. Points in ordination space represent individual plots based on Bray-Curtis dissimilarity indices. Standard deviation ellipses are overlaid on the ordination plots to help visualize the interactive effects of treatment, site, and host on ectomycorrhizal fungal community composition. [Colour figure can be viewed at wileyonlinelibrary.com]

abundance in warmed plots ($F_{1,43}$ = 4.82, $P$ = 0.0336) while the 'Contact-Medium' group had significantly higher relative abundance in the ambient plots ($F_{1,43}$ = 8.32, $P$ = 0.0062). The relative abundance of 'Medium-Long' did not differ significantly between ambient and elevated temperature treatments. Simple linear regression analyses indicated that these responses of EM exploration types to treatment were linked to host photosynthetic performance across sites (Fig. 3b). The relative abundance of 'Contact-Short'

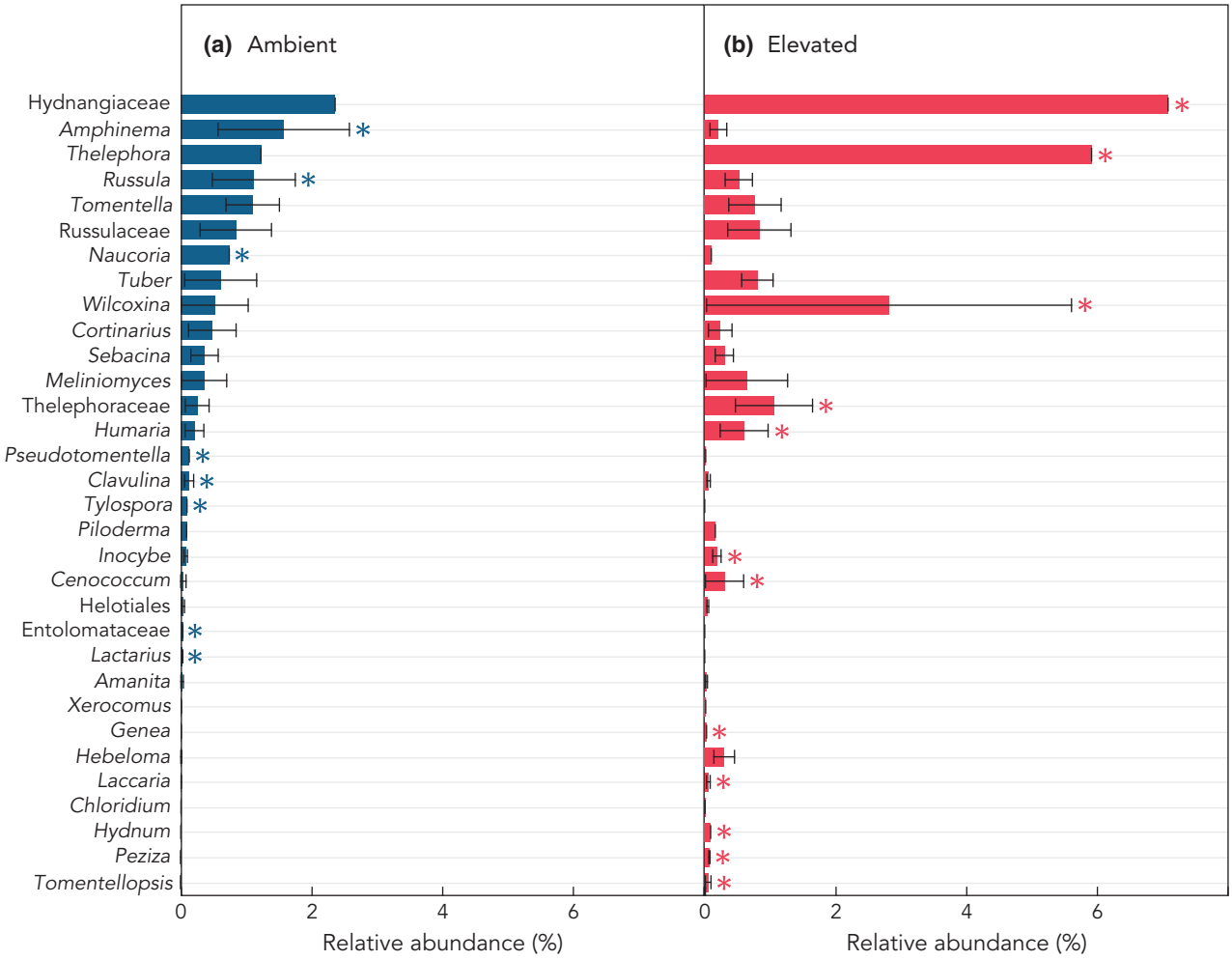


**Fig. 2** Abundances of ectomycorrhizal fungal taxa with >0.0025% relative abundance across all samples. Treatment colors: ambient (blue bars) and elevated (red bars). Values represent means ± one standard error. Asterisks indicate a significantly higher abundance in either ambient (blue asterisk) or elevated (red asterisk) plots as determined by the CLAM test. [Colour figure can be viewed at wileyonlinelibrary.com]

exploration types were significantly negatively correlated with host photosynthetic rate (Cloquet, $P = 0.005$; $R^2 = 0.56$; Ely $P = 0.02$; $R^2 = 0.44$), while 'Contact-Medium' exploration were weakly positively correlated host photosynthetic rate (Cloquet: $P = 0.16$, $R^2 = 0.19$; Ely: $P = 0.09$, $R^2 = 0.26$). Finally, when EM fungal OTUs were grouped by hydrophobic properties we found no significant difference in relative abundance of 'Hydrophilic' or 'Hydrophobic' types between the treatments (Fig. 3c) and found no relationship between their relative abundance and host photosynthetic performance (Table S10).

## Discussion

Consistent with results from the first three years of the B4Warmed experiment (Reich *et al.*, 2015), we found that the photosynthetic performance of the both *A. balsamea* and *B. papyrifera* saplings was significantly suppressed by warming in 2013. This response differs notably from studies at higher latitudes, where experimental warming significantly increased EM host photosynthetic performance (Shaver *et al.*, 2000). The discrepancy between ecosystems likely reflects the extent to which host photosynthetic rates are temperature- and/or moisture-limited. In moist high-latitude systems, where air temperatures rarely reach levels that induce photorespiratory stress and soil moisture is generally high throughout the growing season, warming consistently enhances plant carbon fixation. In contrast, in our study system, which is located at the southern boundary of the boreal forest in well-drained upland soils, air temperatures can be high enough to create leaf thermal stress and dry soil conditions during both the

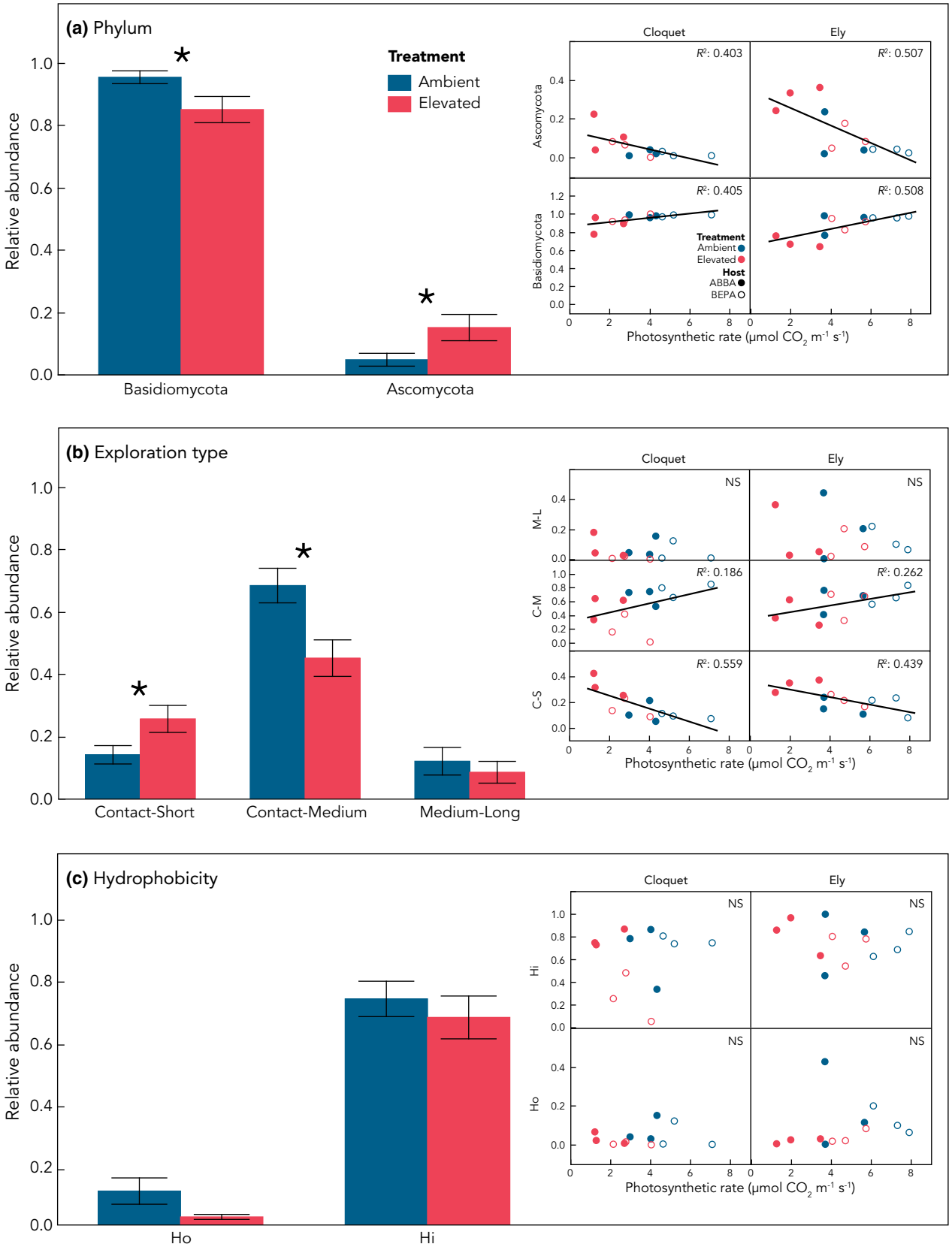
(a) Phylum
Relative abundance
Treatment
Ambient
Elevated
Basidiomycota
Ascomycota
Cloquet
Ely
Ascomycota
Basidiomycota
R²: 0.403
R²: 0.507
R²: 0.405
R²: 0.508
Treatment
Ambient
Elevated
Host
ABBA
BEPA
Photosynthetic rate (μmol CO2 m-1 s-1)
(b) Exploration type
Contact-Short
Contact-Medium
Medium-Long
M-L
C-M
C-S
NS
R²: 0.186
R²: 0.262
R²: 0.559
R²: 0.439
(c) Hydrophobicity
Ho
Hi
NS

**Fig. 3** Mean relative abundance (%) of ectomycorrhizal fungi when grouped by phylum (a), exploration type (b), and hydrophobicity [Hydrophobic (Ho); Hydrophilic (Hi)] (c) by treatment. Treatment colors: ambient (blue bars) and elevated (red bars). Values represent means ± one standard error. Significant differences are indicated with an asterisk. Relationship between relative abundance of the EM fungal groupings (%) and host photosynthetic rates (Anet) by site are shown in sub-panels. Data points in linear regression analyses represent plot-level means. Host species identity is indicated with closed (Abies balsamea) or open (Betula papyrifera) points and treatment is indicated with blue (Ambient) and red (Elevated) colors. [Colour figure can be viewed at wileyonlinelibrary.com]

summer and fall. As such, the addition of above- and belowground heating exacerbates stressful abiotic conditions, causing a reduction of stomatal conductance and carbon fixation for the saplings accustomed to cooler climates (Reich *et al*., 2015). Given the tight link between recently generated plant photosynthate and EM fungal biomass (Högberg *et al*., 2001, 2010; Pena *et al*., 2010), the >40% warming-induced decline in photosynthetic rates of these two boreal sapling hosts likely strongly affected the carbon available to allocate to EM fungi at the time of our sampling.

With regard to experimental warming, some studies have documented significant decreases in EM fungal richness (Geml *et al*., 2015; Morgado *et al*., 2015) in warmed plots, although others have found the opposite (Deslippe *et al*., 2011; Treseder *et al*., 2016). We hypothesize that the lack of a significant effect on OTU diversity in our system may be due to design of the B4Warmed experiment, which included a set of temperate and boreal EM host species planted together at high densities. Since some of the EM host species (*Quercus* and *Populus* spp.) are responding positively to elevated temperatures (see Reich *et al*., 2015), their presence in the warmed plots may help to maintain EM fungal OTU diversity among all of the EM hosts due to the low host specificity observed in this and many EM systems (Horton & Bruns, 1998; Kennedy *et al*., 2003). Similarly, adult *Populus* tree hosts present at the sites, which are not subjected to experimental manipulation, may also buffer against negative responses to warming of sensitive EM fungal taxa. At the community level, given the strong mean-variance relationship present in our data (Fig. S2), we feel more confident in the general linear model than the PERMANOVA model in representing the effect of treatment on EM fungal community composition. Warming significantly affected EM fungal community composition at both sites, but its effect was stronger at Cloquet, which had cooler and drier average soil conditions than Ely. With regard to the significant treatment × host interaction, the change in EM fungal community composition due to warming was greater for *B. papyrifera* than *A. balsamea*. This was somewhat surprising, given the more negative effect of warming on *A. balsamea* photosynthetic rates. It is possible that warming differentially altered C allocation patterns between hosts (Sevanto & Dickman, 2015), although more research is needed to test this possibility. Taken together, it appears that warming has important effects on overall EM fungal community structure, but that its effect is co-mediated by both abiotic and biotic factors.

At both the genus and phylum levels, there were clear positive and negative responses to elevated temperatures among EM fungi. Many of the EM Ascomycete genera (e.g. *Cenococcum*, *Humaria*, *Peziza*, *Wilcoxina*) had significantly greater relative abundance in the warmed plots, suggesting a strong positive response of members of this phylum to the altered abiotic conditions. These results parallel those of Allison & Treseder (2008) and Geml *et al*. (2015), who also found that EM Ascomycetes responded positively to experimental warming in high-latitude systems (but see Deslippe *et al*., 2011 for a different pattern). The mechanism underlying this change may be driven by a direct fungal response, as EM Ascomycetes are often more abundant in drier ecosystems (Smith *et al*., 2007; Gordon & Gehring, 2011). It is also possible that this shift may be related to the EM host responses to warming. Due to their relatively thin mantles and short distance extramatrical mycelium (Agerer, 2001), EM Ascomycetes may be less costly in terms of carbon to their host, which could be beneficial under stress-related reductions in photosynthetic rates (Gordon & Gehring, 2011). This hypothesized linkage is further supported by the significant inverse relationship between EM Ascomycete relative abundance and host photosynthetic rates. Moreover, the relative abundance of 'Contact-Short' distance exploration types, which include some EM Basidiomycetes in addition to the EM Ascomycetes, was significantly higher under elevated temperatures and was strongly negatively correlated with host photosynthetic rates. Conversely, the relative abundance of 'Contact-Medium' exploration types was significantly lower under elevated temperatures and was trended positively with host photosynthetic rates. A similar link between host performance and EM community composition was apparent in studies of the arctic EM host *Betula nana*, where increased host photosynthetic performance corresponded with significant increases in the abundance of *Cortinarius* and carbon costly EM fungi in warmed plots (Shaver *et al*., 2000; Deslippe *et al*., 2011). While we did not find a significant response of 'Medium-Long' exploration types to elevated temperatures and host performance, this may be due to the relatively small proportion of the community

that these fungi comprise at our sites (thereby reducing the power to detect trends in longer distance exploration types).

There is considerable variation in life history strategies among EM fungi and the ecological success of these strategies is thought to correspond, at least in part, with abiotic and biotic conditions. A number of EM Basidiomycete genera possessing 'ruderal' life-history strategies also showed significant positive responses to warming. These taxa included *Hebeloma*, *Laccaria*, and *Thelephora*, all of which typically lack host specificity, have high reproductive rates, and are present in disturbed or early successional stage forests characterized by high soil fertility (Last *et al*., 1987). These EM fungi are also generally incapable of acquiring and utilizing organic forms of nitrogen (Abuzinadah & Read, 1986) and do not provide a strong net benefit to host trees in low fertility soils (Abuzinadah & Read, 1989; Finlay *et al*., 1992; Abuzinadah *et al*., 1986). Similar to EM Ascomycetes, these taxa are also considered to have relatively low carbon cost to hosts (Heinonsalo *et al*., 2010), which may facilitate their positive response to reduced host performance under elevated temperatures. Other warming studies have generally reported positive responses among *Cortinarius* and other 'late-stage' EM fungal genera and have hypothesized this might be the result of increased soil organic matter decomposition rates and organic nutrient acquisition associated with elevated temperatures (Deslippe *et al*., 2011; Treseder *et al*., 2016). In our study, however, many of these 'late-stage' fungi such as *Cortinarius*, *Lactarius*, *Russula*, and *Tomentella* responded negatively to warming. We suggest that in addition to differences in host responses, the lack of correspondence among these studies may be a product of the significantly greater amounts of nutrients found in organic forms in higher-latitude soils (Deslippe *et al*., 2011; Geml *et al*., 2015; Morgado *et al*., 2015), which would more strongly favor EM fungal taxa with stronger organic nutrient acquisition capabilities.

Considered collectively, our results suggest that host photosynthetic performance can have strong effects of EM fungal community composition, and that warming-induced changes, either positive or negative, have cascading effects belowground. By utilizing the B4Warmed experiment, which coupled both above- and belowground warming, our study examined EM fungal community responses under ecological conditions most likely to be present during near-term climate change. It is, to our knowledge, the first to focus on EM fungal communities associated with hosts that are experiencing significant declines in photosynthesis due to warming. We believe that the observed belowground changes in the warmed plots could have important effects on boreal forest dynamics, particularly at latitudes where temperatures are rapidly moving outside host temperature optima. As temperatures rise, shifts in composition towards EM fungal taxa that are relatively less expensive in terms of carbon allocation may benefit boreal saplings experiencing lower photosynthetic rates. However, unless EM fungal community shifts happen concurrently with increases in inorganic nitrogen pools, greater colonization by EM Ascomycete and ruderal Basidiomycete genera may further hinder saplings already negatively affected by warming (due to limited soil exploration capacities). More broadly, our results, combined with previous work in other boreal and arctic systems, suggest that explicit consideration of host physiological responses to warming will significantly improve understanding how both particular fungal taxa as well as whole EM fungal communities will change under the altered climatic conditions that lie ahead.

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## Conflict of interest

The authors declare no conflicts of interest.

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## Supporting Information

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

**Figure S1.** Differences in EM fungal OTU diversity by site and host.
**Figure S2** Relationship between sequence read means and variances of each OTU in the generalized linear model analysis of ectomycorrhizal fungal community composition.
**Table S1.** Sample × OTU matrix for final ectomycorrhizal fungal dataset.
**Table S2.** ANOVA table for effects of site, treatment, and host on sapling photosynthetic rates.
**Table S3.** ANOVA tables for effects of site and treatment on soil temperature, moisture, ammonium, nitrate, and mineralization.
**Table S4.** ANOVA table for effects of treatment, site, and host on ectomycorrhizal fungal OTU diversity.
**Table S5.** PERMANOVA table from the Adonis analysis examining the effects of treatment, site, and host on ectomycorrhizal fungal community composition.
**Table S6.** ANOVA table from the generalized linear model analysis examining the effects of treatment, site, and host on ectomycorrhizal fungal community composition.
**Table S7.** ANOVA tables for effects of treatment on the relative abundance of ectomycorrhizal groups for phylum, exploration type, and hydrophobicity
**Table S8.** Simple linear regression analyses examining the effects of host photosynthetic rates on the relative abundance of ectomycorrhizal Ascomycetes and Basidiomycetes by site.
**Table S9.** Simple linear regression analyses examining the effects of host photosynthetic rates on the relative abundance of ectomycorrhizal exploration types by site.
**Table S10.** Simple linear regression analyses examining the effects of host photosynthetic rates on the relative abundance of hydrophobicity of ectomycorrhizal fungi by site.
**Table S11.** Exploration type, hydrophobicity, and life history strategy assignments for ectomycorrhizal genera.
**Appendix S1.** Plant and soil measurement justifications.