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Resistance of microbial and soil properties to warming treatment seven years after boreal fire

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ABSTRACT

Boreal forests store a large fraction of global terrestrial carbon and are susceptible to environmental change, particularly rising temperatures and increased fire frequency. These changes have the potential to drive positive feedbacks between climate warming and the boreal carbon cycle. Because few studies have examined the warming response of boreal ecosystems recovering from fire, we established a greenhouse warming experiment near Delta Junction, Alaska, seven years after a 1999 wildfire. We hypothesized that experimental warming would increase soil CO_2 efflux, stimulate nutrient mineralization, and alter the composition and function of soil fungal communities. Although our treatment resulted in 1.20 °C soil warming, we found little support for our hypothesis. Only the activities of cellulose- and chitin-degrading enzymes increased significantly by 15% and 35%, respectively, and there were no changes in soil fungal communities. Warming resulted in drier soils, but the corresponding change in soil water potential was probably not sufficient to limit microbial activity. Rather, the warming response of this soil may be constrained by depletion of labile carbon substrates resulting from combustion and elevated soil temperatures in the years after the 1999 fire. We conclude that positive feedbacks between warming and the microbial release of soil carbon are weak in boreal ecosystems lacking permafrost. Since permafrost-free soils underlie 45–60% of the boreal zone, our results should be useful for modeling the warming response during recovery from fire in a large fraction of the boreal forest.

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1. Introduction

Increasing emissions of atmospheric CO_2 are expected to affect high-latitude climate disproportionately (IPCC, 2007). Tundra and boreal ecosystems in northern latitudes have already experienced >1 °C warming, and may warm by 4–7 °C over the next century (Moritz et al., 2002; ACIA, 2004). Climate warming in these systems may have biogeochemical consequences because they contain large stocks of soil organic matter. Recent estimates place these stocks at >1600 Pg carbon (C), more than double the amount of C in the atmosphere (Schuur et al., 2008). As the climate warms in high latitudes, rates of decomposition may also increase, leading to greater soil CO_2 respiration, increased nutrient mineralization, and a positive feedback to global warming (Lükewille and Wright, 1997; Rustad et al., 2001; Schuur et al., 2009).

One potential impact of climate change in boreal ecosystems is increased frequency of wildfire (Kasischke and Stocks, 2000). In boreal Asia and North America, fires burn an average of 11.1 million ha yr^{-1} (Giglio et al., 2006) and drive major ecosystem impacts such as increased albedo, reduced C storage, and drastic changes in vegetation and microbial communities (Bond-Lamberty et al., 2004; Randerson et al., 2006; Waldrop and Harden, 2008). In Alaskan boreal forest, wildfire triggers rapid ecological succession and further changes in ecosystem properties (Mack et al., 2008). Within a few years after fire, grasses and shrubs dominate the vegetation, followed by deciduous *Populus tremuloides* trees that dominate the canopy ~15–50 years post-fire (Treseder et al., 2004; Mack et al., 2008). After >80 years, black spruce (*Picea mariana*) becomes the dominant canopy tree, with an understory of primarily shrubs and mosses. If fire frequency increases with climate change, a larger fraction of the landscape will be occupied by early-successional systems, and it becomes important to understand their responses to environmental changes, such as climate warming.

Losses of soil C are primarily determined by the metabolic rates of decomposer microbes, which often increase with rising

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temperature (Lloyd and Taylor, 1994). In boreal ecosystems, fungi are important drivers of soil C loss because they tolerate acidic soils and, along with bacteria, produce extracellular enzymes required to degrade recalcitrant litter inputs of the dominant plant species (Högberg et al., 2007; Allison et al., 2009). Additionally, most of the dominant plants in boreal forests associate with symbiotic mycorrhizal fungi that contribute to soil CO_2 efflux, and may drive turnover of soil organic C through the production of extracellular enzymes (Read et al., 2004; Talbot et al., 2008; Bödeker et al., 2009).

Although microbial metabolic rates usually increase with warming, the impact of climate change on boreal C cycling is far from certain. For example, soil drying or reductions in snow depth may counteract warming effects on microbial metabolism (Monson et al., 2006; Allison and Treseder, 2008). In addition, microbial communities may adapt to warming through physiological mechanisms (Malcolm et al., 2008) or changes in community composition (Clemmensen et al., 2006), such that temperature effects diminish over time. This uncertainty is compounded by a paucity of direct experimental manipulations of soil temperature in boreal ecosystems. Only two boreal sites were included in a 2001 meta-analysis of soil warming studies (Rustad et al., 2001), and there have been few recent studies (Bergner et al., 2004; Niinistö et al., 2004; Allison and Treseder, 2008; Bronson et al., 2008). There is considerable variability in the warming response across these studies, with most showing higher rates of soil CO_2 efflux but some showing lower rates (Allison and Treseder, 2008; Bronson et al., 2008).

The objective of our current study was to determine how climate warming would affect soil properties and the fungal community in a boreal ecosystem recovering from wildfire. Our work follows up on the Bergner et al. (2004) study, which assessed warming responses immediately after a 1999 wildfire near Delta Junction, Alaska. We hypothesized that warming would increase the metabolic rates of decomposer microbes, thereby increasing soil CO_2 efflux and the mineralization of organic nutrients (Rustad et al., 2001). In turn, we expected that greater C and nutrient mineralization would support more microbial biomass and extracellular enzyme production. Finally, we predicted that warming would alter the composition of fungal communities because laboratory studies suggest that higher nutrient availability should favor the growth of Ascomycete and Zygomycete fungi over Basidiomycetes (Zadražil and Brunnert, 1980; Fog, 1988).

2. Materials and methods

2.1. Site description

We conducted our experiment in an upland boreal ecosystem that experienced a severe fire that killed all of the canopy trees in 1999. The site is located on the Fort Greely military base (63° 55′ N, 145° 44′ W) near Delta Junction, Alaska. Permafrost is discontinuous in this region and was not present in the site. The dominant plants were herbaceous perennials, deciduous trees, and shrubs; major plant taxa included *Betula*, *Calamagrostis*, *Festuca*, *Ledum*, *Lupinus*, *Populus*, *Salix*, and *Vaccinium* as described by Mack et al. (2008). Before the 1999 fire, a forest of *P. mariana* occupied the area. The climate is dry and cold, with a precipitation rate of 303 mm y^{-1} and a mean annual temperature of −2 °C (http://weather.noaa.gov/). Soils are well-drained and consist of a shallow organic horizon (<5 cm depth) underlain by a silt-loam mineral soil (A horizon) derived from gravelly glacial till and outwash (Harden et al., 2006; Waldrop and Harden, 2008). The 1999 fire removed ~60% of the surface organic C, leaving behind an unburned forest floor horizon approximately 0–3 cm thick (King et al., 2002). The unburned organic horizon (where present) was overlain by several centimeters of char and ash immediately after the fire, but little of this burned material remained by 2006.

For the warming manipulation (see Allison and Treseder, 2008 for details), we established 5 pairs of 2.5 m × 2.5 m plots in a ~1 km^2 area of burned forest. One plot in each pair served as a control, and was located in a visually similar area within 10 m of the treatment plot to minimize any effects of spatial variability. The treatment plot was passively warmed with a greenhouse constructed of two-by-fours in an A-frame covered with clear 6 mil plastic sheeting (AT Plastics, Inc, Edmonton, Canada). The plastic transmits 60–70% of photosynthetically active radiation (Krizek et al., 2005). The dominant plants at the site (i.e. mosses, grasses, forbs, shrubs) were fully enclosed in the greenhouses. We applied the warming treatment from mid May to mid September each year (starting in 2006) by attaching a top plastic panel to the greenhouse frame. Therefore snowfall could reach the greenhouse plots during the winter. Precipitation during the growing season was captured with gutters on the greenhouses and redistributed via tubing to the plot surface at 8 locations separated by at least 50 cm. Gaps between the plastic and the frame or ground allowed air to circulate within the greenhouses. Large animals occasionally added additional air vents to the plastic panels. We did not include a chamber control (i.e. frames with no plastic) because a prior open-top chamber study at this site showed there was no chamber effect (Bergner et al., 2004).

2.2. Temperature and soil water content

We measured soil temperatures using Onset HOBO dataloggers placed 5 cm beneath the soil surface in control and warmed plots. Temperatures in 4–5 experimental blocks were recorded every 30 min during 3 growing seasons: May 13–September 18, 2006; May 10–September 18, 2007; and May 30–September 15, 2008. Soil water content was determined gravimetrically (oven-dry, 65 °C) on samples collected for extracellular enzyme assays. These cores generally included 1–3 cm of organic soil from the surface with the remainder derived from the mineral A horizon.

2.3. Soil CO_2 efflux

Soil CO_2 effluxes were measured with chambers and an infrared gas analyzer (PP Systems EGM-4) as described previously (Allison and Treseder, 2008). Chamber bases 25 cm in diameter were inserted 2 cm into the soil in May 2006. At 3–4 time points during the 2006–2008 growing seasons, we placed an opaque lid on the chamber base and measured the rate of increase in CO_2 concentration in the chamber for 5–10 min. We also measured soil temperature next to the chamber with a temperature probe. Fluxes were calculated according to the ideal gas law using the chamber volume, ambient air temperature, and 1 atm pressure. Litter, grasses, mosses, and other plants were generally left in the chambers to avoid disrupting root and litter inputs, except that we clipped aboveground plant biomass in chambers on August 21, 2006. However, before and after measurements indicated that clipping had no significant effect on soil CO_2 efflux.

2.4. Resin nutrients and microbial biomass

The availabilities of NH_4^+, NO_3^-, and PO_4^{3-} were measured as reported previously using resin bags (Allison et al., 2008). We placed 4 anion and 4 cation bags 5 cm below the soil surface and at least 25 cm apart in each warmed and control plot. One set of bags was placed in the field from May 14, 2006 to September 17, 2006, and a second set was placed in the field from May 11, 2007 to September 19, 2007. After collection, bags were rinsed in deionized

water and extracted in 0.1 M HCl/2.0 M NaCl. We analyzed the cation extracts for NH_4^+ using a modified Berthelot–salicylate method (Weatherburn, 1967) and the anion extracts for NO_3^- using the vanadium method (Doane and Horwath, 2003) and PO_4^{3-} using the malachite green method (Lajtha et al., 1999). Nutrient availabilities from bags within the same plot were averaged prior to statistical analysis, and data are presented as ng nutrient (N or P) g^{-1} resin d^{-1}.

We measured microbial biomass C and N with the chloroform fumigation-direct extraction technique (Brookes et al., 1985; Vance et al., 1987) on soils collected for extracellular enzyme assays in July 2006 (see below). Soils from each plot were subsampled to determine initial gravimetric water content and K_2SO_4-extractable C and N concentrations before and after fumigation. We calculated microbial C and N pools assuming a fumigation–extraction efficiency of 0.45 for C (Vance et al., 1987) and 0.54 for N (Brookes et al., 1985).

2.5. Extracellular enzyme activities

The activities of extracellular enzymes involved in C and N cycling were determined colorimetrically as previously reported (Allison and Jastrow, 2006; Allison et al., 2008). At 7 time points during the 2006 and 2007 growing seasons, we collected 3–5 soil cores (5 cm depth, 2.5 cm diameter) in each plot. Cores were combined and homogenized by hand, then subsampled to determine gravimetric water content and enzyme activities. We assayed the activities of β-glucosidase which releases glucose from cellulose metabolites, polyphenol oxidase which degrades lignin, *N*-acetylglucosaminidase which degrades chitin metabolites, and glycine aminopeptidase which breaks down proteins and polypeptides. For these assays, the following substrates (respectively) were dissolved in 50 mM sodium acetate buffer, pH 5.0: 5 mM *p*-nitrophenyl-β-glucopyranoside, 50 mM pyrogallol/50 mM EDTA, 2 mM *p*-nitrophenyl-β-*N*-acetylglucosaminide, and 5 mM glycine *p*-nitroanilide. We combined 150 μl of each substrate with 50 μl soil slurry and measured the formation of colored product with a microplate reader after 1–4 h. Activities are expressed in μmol g^{-1} dry soil h^{-1}.

2.6. Fungal community analysis

Three soil samples were collected and composited from each warming and control plot on May 29, 2008. Soil samples were immediately frozen at −20 °C and total DNA was extracted using the PowerSoil DNA kit (Mo Bio Laboratories, Inc) within one month of soil collection. DNA extracts were pooled from three 0.25 g soil subsamples, and fungal DNA was amplified using the ITS-F and TW13 primer pair (Gardes and Bruns, 1993; Taylor and Bruns, 1999). These primers target the ITS and 28S regions of fungal rDNA yielding approximately 1200 bp amplicons. Template DNA (0.13 μl template DNA μl^{-1} reaction volume) was combined in 30 μl PCR reaction volumes with 200 mM Tris–HCl PCR buffer, 0.2 mM each dNTP, 0.5 μg μl^{-1} BSA, 0.1 μM each primer, 1.23 mM $MgSO_4$, and 0.01 U μl^{-1} Platinum *Taq* DNA Polymerase (Invitrogen). PCR reactions were carried out using 5 min initial denaturation at 95 °C, followed by 35 cycles of 30 s at 95 °C, 45 s of annealing at 50 °C, 6 min of elongation at 72 °C, and a final elongation for 10 min at 72 °C on an iCycler thermocycler (BioRad). We used a pCR®2.1-TOPO® vector to clone PCR products in half-volume reactions from a TOPO TA Cloning kit (Invitrogen). Fifty clones per plot were selected to create 10 clone libraries in total. Clones were bi-directionally sequenced by Agencourt Bioscience Corporation.

We quality-checked and analyzed fungal DNA sequences from clone libraries according to procedures reported previously (Allison et al., 2008, 2010). Forward and reverse reads were assembled into contiguous sequences using CodonCode Aligner (CodonCode, Inc). We then selected a ~425 bp segment of the 28S rRNA gene to construct an alignment using CLUSTALW (Chenna et al., 2003). This segment started ~625 bp upstream of the reverse primer and contained enough variation to distinguish fungal taxa at roughly the genus level. We removed sequences that aligned poorly, were low quality, and/or did not match fungal taxa in BLAST searches. Reverse reads that did not form contiguous sequences but matched 28S rRNA genes of fungal taxa were left in the alignment.

We used our alignment (426 total sequences) and the Phylip program DNADIST (Felsenstein, 2005) to construct a matrix of evolutionary distances between sequences. We then input this matrix to DOTUR (Schloss and Handelsman, 2005) to assign sequences to operational taxonomic units (OTUs) defined by 80–100% similarity. There were 7 clones that were not included in the alignment because of poor-quality reverse reads, but did have good quality forward (ITS) reads that matched other contiguous sequences with >97% similarity. These 7 clones could therefore be reliably assigned to OTUs and were included in subsequent analyses. To test for warming effects, we analyzed fungal community structure with non-metric multidimensional scaling (NMS) ordinations and multi-response permutational procedures (MRPP) (McCune and Grace, 2002; Allison et al., 2010). All sequences are available in GenBank under the accession numbers GU211934–GU212366.

2.7. Statistical analyses

Temperature data, soil water contents, CO_2 effluxes, resin nutrients, and extracellular enzymes were analyzed with repeated-measures ANOVA with block as a random factor and warming treatment as a fixed effect. To conduct the analyses, we used SAS PROC MIXED with a covariance structure (first-order autoregressive or compound symmetry) that yielded the lowest Akaike Information Criterion (SAS Institute, 2004). Where necessary, post-hoc *t*-tests of warming effects within dates were conducted with the SLICE option in SAS. Treatment effects on microbial C and N pools were analyzed with paired *t*-tests. Temperature data were converted to daily means and square-root transformed to improve normality prior to analysis. Glycine aminopeptidase and polyphenol oxidase activities were also square-root transformed. All other data except microbial C:N ratios were log-transformed to improve normality.

We used stepwise multiple regression to determine the effect of soil temperature (from the probe data) and log-transformed soil water content on log-transformed CO_2 efflux rates ($n = 69$). We also tested temperature and moisture effects on CO_2 effluxes within the warmed ($n = 35$) and control ($n = 34$) plots separately. The significance criterion for variables to enter and to stay in each model was $P < 0.05$. We then confirmed the temperature relationship with a second simple regression of CO_2 effluxes versus soil temperatures with all data points ($n = 99$), since we did not collect soil water content data in 2008.

3. Results

3.1. Temperature and moisture dynamics

The warming treatment was effective, with a significant ($P < 0.05$) temperature increase of 1.20 °C in warmed soils (Table 1). The diurnal temperature range was ~15 °C, and mean daily temperatures ranged from 5 °C to 21 °C across the growing season. Minimum soil temperatures were ~1 °C, and soils occasionally reached temperatures >30 °C.

Table 1
Soil and microbial properties in control and greenhouse-warmed plots sampled to 5 cm depth.

	Year	Control		Warming		*P*-value
		Mean ± SE	*n*	Mean ± SE	*n*	
Temperature (°C)	2006–08	13.07 ± 0.08	1592	14.27 ± 0.09	1460	0.019
Resin NH_4^+ (ng N g^{-1} resin d^{-1})	2006–07	56 ± 30	10	69 ± 26	10	NS
Resin NO_3^- (ng N g^{-1} resin d^{-1})	2006–07	3.4 ± 1.2	10	3.9 ± 1.8	10	NS
Resin PO_4^{3-} (ng P g^{-1} resin d^{-1})	2006–07	77 ± 29	10	89 ± 49	10	NS
Microbial C (μg g^{-1} soil)	2006	349 ± 81	5	358 ± 34	5	NS
Microbial N (μg g^{-1} soil)	2006	26.7 ± 8.8	5	25.8 ± 2.5	5	NS
Microbial C:N	2006	15.5 ± 1.8	5	14.1 ± 1.2	5	NS
Glycine aminopeptidase (μmol pNA g^{-1} soil h^{-1})	2006–07	0.513 ± 0.070	35	0.376 ± 0.054	35	NS
Polyphenol oxidase (μmol pyrogallol g^{-1} soil h^{-1})	2006–07	15.3 ± 1.3	35	13.1 ± 1.1	35	NS

Soil water content declined significantly in the warmed soils (Fig. 1), and there was a significant warming × date interaction ($P < 0.05$). Soil water content was significantly lower in warmed plots on August 21, 2006 ($P < 0.001$), August 14, 2007 ($P < 0.05$), and September 17, 2007 ($P < 0.01$), with reductions up to 30% observed.

3.2. CO_2 effluxes

Although there was significant variation in soil CO_2 efflux across the growing season ($P < 0.001$, Fig. 2), there was no significant effect of the warming treatment. However, according to multiple regression ($n = 69$), soil temperature explained 54% of the data variance ($P < 0.001$) and soil moisture explained 6% ($P < 0.01$). Both relationships were positive. When the data were analyzed separately within warmed and control plots, both temperature and soil moisture were significant ($P < 0.01$) in the control plots, but there was no significant effect of moisture on CO_2 efflux from the warmed plots. The slope of the temperature relationship did not differ significantly in warmed versus control plots. A simple regression with all of the temperature data ($n = 99$) also showed a significant ($P < 0.001$; $R^2 = 0.49$) positive relationship between CO_2 efflux and soil temperature (Fig. 3). Because the CO_2 data were log-transformed, this relationship implies that CO_2 efflux increases exponentially with increasing temperature, as expected from the metabolic response of soil organisms (Lloyd and Taylor, 1994).

3.3. Nutrient pools

Resin-available NH_4^+ ranged from 56 ng g^{-1} resin d^{-1} in the control to 69 ng g^{-1} resin d^{-1} in the warmed plots, but the difference was not statistically significant (Table 1). Resin-available NO_3^- was an order of magnitude lower and also did not vary significantly with warming treatment. Likewise, we did not find any significant differences in resin-available PO_4^{3-} or microbial pools of C and N (Table 1).

3.4. Extracellular enzyme activities

The activities of β-glucosidase and *N*-acetyl-glucosaminidase increased significantly with warming (Fig. 4). Averaged across all time points, β-glucosidase increased from 1.38 ± 0.08 to 1.59 ± 0.09 μmol pNP g^{-1} soil h^{-1} ($P < 0.05$) and *N*-acetyl-glucosaminidase increased from 0.54 ± 0.04 to 0.73 ± 0.06 μmol pNP g^{-1} soil h^{-1} ($P < 0.01$). Although there were no interactions between warming treatment and date, post-hoc *t*-tests showed that the effect of warming within dates was only significant ($P < 0.05$) on 7/2/2006 for β-glucosidase, and there were no significant effects within dates for *N*-acetyl-glucosaminidase. Warming had no significant effect on glycine aminopeptidase or polyphenol oxidase activities (Table 1).

3.5. Fungal community dynamics

We obtained a total of 433 fungal sequences belonging to 113 OTUs at the 99% similarity level. At 80% sequence similarity, we observed an OTU (OTU 1; see Supplementary Appendix A) that corresponded to the Ascomycota and contained 40% of the sequences. OTU 2 at the 80% level corresponded to the Basidiomycota and contained 33% of the sequences. Two more OTUs (OTUs 3 and 6) were identified as Zygomycota and represented 10% of the sequences. The remaining OTUs representing <17% of the total sequences could not be reliably identified. Based on NMS ordinations and MRPP analyses, we found no significant shifts in fungal community structure with warming.

4. Discussion

Overall we found little support for our hypothesis that warming would stimulate microbial activity and rates of soil CO_2 efflux. Furthermore, warming had no significant effect on the composition of fungal communities. The only parameters that responded to warming were the activities of cellulose- and chitin-degrading enzymes, which increased as predicted. However, these enzymatic changes were small and did not affect rates of CO_2 efflux or nutrient pools. Our results contrast with a meta-analysis (Rustad et al., 2001) showing that CO_2 efflux and nutrient mineralization increase with temperature in a majority of warming studies. Our findings also contrast with a prior warming study conducted at the same site in which soil respiration increased 20% with 0.9 °C warming (Bergner et al., 2004). The earlier study used open-top

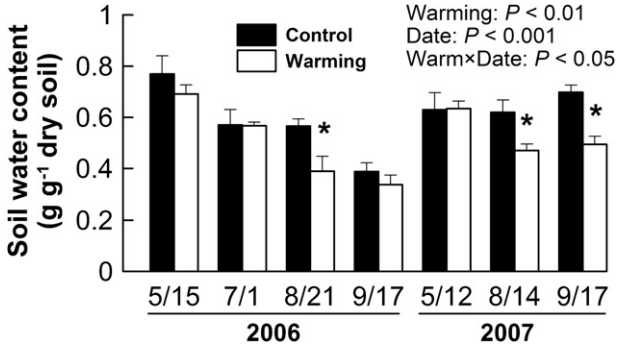

Fig. 1. Mean (±SE) water content in greenhouse-warmed and control soils at 3–4 time points in each of the 2006 and 2007 growing seasons ($n = 5$). Asterisk (*) denotes a significant ($P < 0.05$) treatment effect within date. Statistics from repeated-measures ANOVA.

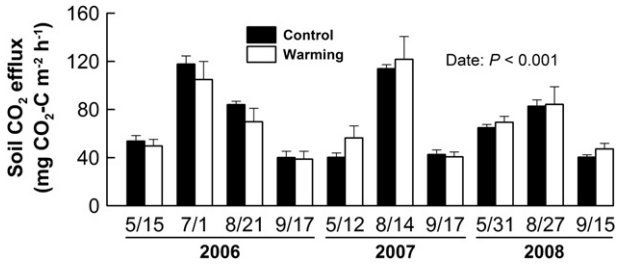

Fig. 2. Mean (±SE) CO_2 efflux in greenhouse-warmed and control soils at 3–4 time points in each of the 2006, 2007, and 2008 growing seasons ($n = 5$). Statistics from repeated-measures ANOVA.

chambers and took place during the 2000–2002 growing seasons, shortly after the 1999 wildfire at the site. Since our treatment generated 1.2 °C warming, the differences between studies cannot be explained by a weaker warming effect.

It is possible that warming in our study resulted in soil drying that constrained CO_2 efflux, as we have observed in a nearby mature boreal forest site (Allison and Treseder, 2008). However, several analyses suggest that moisture did not limit the warming response of soil respiration in our current study. Although warmed soils were significantly drier overall, the differences were statistically significant only on three of seven dates (Fig. 1). Furthermore, regression analyses showed that soil moisture explained only 6% of the variance across all soil respiration data, and moisture showed no significant relationship with soil respiration in the warmed plots. In addition, the level of drying we observed probably did not reduce soil water potential enough to negatively affect soil respiration. Our lowest gravimetric moisture content (Fig. 1) corresponds to a volumetric moisture content of ~0.25 cm^3 cm^{-3}, assuming a soil bulk density of 0.69 g cm^{-3} (King et al., 2002). For a silt-loam soil such as ours, this moisture content corresponds to a soil matric potential of approximately −0.1 MPa (Saxton et al., 1986), which is well above the threshold of −0.5 MPa reported by Davidson et al. (1998) to constrain soil respiration in forest soils. Finally, we only measured moisture content in the surface soil, and

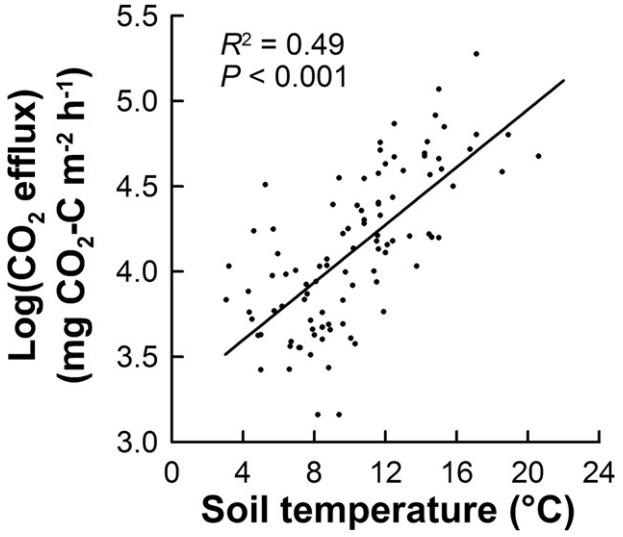

Fig. 3. Log-transformed soil CO_2 efflux increases significantly with soil temperature as recorded by a temperature probe during flux measurements (linear regression; $n = 99$). Points represent the mean value of 1–2 soil CO_2 efflux measurements from each experimental plot on a single date.

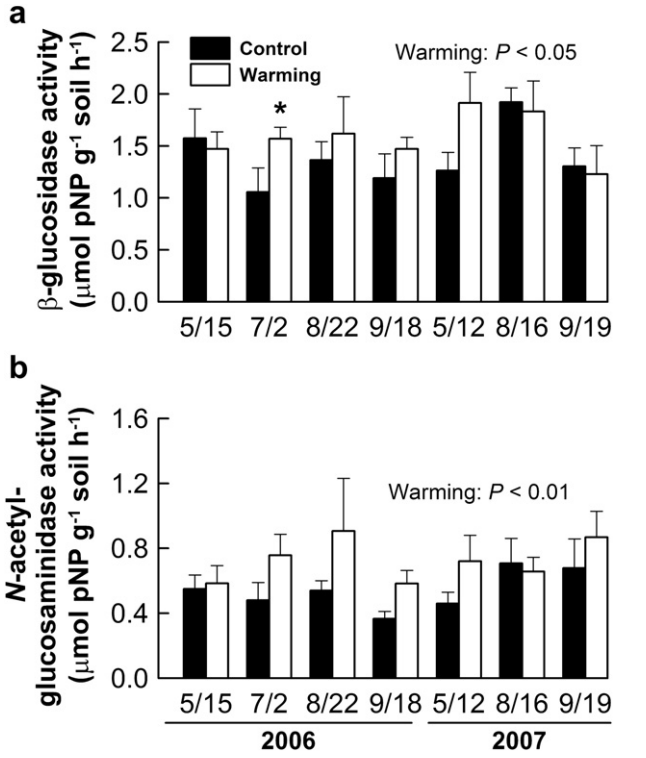

Fig. 4. Mean (±SE) β-glucosidase (a) and *N*-acetyl-glucosaminidase (b) activities in greenhouse-warmed and control soils at 3–4 time points in each of the 2006 and 2007 growing seasons ($n = 5$). Asterisk (*) denotes a significant ($P < 0.05$) treatment effect within date. Statistics from repeated-measures ANOVA.

drying effects may have been minimal in deeper soil horizons that contribute a substantial fraction of soil respiration.

There are other possible explanations for the resistance to warming in our present study. Since this work was conducted more than seven years after fire, changes in soil properties or the microbial community (Treseder et al., 2004) may have reduced the temperature sensitivity of soil respiration. In particular, labile forms of soil C may have been lost during and after the 1999 fire, leaving behind more recalcitrant C forms with weak temperature responses on short time scales (Kirschbaum, 2004; Eliasson et al., 2005). This mechanism could explain why Bergner et al. (2004) found a positive response of soil respiration to warming at our site: that study was conducted only 3 years after fire, potentially before labile C pools had been strongly depleted.

Labile C losses may have been accelerated by higher temperatures in the burned soil. In the growing season of 2002 (3 years post-fire), temperatures were 5 °C warmer in burned compared to unburned soils at 11 cm depth (Treseder et al., 2004). During the 2006–2007 growing seasons, we found that soil temperatures at 5 cm depth averaged 9.3 °C at the unburned site (Allison and Treseder, 2008) versus 13.1 °C at the burned site (Table 1). Consistent with higher temperatures in the burned site, we found a trend toward declining soil C stocks since the 1999 fire (Allison et al., 2010). In addition, average soil CO_2 effluxes were only 68 ± 4 mg CO_2–C m^2 h^{-1} from burned soils (Fig. 2) compared to 106 ± 9 mg CO_2–C m^2 h^{-1} from nearby unburned soils (Allison and Treseder, 2008), suggesting lower C availability in the burned soil.

Another alternative explanation for our results is that aboveground warming in the greenhouses caused a reduction in belowground plant inputs that compensated for an increase in microbial metabolic rates. Bergner et al. (2004) found that experimental warming significantly reduced root biomass by 33%. If this

biomass change is correlated with a decline in total belowground inputs, the effect on CO_2 efflux may now be sufficient to offset any microbial response to warming because plant biomass has increased since Bergner et al.'s (2004) study. Light attenuation due to the greenhouse plastic could have further contributed to reductions in belowground C allocation.

Experimental limitations may have also contributed to the lack of temperature response that we observed. It is possible that we would have detected warming effects if our treatments had begun immediately after the 1999 fire and continued for 7–10 years. Climate change is a gradual process that should affect boreal ecosystems across successional stages, so our imposition of a warming treatment seven years after fire is somewhat artificial, as are most warming manipulations (Rustad et al., 2001). In addition, we only applied the warming treatment during the growing season, so we missed any warming responses that might have occurred during the winter season, which can account for 10–20% of annual soil respiration (Mast et al., 1998; Welker et al., 2000). Finally, we might have observed stronger warming effects if the magnitude of the temperature increase was greater than 1.2 °C.

We were surprised that warming had no effect on fungal community composition or microbial biomass, although there was also no response in Bergner et al.'s earlier study. It is possible that the microbial community may require more than two or three years to respond to warming treatment; in a subarctic heath system, warming effects on fungal communities did not appear until 15 years after treatments began (Rinnan et al., 2007). Our study in a nearby mature boreal forest showed only minor warming effects on fungal communities after two years of warming, although microbial biomass declined sharply (Allison and Treseder, 2008). In our current study, we may have missed some microbial responses to warming because we did not measure bacterial communities, or because we assessed fungal communities too early in the third growing season of the experiment.

5. Conclusions

Taken together with results from similar greenhouse experiments, our current study suggests a dynamic pattern of ecosystem response to warming across successional gradients. In boreal ecosystems without permafrost, positive feedbacks between warming and CO_2 mineralization may not be uniform or sustained. In the first few years after wildfire, warming increases soil CO_2 losses (Bergner et al., 2004), but this positive feedback may then disappear after 7–10 years (the timeframe of our current study), and ultimately reverse as the forest matures (Allison and Treseder, 2008). We emphasize that temperature responses may differ in ecosystems underlain by permafrost, where warming causes more sustained and consistent C losses (Van Cleve et al., 1990; Stokstad, 2004; Schuur et al., 2008). However, our study is still relevant for climate feedbacks because 45–60% of the boreal zone may be permafrost free, based on the areal extent of the boreal forest (Larsen, 1980) and the distribution and continuity of permafrost (Zhang et al., 2008). We acknowledge that our study has limitations, such as lack of warming during the winter and artifacts due to the design of the greenhouse chambers (i.e. light attenuation, changes in water balance, etc.). Nonetheless, our work implies that ecosystem properties such as soil respiration and fungal communities may become resistant to warming within a decade after wildfire in Alaskan boreal forest. Exactly how long this resistance will last is currently unknown, but our study suggests that large-scale models may need to represent successional processes and permafrost distributions in boreal ecosystems in order to accurately predict climate-C cycle feedbacks.

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Appendix. Supplementary material

A Microsoft Excel file containing clone IDs, GenBank accession numbers, treatment origin, and operational taxonomic unit assignments can be found in the online version at doi:10.1016/j.soilbio.2010.07.011.

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