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Warming and drying suppress microbial activity and carbon cycling in boreal forest soils

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Abstract

Climate warming is expected to have particularly strong effects on tundra and boreal ecosystems, yet relatively few studies have examined soil responses to temperature change in these systems. We used closed-top greenhouses to examine the response of soil respiration, nutrient availability, microbial abundance, and active fungal communities to soil warming in an Alaskan boreal forest dominated by mature black spruce. This treatment raised soil temperature by 0.5 °C and also resulted in a 22% decline in soil water content. We hypothesized that microbial abundance and activity would increase with the greenhouse treatment. Instead, we found that bacterial and fungal abundance declined by over 50%, and there was a trend toward lower activity of the chitin-degrading enzyme *N*-acetyl-glucosaminidase. Soil respiration also declined by up to 50%, but only late in the growing season. These changes were accompanied by significant shifts in the community structure of active fungi, with decreased relative abundance of a dominant Thelephoroid fungus and increased relative abundance of Ascomycetes and Zygomycetes in response to warming. In line with our hypothesis, we found that warming marginally increased soil ammonium and nitrate availability as well as the overall diversity of active fungi. Our results indicate that rising temperatures in northern-latitude ecosystems may not always cause a positive feedback to the soil carbon cycle, particularly in boreal forests with drier soils. Models of carbon cycle-climate feedbacks could increase their predictive power by incorporating heterogeneity in soil properties and microbial communities across the boreal zone.

Keywords: bacteria, boreal forest, climate change, extracellular enzyme, microbial community, mycorrhizal fungi, nitrogen availability, nucleotide analog, soil respiration, warming

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Introduction

High latitude ecosystems, including tundra and boreal forest, are predicted to warm substantially over the 21st century due to anthropogenic climate change (IPCC, 2007). Already, these systems have warmed by ~1.5 °C (Moritz *et al*., 2002), and an additional 4–7 °C increase is expected by 2100 (ACIA, 2004). Warming temperatures may also be associated with changes in ecosystem water balance via increased rates of evapotranspiration and altered precipitation regimes (IPCC, 2007). Because high-latitude systems store up to 30% of global terrestrial carbon (C) in soils and plant biomass (Gorham, 1991; Jobbagy & Jackson, 2000; Kasischke, 2000), feedbacks between climate and the C cycle could have strong impacts on atmospheric CO_2 concentrations. Thus, understanding the mechanisms that link temperature change and the C cycle are important for predicting future global change.

Despite the importance of this feedback, experiments that directly test the impact of warming on ecosystem processes are relatively scarce. A meta-analysis by Rustad *et al*. (2001) found that warming increased soil respiration by 20% ($n = 17$ sites) and net nitrogen (N) mineralization by 46% ($n = 12$ sites). Additional studies in the Alaskan boreal zone have produced similar results (Van Cleve *et al*., 1990; Bergner *et al*., 2004). However, these analyses do not represent the full range of ecosystems in these biomes, and the mechanisms by

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which warming alters soil processes remain unclear. For example, a recent study in Canadian boreal forest found that soil respiration increased in response to direct soil warming, but declined if the air above the plots was also heated (Bronson *et al*., 2008). Given the relevance of arctic and boreal ecosystems for the global C cycle and the heterogeneity of northern-latitude vegetation and soils, additional mechanistic experiments are warranted.

Microbial communities are likely to play a major role in regulating climate feedbacks to the C cycle. Because microbes degrade and mineralize organic material, warming effects on microbial communities may alter ecosystem C and nutrient balance. In boreal ecosystems, fungi are particularly important drivers of these processes because they are adapted to low soil pH (Högberg *et al*., 2007) and commonly form mycorrhizal associations that help boreal plants acquire nutrients (Read *et al*., 2004). Both mycorrhizal and nonmycorrhizal fungi produce extracellular enzymes that release labile C and nutrients from organic matter (Read & Perez-Moreno, 2003).

Fungal communities and processes are known to respond to changes in temperature (Robinson, 2002). In Alaskan arctic tundra, warming increased the soil concentration of the fungal biomarker ergosterol (Clemmensen *et al*., 2006). Similarly, warming increased the abundance of arbuscular mycorrhizal fungi in a California annual grassland (Rillig *et al*., 2002). In a laboratory microcosm experiment with wood-decay fungi, fungal diversity was higher under a fluctuating temperature regime than at constant temperature (Toljander *et al*., 2006). Increasing temperatures due to climate change in England have resulted in a longer period of sporocarp production, with some taxa fruiting twice during the growing season (Gange *et al*., 2007). Although these studies demonstrate that warming affects fungal communities, they do not address whether specific fungal taxa control ecosystem responses to warming (Robinson, 2002).

Our objective was to assess ecosystem and microbial responses to warming in a well-drained, black spruce forest in central Alaska. Previous warming manipulations in this region have focused on early successional ecosystems following fire (Bergner *et al*., 2004), or systems underlain by permafrost (Van Cleve *et al*., 1990). Our study is unique because we examine the warming effect on boreal soils in a mature spruce ecosystem with no permafrost. Based on prior studies, we hypothesized that warming would increase the abundance and activity of soil microbial taxa, particularly fungi. In turn, we predicted that warming would stimulate organic matter turnover, soil respiration, and nutrient mineralization.

Materials and methods

Site description

We established the warming experiment in a mature black spruce [*Picea mariana* (P. Mill.) B.S.P] forest located in central Alaska, USA, (63°55′N, 145°44′W) and described in detail by Treseder *et al*. (2004). Soils are well-drained with an O-horizon thickness of $\sim$ 9.8 cm (King *et al*., 2002) and have a pH of 4.9 (2 : 1 DI water : soil; S. D. Allison, unpublished data). The vegetation understory is dominated by mosses, lichens, and the ericoid shrubs *Vaccinium uliginosum*, *V. vitis-idaea*, *Ledum groenlandicum*, and *Empetrum nigrum*, as well as the ectomycorrhizal shrub *Betula glandulosa* (Treseder *et al*., 2004). The growing season begins with bud break in mid-May and extends until leaf senescence in mid-September.

Five pairs of 2.5 m $\times$ 2.5 m plots were established in a 1 km^2 area of forest. The experiment should be considered mainly a soil manipulation, as the dominant plants (black spruce) were not included in the plots. However, there were spruce trees present within 1 m of each of the plots, so spruce roots were very likely present in the plots. One plot from each pair was assigned to the warming treatment while the other served as a control. Plots in each pair were located 3–5 m apart and contained visually similar vegetation communities. Soils were warmed passively with closed-top chambers (greenhouses; Plate 1) constructed of two-by-fours and 6 mil greenhouse plastic (AT Plastics, Inc., Edmonton, Canada), which transmits 60–70% of photosynthetically active radiation (Krizek *et al*., 2005). Gaps between the plastic and the frame or the ground allowed air to circulate through the greenhouse. We installed gutters and tubing to allow precipitation to reach the plot during the warming treatment. The tubing terminated

Plate 1 Greenhouse constructed to passively warm boreal forest soils in central Alaska.

at eight points separated by ~50 cm in order to distribute precipitation evenly within each greenhouse. The treatment was applied during the 2005–2007 growing seasons; greenhouses were left in place between growing seasons, but the top plastic panel was removed to allow snow fall to reach the plot. We did not include a chamber control without plastic panels because a recent study at a nearby site found no differences between chamber controls and control plots without chambers (Bergner *et al*., 2004).

We measured soil temperatures in paired control and warmed plots using Onset HOBO dataloggers. Three pairs of dataloggers were buried at 5 cm depth and recorded temperature every 30 min. Dataloggers were moved to a different subset of the five experimental blocks every 1–2 months. Only five plots were monitored during the first 3 months of 2005 due to datalogger failure. Most of the dataloggers were moved to a different experiment after 2006, so 2007 measurements are based on one pair of dataloggers that were left in a single experimental block for the entire growing season.

Soil respiration

We measured soil respiration rates with an infrared gas analyzer (PP Systems EGM-4, Amesbury, MA, USA) to monitor the change in CO_2 concentration over time in flux chambers. Two 25 cm diameter chamber bases were installed in each plot on May 25, 2005, to a depth of 2–3 cm. Surface vegetation was left intact in the chambers. Fluxes were measured every 1–2 months during the 2005, 2006, and 2007 growing seasons starting in July 2005, for a total of 11 time points. For each measurement, we monitored CO_2 concentrations for 5–10 min after placing a lid over the chamber base (Allison *et al*., 2008). CO_2 concentrations in the chambers generally did not exceed 600 ppm during the measurement interval. Chamber volumes were corrected for moss and litter content, and the flux was calculated as

$$f = \frac{mV}{ART},$$

where m is the change in CO_2 concentration in the chamber with time, V the chamber volume, A the cross-sectional area of the chamber, R the ideal gas constant, and T is the chamber air temperature in Kelvin. Atmospheric pressure was assumed to be 1 atm. We also inserted a temperature probe at 5 cm depth next to each flux chamber to measure soil temperature during respiration measurements.

Nutrient availability

We measured the availability of NH_4^+, NO_3^-, and PO_4^{3-} using resin bags as described by Allison *et al*. (2008). Four anion and four cation resin bags were placed in each warmed and control plot at 5 cm depth on May 28, 2005. Bags were collected and replaced on July 27, 2005; the new batch was retrieved on September 15, 2005. In 2006, resin bags were placed in the soil from May 14 until September 17. Collected bags were rinsed in DI water and extracted in 0.1 M HCl/2.0 M NaCl. We measured NH_4^+ concentrations using a modified Berthelot-salicylate method (Weatherburn, 1967) and NO_3^- concentrations with the vanadium method of Doane & Horwath (2003). PO_4^{3-} concentrations were determined spectrophotometrically at 630 nm according to the malachite green method of Lajtha *et al*. (1999) adapted for microplates. Nutrient availabilities were expressed as ng nutrient (N or P) g^{-1} resin day^{-1}.

Microbial community structure

We used nucleotide analog labeling with bromodeoxyuridine (BrdU) to examine the structure and diversity of active fungal communities (Borneman, 1999; Allison *et al*., 2007). In each control and warmed plot, we injected 10 mL of 2.5 mM BrdU solution at each of the five points in an 'X' pattern (~ 1 m^2) on August 17, 2006. After a 24-h incubation period, we collected a 2 cm diameter × 5 cm depth core from each injection point and combined cores within a plot. We froze the cores within 1 h, shipped them back to UCI frozen, and stored them at −80 °C until DNA extraction.

Before DNA extraction, cores from each plot were homogenized in a blender for ~ 10 s. We extracted DNA from a ~ 0.15 g subsample of soil from each plot using the MoBio PowerSoil (MO BIO Laboratories, Carlsbad, CA, USA) DNA kit. We did not attempt to remove roots or ectomycorrhizal root tips from the soil samples because we were interested in warming effects on the entire fungal community.

We used the immunocapture procedure of Yin *et al*. (2004) to isolate BrdU-labeled DNA from the total DNA extract. This procedure allowed us to examine the community structure of active fungi because only actively growing microbes incorporate BrdU into DNA. We also subjected unlabeled soil DNA extracts from the same site to the same procedure as a control for immunocapture of unlabeled DNA.

To identify fungal taxa, we amplified a ~ 760 bp region of the 18S ribosomal gene using the general fungal primers nu-SSU-0817-5′ and nu-SSU-1563-3′ (Borneman & Hartin, 2000). The PCR contained 2.5 mM $MgSO_4$, 0.5 μg μL^{-1} BSA, 250 μM of each dNTP,

400 nM of each primer, 0.04 U μL^{-1} Invitrogen High Fidelity Platinum Taq (Carlsbad, CA, USA), 1 × Platinum Taq buffer (Invitrogen, Carlsbad, CA, USA), and 0.1 μL template DNA μL^{-1} reaction mixture. PCR was carried out on a BioRad iCycler thermocycler (Bio-Rad Laboratories, Hercules, CA, USA) with a 3 min initial denaturation step at 94 °C; 32–35 cycles of 1 min denaturation at 94 °C, 1 min primer annealing at 49 °C, and 2 min elongation at 72 °C; and a final 2 min elongation step at 72 °C.

Amplified PCR products were purified by gel extraction and used to create clone libraries for control and warmed treatments (Allison *et al*., 2007). PCR amplifications from no-BrdU controls produced very weak bands, so we assume that contamination from unlabeled (nonactive) DNA is minimal. Clones were sequenced by Agencourt Bioscience Corporation (Beverly, MA, USA), resulting in 79–95 clones $plot^{-1}$.

BIOEDIT (Hall, 1999) and CLUSTALW (Chenna *et al*., 2003) were used to edit and align ~700 bp of the 18S ribosomal gene. We removed bad sequence reads, nonfungal sequences, and potential chimeras from the alignment and used it to create distance matrices in the PHYLIP program DNADIST (Felsenstein, 2005). The matrices were generated with an F84 evolutionary model and a transition : transversion ratio of 1.0.

To group fungal DNA sequences into operational taxonomic units (OTUs), we used the software program DOTUR (Schloss & Handelsman, 2005) with distance matrices from our alignment as inputs. We defined OTUs with sequence similarity cutoffs of ≥ 80%, ≥ 90%, ≥ 95%, ≥ 97%, and ≥ 99% to examine community structure at different levels of taxonomic resolution. We also used DOTUR to calculate Chao1 estimates of OTU richness (Chao, 1984) and the Shannon diversity index (Magurran, 1988) for each clone library at ≥ 99% sequence similarity. OTUs were identified by comparing our sequences with known organisms in the National Center for Biotechnology Information database using BLAST searches (see Supporting Information). Sequences generated from this study are available in GenBank under accession numbers EU234609–EU235739.

As an index of microbial biomass, we used quantitative PCR to measure the abundance of fungal and bacterial rRNA genes in our total DNA samples. For fungi, we used the primers nu-SSU-0817-5′ and nu-SSU-1196-3′ (Borneman & Hartin, 2000), and for bacteria we used Eub338 and Eub518 (Fierer *et al*., 2005). Quantitative PCR conditions are described in Allison *et al*. (2008). Sample extracts were analyzed in triplicate, and melting curve analyses were used to verify the specificity of the PCR reaction. We used bulk DNA extracts from our soil samples to construct standard curves, and expressed fungal and bacterial abundances in warmed plots as a fraction of the mean abundances in control plots.

Enzyme activities and soil water content

In order to assess the functional capacity of the microbial community, we assayed the activities of four extracellular enzymes involved in soil C and nutrient cycling: *β*-glucosidase (BG) catalyzes one of the later steps of cellulose degradation, polyphenol oxidase (PPO) degrades complex polymers such as lignin and humic acids, *N*-acetyl-glucosaminidase (NAG) is involved in chitin and fungal cell wall breakdown, and glycine-aminopeptidase (GAP) breaks down polypeptides. Soil samples were collected by taking at least three 2 cm × 5 cm cores from each plot at a distance of 30–100 cm from soil respiration chambers. We took samples every 1–2 months throughout the growing seasons of 2005–2006 for a total of seven sampling dates. Cores were kept cold and shipped back to UC Irvine where they were combined within a plot and homogenized by hand. These cores were also used to determine soil water content by drying a subsample at 65 °C for 48 h. Soil processing for enzymes and water content occurred within 1 week of sampling. Potential enzyme activities were assayed colorimetrically on a microplate reader as reported elsewhere (Allison & Jastrow, 2006; Allison *et al*., 2008). Activities are reported as μmol p-nitrophenol formed g^{-1} dry soil h^{-1} for BG and NAG, μmol p-nitroaniline formed g^{-1} dry soil h^{-1} for GAP, and μmol pyrogallol substrate consumed g^{-1} dry soil h^{-1} for PPO.

Statistics

We examined fungal community structure with nonmetric multidimensional scaling (NMS) of relative abundance data (Allison *et al*., 2007). For each level of sequence similarity, we calculated the relative abundance of each OTU in each experimental plot. These abundance distributions were used to calculate a matrix of community distances between plots using a Bray–Curtis coefficient (SAS PROC DISTANCE, NONMETRIC option) (SAS, Version 9.0; SAS Institute, Inc., Cary, NC, USA). The distance matrices were input to SAS PROC MDS to generate two-dimensional NMS ordinations of the fungal communities. We tested for shifts due to warming along either dimension using paired *t*-tests. Nonparametric Kruskal–Wallis tests were also used to test for warming effects on the relative abundances of individual fungal OTUs. Because this test is conservative, we did not correct for multiple comparisons, and we report *P*-values of 0.05–0.10 as marginally significant.

Effects of warming on soil water content, soil respiration, nutrient availability, and enzyme activities were tested with repeated measures ANOVAs in SAS PROC MIXED. We used a compound symmetry covariance structure and included block as a random effect. If the date × warming interaction was significant, we tested for significant warming effects within each date using the SLICE option in SAS. We used multiple linear regression to examine relationships between soil respiration, soil water content, and temperature. To improve normality, soil water content and respiration data, resin nutrient availabilities, BG activities, and NAG activities were log transformed, and PPO and GAP activities were square-root transformed. One resin NH_4^+ value and one NO_3^- value were obvious outliers and were removed from the dataset. The significance of the warming effect on fungal and bacterial abundances was determined by ANOVA on log-transformed data with block as a random effect. A significance level of $\alpha = 0.05$ was used for all analyses except where noted.

Results

Soil temperature and water content

On average across all 3 years, surface soil temperatures in the warming treatment were 0.5 °C higher than controls based on datalogger measurements (Fig. S1, Supporting Information). Mean soil temperatures as measured by the temperature probe during soil respiration measurements during 2005–2007 were also 0.5 °C higher in the warmed plots (9.83 vs. 9.34 °C). Throughout 2005, warming increased average soil temperature from 9.3 to 10.1 °C, with increases in daily maximum, minimum, and mean temperatures (Fig. S1). The warming effect was less evident in 2006 (mean increase from 8.5 to 8.8 °C), although minimum soil temperatures were elevated throughout most of the growing season in the warmed plots. Small variations in the depth or placement of the six dataloggers from year to year may have contributed to the weaker warming effect in 2006. Soil temperatures in 2007 were based on a single pair of dataloggers, but the measured temperature increase was consistent with the prior 2 years (10.1 °C control mean vs. 10.5 °C warming mean). The diurnal range of soil temperature was ~ 10 °C (Fig. S1). Mean (± SE) soil water content declined significantly in the warmed plots from 0.95 ± 0.10 to $0.74 \pm 0.08\,g\,g^{-1}$ ($P = 0.025$; Fig. 1).

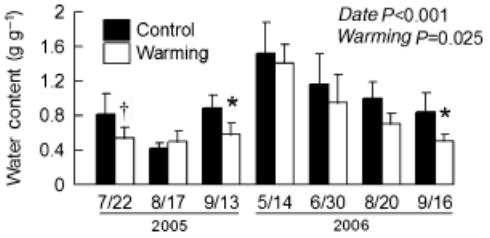

Fig. 1 Soil water content in warmed and control plots during the 2005–2006 growing seasons ($n = 5$). Bars show mean and SE on each date. Significant effects from repeated-measures ANOVA are show in italicized text. $^{\dagger}P < 0.10$, $^{*}P < 0.05$ for warming effect within date.

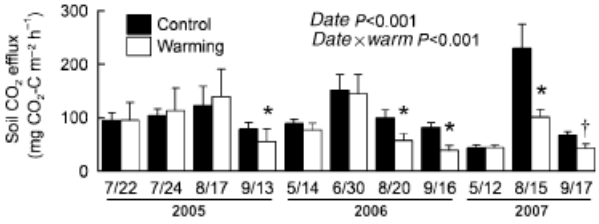

Fig. 2 Soil CO_2 fluxes based on chamber measurements in warmed and control plots during the 2005–2007 growing seasons ($n = 5$). Bars show mean and SE on each date. Significant effects from repeated-measures ANOVA are shown in italicized text. $^{\dagger}P < 0.10$, $^{*}P < 0.05$ for warming effect within date.

Soil respiration

Soil respiration declined with warming late in the growing season in all 3 years of measurement (date × warming, $P < 0.001$; Fig. 2). This interaction was significant even if soil water content and temperature were included in the ANOVA model as covariates. Across all three growing seasons, warming reduced soil respiration from 105.8 ± 8.8 (mean ± SE) to 82.8 ± 8.9 mg CO_2-C $m^{-2}\,h^{-1}$, although warming as a single factor was not significant. The warming effect was most pronounced late in the growing seasons of 2006 and 2007 when soil respiration declined by nearly 50% (Fig. 2). Although warming reduced soil respiration at certain time points, overall there was a significant positive relationship between soil respiration and temperature ($P = 0.005$, partial R-square = 0.11, $n = 70$). There was also a significant positive relationship between soil respiration and soil water content ($P < 0.001$, partial R-square = 0.15, $n = 70$).

Nutrient availability

Warming marginally increased NH_4^+ availability, especially in late 2005 when values increased from 23.4 ± 16.7 to $33.0 \pm 10.0\,ng\,N\,g^{-1}\,resin\,day^{-1}$ ($P = 0.048$, Table 1; $P = 0.076$ for date × warming interaction). We observed a similar pattern for NO_3^- availability, which showed a marginally significant increase overall (Table 1; $P = 0.060$ for warming effect). Although this pattern was driven by

Table 1 Mean nutrient availabilities and microbial abundances in control and warmed soils

		Control ± SE	Warming ± SE
Resin NH_4^+ (ng N g^{-1} resin day^{-1})	Early 2005	13.6 ± 5.8	15.3 ± 3.5
	Late 2005	23.4 ± 16.7	33.0 ± 10.0*
	2006	23.6 ± 7.0	18.9 ± 7.7
Resin NO_3^- (ng N g^{-1} resin day^{-1})	Early 2005	18.4 ± 5.3	13.0 ± 4.8
	Late 2005	2.1 ± 0.6	6.7 ± 2.4
	2006	1.9 ± 1.3	6.2 ± 3.9
Resin PO_4^{3-} (ng P g^{-1} resin day^{-1})	Early 2005	18.4 ± 8.1	6.8 ± 5.2
	Late 2005	59.9 ± 40.0	31.9 ± 23.2
	2006	50.1 ± 22.4	5.6 ± 2.2†
Bacterial relative abundance (qPCR)		1.00 ± 0.04	0.56 ± 0.06**
Fungal relative abundance (qPCR)		1.00 ± 0.29	0.55 ± 0.19*

†$P<0.10$, *$P<0.05$, **$P<0.01$ for comparison of control vs. warming within each date (ANOVA).

Table 2 Active fungal taxa in control and warming clone libraries with relative abundances >2%

OTU Designation*					
Number	Class	Order	Family	Control ± SE	Warming ± SE
2	Basidiomycetes	Agaricales	Cortinariaceae	26.0 ± 16.1	18.5 ± 13.3
4	Basidiomycetes	Thelephorales		41.1 ± 16.8	2.5 ± 2.4
3	Basidiomycetes	Cantharellales	Hydnaceae	7.8 ± 4.7	16.7 ± 12.8
15	Basidiomycetes	Agaricales	Tricholomataceae	3.4 ± 3.4	10.0 ± 10.1
7	Basidiomycetes	Agaricales		2.3 ± 1.0	9.0 ± 3.8
10	Basidiomycetes	Agaricales		6.6 ± 5.2	3.2 ± 1.6
9	Basidiomycetes	Polyporales	Albatrellaceae	0.7 ± 0.7	6.0 ± 6.0
13	Basidiomycetes	Agaricales		3.9 ± 4.0	2.5 ± 2.3
11	Basidiomycetes	Russulales	Russulaceae	0.0 ± 0.0	4.9 ± 2.0

*Operational taxonomic unit (OTU) from DOTUR analysis (≥ 99% sequence similarity; see Supporting Information), followed by taxonomic designation based on BLAST matches to known sequences in NCBI databases.

higher NO_3^- availability in late 2005 and 2006, the warming effect was not significant when tested within dates. Including outliers (see Methods) would increase the magnitude and significance of the positive effects of warming on NH_4^+ and NO_3^- availability. In contrast to N availability, resin-available PO_4^{3-} consistently declined with warming ($P = 0.013$ for warming effect).

Microbial community structure

Active fungal communities were strongly dominated by Basidiomycete fungi, which accounted for 93% of all sequences. All fungal OTUs (≥ 99% sequence similarity level) with overall relative abundances >2% were Basidiomycetes (Table 2). Most Basidiomycetes belonged to the orders Agaricales (48% of total community), Thelephorales (24%), and Cantharellales (12%). Ascomycetes accounted for only 5% of the total sequences, with the most abundant Ascomycete order being Eurotiales (2% of total community). Overall, we observed 65 fungal OTUs at ≥ 99% sequence similarity from 870 cloned sequences.

Warming had significant effects on microbial abundance and the composition of active fungal communities. The abundance of both fungal and bacterial ribosomal DNA declined by nearly 50% according to quantitative PCR measurements (Table 1). Compared with paired controls, all warming plots were shifted to the left along the first dimension of the NMS plot for OTUs defined by ≥ 99% sequence similarity (Fig. 3a, $P = 0.049$, paired t-test). Warming was also linked with a significant negative shift along the second dimension of the NMS plot for OTUs defined by ≥ 95% similarity (Fig. 3b, $P = 0.025$).

Changes in community structure were driven by shifts in the relative abundance of certain fungal taxa. At ≥ 99% sequence similarity, OTU 4 (order Thelephorales) declined from 41 ± 16.8% to 2.5 ± 2.4% rela-

tive abundance, while OTUs 7 and 11 (both Basidiomycete groups) increased (Table 3). OTU 29, a relatively rare Ascomycete (family Trichocomaceae), declined from 0.7 ± 0.3% to 0.0% relative abundance with warming. This pattern differed from other Ascomycete OTUs, which tended to increase in relative abundance with warming (see Ascomycete OTUs at 80–97% sequence similarity, Table 3). Also, a taxon dominated by the family Russulaceae increased in relative abundance from 0.0% to ~5% with warming (OTUs 9 and 11, Table 3).

Warming had a positive effect on fungal taxonomic richness and diversity. The number of OTUs at ≥ 99% sequence similarity increased from 27 to 51 with warming. Rarefaction curves of OTU richness vs. number of sequences sampled had shallow slopes (Fig. S2), indicating that fungal diversity was adequately sampled in the plots. There was also a significant increase in diversity from 1.86 [95% CI = (1.73, 1.98)] to 2.86 [95% CI = (2.74, 2.98)] as measured by the Shannon diversity index. Although the trend was similar, the Chao1 estimate of diversity did not increase significantly [control = 46.5 OTUs, 95% CI = (32.3, 99.9); warming = 84 OTUs, 95% CI = (63.3, 141.4)]. The increase in fungal richness was largely driven by the appearance of Ascomycete and Zygomycete taxa that were not active in the control plots (Table 3).

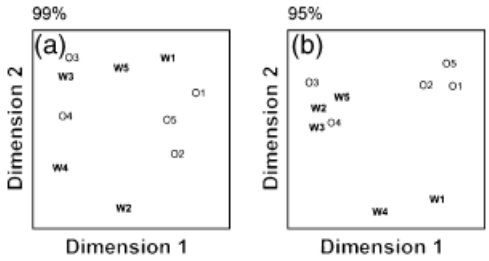

Fig. 3 Nonmetric multidimensional scaling plots of active fungal communities comprised of operational taxonomic units defined by ≥ 99% sequence similarity (a) or ≥ 95% sequence similarity (b). W, warming plots; O, control plots; numbers represent blocks. Warming resulted in a significant negative shift along dimension 1 ($P = 0.049$) in part (a) and a significant negative shift along dimension 2 ($P = 0.025$) in part (b) (paired *t*-tests).

Enzyme activities

Warming had relatively little effect on soil enzyme activities, although there was a weak trend toward lower activity of the chitin-metabolizing enzyme NAG with warming (Fig. 4, $P = 0.109$). The other enzymes varied substantially with sampling date and had mean activities of 1.8–7.8 μmol pNP g^{-1} soil h^{-1} for BG, 0.2–1.5 μmol pNA g^{-1} soil h^{-1} for GAP, and 9.7–33.9 μmol pyrogallol g^{-1} soil h^{-1} for PPO.

Discussion

Contrary to our initial hypothesis, we found that warming suppressed soil respiration, particularly late in the growing season. This response was probably driven by

Table 3 Mean relative abundances (%) of active fungal taxa that responded to warming

Sequence similarity	OTU designation*		Control ± SE	Warming ± SE	*P*-value
99%					
	4	Thelephorales	41.1 ± 16.8	2.5 ± 2.4	0.058
	7	Agaricales	2.3 ± 1.0	9.0 ± 3.8	0.028
	11	Russulaceae	0.0 ± 0.0	4.9 ± 2.0	0.054
	29	Trichocomaceae	0.7 ± 0.3	0.0 ± 0.0	0.050
97%					
	7	Tricholomataceae	0.2 ± 0.2	2.8 ± 1.1	0.034
	9	Russulaceae	0.0 ± 0.0	5.1 ± 1.8	0.019
	18	Ascomycetes	0.0 ± 0.0	1.6 ± 0.7	0.053
95%					
	9	Russulaceae	0.0 ± 0.0	5.1 ± 1.8	0.019
	13	Ascomycetes	0.0 ± 0.0	1.6 ± 0.7	0.053
90%					
	1	Ascomycetes	2.5 ± 0.6	6.3 ± 2.8	0.042
	6	Ascomycetes	0.0 ± 0.0	1.6 ± 0.7	0.053
80%					
	1	Ascomycetes	2.5 ± 0.6	8.1 ± 3.3	0.026
	3	Zygomycetes	0.0 ± 0.0	0.9 ± 0.5	0.053

*Operational taxonomic unit (OTU) from DOTUR analysis (≥ 99% sequence similarity; see Supporting Information) followed by taxonomic designation based on BLAST matches to known sequences in NCBI databases.

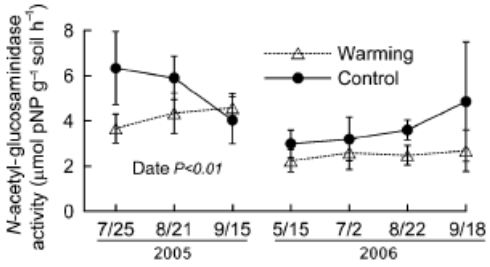

Fig. 4 Activity of the chitin-degrading extracellular enzyme *N*-acetyl-glucosaminidase in warmed and control plots during the 2005 and 2006 growing seasons. Symbols represent mean ± SE ($n = 5$). The date effect was significant (repeated-measures ANOVA) and there was a trend toward lower enzyme activity in the warming treatment ($P = 0.109$).

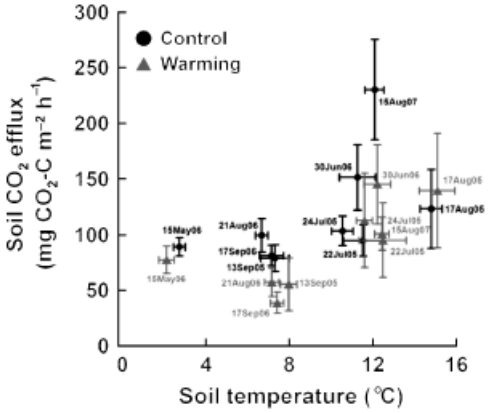

Fig. 5 Soil respiration as a function of soil temperature for dates when both parameters were measured simultaneously. Symbols represent mean ± SE ($n = 5$) for control (circles) and warming (triangles) plots.

changes in the activity of microbes or plant roots that contribute to soil respiration. Warming has been shown to reduce root biomass in boreal soils (Bergner *et al*., 2004; Bronson *et al*., 2008), and a similar response at our site might have caused a decline in root respiration. Importantly, we found clear evidence for a decline in fungal and bacterial abundance as indicated by qPCR (Table 1), suggesting a reduced potential for the microbial community to metabolize C. This result is consistent with the trend toward lower NAG activity in warmed soils (Fig. 4), as NAG is involved with the metabolism of chitin, a compound abundant in fungal cell walls. In contrast to our results, a recent study in arctic tundra found that warming increased fungal biomass (Clemmensen *et al*., 2006).

In our study, it is likely that reduced soil moisture played a role in suppressing soil CO_2 fluxes, perhaps in combination with temperature changes at the end of the growing season. Other warming studies have found that warming reduces soil moisture, and this effect may suppress decomposition rates (Verburg *et al*., 1999). If we plot soil respiration vs. soil temperature by date and treatment, there is an overall positive relationship (simple linear regression, $P < 0.001$, R-square = 0.14, $n = 90$), but respiration is lower in the warming treatment on most dates that fall late in the growing season (Fig. 5). On these dates, soil moisture was also significantly reduced (Fig. 1). Thus, the indirect effect of our warming treatment on soil moisture probably contributed to the negative responses of microbial biomass and soil respiration.

The relationships between soil moisture, temperature, and respiration are often nonlinear, and soil moisture has been shown to constrain the respiration response to temperature in boreal soils (Gulledge & Schimel, 2000). Below a certain threshold, moisture may constrain soil respiration more strongly than temperature (Davidson *et al*., 1998). In our greenhouses, soil moisture may have reached such a threshold by the end of the growing season due to increased evaporation associated with elevated air temperatures. Although we did not assess within-plot heterogeneity explicitly, the soil cores composited within each greenhouse appeared consistently drier than those from control plots late in the growing season.

In addition to higher mean soil temperature and reduced moisture, declines in microbial abundance and activity may have been related to elevated temperature minima or maxima. Fungi may be sensitive to different portions of the diel cycle, and there is some evidence from culture studies that temperature maxima reached in soils may limit fungal growth (Gleason *et al*., 2005). Higher minimum soil temperatures may have also inhibited fungal growth, although we are unaware of any experiments that test this mechanism.

It is also possible that changes in plant community composition or light attenuation by the greenhouses reduced plant photosynthesis and the allocation of C belowground. However, indirect warming effects mediated by changes in plant community structure are unlikely because we observed treatment responses within 1 year, which is probably faster than the response time for plant communities (Chapin *et al*., 1995). Light attenuation was not likely to be a major issue because the dominant plant in the community (black spruce) was not present in the greenhouses. Also, the reduction in soil respiration occurred at the end of the growing season, whereas light attenuation was constant in the greenhouses all season long.

Our results are somewhat unusual because most studies have found that warming increases rates of soil C cycling (Rustad *et al*., 2001). However, field manipulations of soil temperature are surprisingly rare in boreal forests, given the amount of C sequestered in boreal soils and the number of modeling studies that

examine boreal C cycle-climate feedbacks. Furthermore, there is evidence for heterogeneity in soil responses to warming among the boreal sites that have been studied. In a black spruce forest in Manitoba, soil respiration increased in response to warming by heating cables, but declined if the air above the cables was also heated (Bronson *et al*., 2008). The only other warming study conducted in Alaskan boreal forest found that forest floor biomass decreased with warming by heating cables, suggesting an increase in soil C mineralization (Van Cleve *et al*., 1990). In a Scots pine forest in Finland, warming with closed chambers increased soil CO_2 fluxes (Niinistö *et al*., 2004), whereas warming with heating cables in the CLIMEX experiment had no effect on litter decomposition in Norwegian boreal forest (Verburg *et al*., 1999). Some of the variation in warming responses across studies could have been due to methodological differences (Bronson *et al*., 2008), although this explanation is not consistent with the findings of a broader meta-analysis (Rustad *et al*., 2001).

These studies suggest that warming effects are mediated by other edaphic factors, such as soil moisture and the presence or absence of permafrost. In boreal forests with well-drained soils that lack permafrost, such as our site, warming may reduce soil C cycling if microbial activity becomes more limited by moisture than by temperature. The other boreal studies support this contention: Van Cleve *et al*.'s (1990) site was underlain by permafrost, and warming plots at the Manitoba and Finnish sites were irrigated (Niinistö *et al*., 2004; Bronson *et al*., 2008). At these sites, temperature probably limited microbial activity more than moisture, consistent with positive effects of warming on soil respiration.

Our warming treatment not only reduced total microbial abundance, but also suppressed the relative activity of certain groups of fungi. In particular, the relative abundance of OTU 4 ($\geq$ 99% sequence similarity level) showed a marginally significant decline (Table 3). This OTU belongs to the order Thelephorales, and is probably dominated by the ectomycorrhizal genus *Sarcodon*, which produced a large number of sporocarps at the site during the 2005 growing season (personal observation). In August 2005, we found that soil CO_2 fluxes were twice as high in chambers containing *Sarcodon* sporocarps relative to chambers without sporocarps (K. K. Treseder, unpublished data). Taken together, these results suggest that warming and drying may alter soil respiration by suppressing the activity of a fungal taxon known to be important for CO_2 fluxes in this site.

Particularly at the $\geq$ 99% sequence similarity level, there was evidence that fungal community structure differed among blocks (Fig. 3). Although this variation was not obviously related to a particular soil parameter, there are differences in soil moisture and plant community composition across the site. For example, block 4 is noticeably wetter than the others during the early part of the growing season. Additionally, the plant community in this block is somewhat distinct because it is dominated by *Vaccinium* and mosses, which could affect fungal community structure through plant litter inputs. Based on the clustering of fungal communities within a block (Fig. 3a), spatial heterogeneity was probably at least as important as the warming treatment for determining fungal community structure.

In addition to affecting community structure, the warming treatment increased the diversity of active fungi. A likely explanation for this increase is that warming and drying suppressed the activity of dominant fungi, such as Thelephorales OTU 4, resulting in reduced competition for other fungal groups. In particular, several taxa of Ascomycetes and Zygomycetes increased significantly with warming (Table 3). These fungi are likely to be saprotrophic (Alexopoulos *et al*., 1996), and may respond differently to changes in temperature, moisture, and nutrient availability than the ectomycorrhizal taxa that were most abundant in our plots.

We observed a slight increase in soil N availability with warming, in contrast to declines in soil respiration and P availability (Table 1). Most other warming manipulations have resulted in higher soil N availability (Rustad *et al*., 2001), and the hypothesized mechanism for this response is increased mineralization of soil organic matter (Hobbie *et al*., 2002). However, our results suggest that N availability may be decoupled from C and P cycling (e.g. late 2005; Table 1). C and N mineralization are typically linked (McGill & Cole, 1981), so a coincidence of greater N availability and lower soil respiration was unexpected and may have resulted from reduced N immobilization. The factors regulating P cycling in these soils are even less clear than for N, but reduced P availability could be due to lower P mineralization, or increased P uptake by roots or microbes. Nonetheless, there does appear to be a link between soil respiration and P availability, as P fertilization increases soil respiration by $>$50% at this site (S. D. Allison, unpublished data).

Because warming altered nutrient availability, it is possible that changes in microbial activity and soil respiration were driven by nutrient feedbacks. Specifically, increasing N availability could suppress fungal activity (Fog, 1988). We tested this hypothesis as part of an N fertilization experiment at the same site, but did not observe a reduction in overall fungal abundance or activity (Allison *et al*., 2008). We found that active Cortinariaceae were replaced by fungi from a different taxonomic group of Agaricales, and that both groups were probably ectomycorrhizal. These fungal responses to N differ from our current warming study, where

the relative abundance of Cortinariaceae did not change significantly with warming. Thus, warming effects on the fungal community are probably not due to an indirect feedback mediated by N availability at this site.

Our results should be scaled up with caution, as they represent mainly growing-season responses in mature boreal forests. Climate warming is expected to increase the length of the growing season (ACIA, 2004), which may have a positive effect on annual fluxes of CO_2 from the soil. In addition, a high degree of winter warming is expected under climate change and may differ in its effect on soil respiration compared with growing-season warming. Because of frequent fires, many areas of the boreal zone are in early stages of succession, and may respond differently to warming than mature forests. For example, Bergner *et al*. (2004) found that warming with open-top chambers had a positive effect on soil respiration and did not affect microbial biomass. Finally, we assessed fungal community structure only once in 2006 when fungi may have been responding to increased soil temperature, lower soil moisture, or both parameters. Communities may respond differently in other years and seasons, or if temperature and moisture change independently.

Conclusion

Warming and associated drying had a clear negative impact on microbial abundance and soil respiration in our boreal forest site. Although these parameters typically show positive responses to warming, microbial processes in well-drained boreal soils may be more strongly constrained by declining soil moisture than by temperature. We found that N availability increased with warming, suggesting that the N cycle may be partially decoupled from C and P cycling in this system. Furthermore, fungi known to be important for CO_2 efflux responded negatively to the warming treatment, while other fungal taxa responded positively and may have contributed preferentially to N cycling. Our results suggest that soil respiration in drier boreal ecosystems may not increase with climate warming, as occurs in some boreal systems with wet soils (Van Cleve *et al*., 1990; Niinistö *et al*., 2004). Therefore, ecosystem models in the boreal zone should consider how spatial heterogeneity in soil properties and microbial communities may affect the direction of the climate warming-C cycle feedback.

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Supporting Information

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

Fig. S1. Daily maximum, average, and minimum soil temperatures at 5 cm depth during the 2005–2007 growing seasons in warmed and control plots ($n = 2$–3 for 2005–2006; $n = 1$ for 2007).

Fig. S2. Rarefaction curve for fungal taxa defined by $\geq$ 99% DNA sequence similarity of the 18S rRNA gene.

Appendix S1. The Online Appendix contains BLAST hits, taxonomic designations, and operational taxonomic units of 18S ribosomal RNA gene sequences from soil fungi.

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