

Interactive effects of wildfire and permafrost on microbial communities and soil processes in an Alaskan black spruce forest

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Abstract

Boreal forests contain significant quantities of soil carbon that may be oxidized to CO₂ given future increases in climate warming and wildfire behavior. At the ecosystem scale, decomposition and heterotrophic respiration are strongly controlled by temperature and moisture, but we questioned whether changes in microbial biomass, activity, or community structure induced by fire might also affect these processes. We particularly wanted to understand whether postfire reductions in microbial biomass could affect rates of decomposition. Additionally, we compared the short-term effects of wildfire to the long-term effects of climate warming and permafrost decline. We compared soil microbial communities between control and recently burned soils that were located in areas with and without permafrost near Delta Junction, AK. In addition to soil physical variables, we quantified changes in microbial biomass, fungal biomass, fungal community composition, and C cycling processes (phenol oxidase enzyme activity, lignin decomposition, and microbial respiration). Five years following fire, organic surface horizons had lower microbial biomass, fungal biomass, and dissolved organic carbon (DOC) concentrations compared with control soils. Reductions in soil fungi were associated with reductions in phenol oxidase activity and lignin decomposition. Effects of wildfire on microbial biomass and activity in the mineral soil were minor. Microbial community composition was affected by wildfire, but the effect was greater in non-permafrost soils. Although the presence of permafrost increased soil moisture contents, effects on microbial biomass and activity were limited to mineral soils that showed lower fungal biomass but higher activity compared with soils without permafrost. Fungal abundance and moisture were strong predictors of phenol oxidase enzyme activity in soil. Phenol oxidase enzyme activity, in turn, was linearly related to both ¹³C lignin decomposition and microbial respiration in incubation studies. Taken together, these results indicate that reductions in fungal biomass in postfire soils and lower soil moisture in nonpermafrost soils reduced the potential of soil heterotrophs to decompose soil carbon. Although in the field increased rates of microbial respiration can be observed in postfire soils due to warmer soil conditions, reductions in fungal biomass and activity may limit rates of decomposition.

Keywords: Alaska, carbon cycling, enzymes, fungi, lignin, permafrost, wildfire

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Introduction

Boreal forest ecosystems cover nearly 1.2 billion hectares of the surface of the Earth, equivalent to 17% of the total land surface and contain approximately 90 Gt carbon (C) in vegetation and 470 Gt C in soil organic

matter (SOM) and forest floor litter (Schlesinger, 1977). Such large pools of C within the boreal forest ecosystem, equivalent to half of the total carbon within all forest ecosystems, make it particularly important to the global carbon cycle and global climatic change. If the carbon balance in boreal forest ecosystems is significantly altered, this can lead to important changes in the rate of accumulation of carbon within the atmosphere,

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resulting in positive or negative feedbacks to climate change (Trumbore & Harden, 1997; Harden *et al.*, 2006).

There are several ways in which the carbon balance of boreal forests is being affected on a global scale. The projected climate change for the boreal forest region includes increases in temperature between 1 and 3°C within 50 years and drier soils in summer (Watson *et al.*, 1995). Warmer and drier expected climate patterns are expected to increase decomposition rates and soil respiration (Dioumaeva *et al.*, 2003) and the frequency and intensity of wildfires with a resulting net loss of carbon to the atmosphere (Harden *et al.*, 2000) and a positive feedback to global climate change. Climate warming is also expected to increase the rate of permafrost thawing, which may interact with wildfire behavior and biogeochemical process rates. For example, the presence of permafrost at depth may reduce the extent and intensity of wildfire because permafrost can reduce soil drainage resulting in wetter soil conditions (Harden *et al.*, 2001). Increased soil drainage resulting from permafrost thaw can also lead to reductions in SOM storage, due in part to increases in microbial decomposition rates (Harden *et al.*, 2001).

An important debate in global change biology is the extent to which changes in microbial communities affects the decomposition rate of SOM (Schimel & Gulledge, 1998). Decomposition in wildfire affected soils is the net result of interactions between temperature, moisture, carbon 'quality', and the types and amounts of enzymes produced by soil micro-organisms, with little influence of mineral protection in the dominantly organic soils (Sollins *et al.*, 1996). Soil temperatures are often elevated postfire, which often results in increased CO₂ fluxes in the short term (Richer *et al.*, 2000; O'Neil *et al.*, 2003; Bergner *et al.*, 2004); however, lower heterotrophic respiration rates are also observed (Bond-Lamberty *et al.*, 2004; Certini, 2005). Reductions in carbon quality and losses of microbial decomposer organisms can result from wildfire, and this has the potential to reduce soil respiration rates. Carbon quality is reduced postfire due to the absence of roots and root exudates, the presence of charcoal, and oxidation of labile carbon relative to recalcitrant soil carbon (Pietikainen *et al.*, 2000; Neff & Hooper, 2002; Certini, 2005), although a modeling study suggests that the remaining organic matter may still be very decomposable (Carrasco *et al.*, 2006). A consequence of reductions in labile carbon sources is less energy availability to micro-organisms, which reduces microbial abundance, alters community composition, and modifies the types of enzymes produced for decomposition (Certini, 2005; Hart *et al.*, 2005). This cascade may affect rates of SOM decomposition, potentially reducing the temperature sensitivity of decomposition in burned soils.

Importantly, the effect of wildfire on soil microbial communities is usually transient, lasting until plant communities develop and bring new resources to the microbial community (Hart *et al.*, 2005).

Permafrost is a common feature of the Alaskan interior and strongly influences soil moisture by acting as a barrier to water movement. Soil moisture is an important driver of vegetation structure, as well as the composition and functioning of the soil microbial community in northern soils (Zak & Kling, 2006). Permafrost varies spatially in relation to subsurface and topographic conditions (Jorgenson *et al.*, 2001) and permafrost thaw may be promoted by warmer soil temperatures in wildfire-affected soils and by regional climate warming (Zhuang *et al.*, 2002; Euskirchen *et al.*, 2006). It is important to consider the extent to which microbial communities vary among different permafrost regimes because, over the long term, permafrost thaw will alter moisture characteristics and carbon dynamics of interior Alaska (Harden *et al.*, 2001).

In a field experiment near Delta Junction, Alaska, we examined how microbial communities, particularly fungal communities, are impacted by wildfire and the interactive effect of near-surface permafrost by sampling control and burned black spruce [*Picea mariana* Mill.) *B.S.P.*] forest soils with and without near-surface permafrost. We particularly focused on fungi because of their high abundance in boreal soil environments and their central role in lignin decomposition. We analyzed the decomposition process at multiple scales of resolution, from microbial respiration at the field scale, lignin decomposition in soil samples, soil enzyme activities, and finally quantifying fungal-specific gene sequences to estimate fungal biomass. We focused particularly on lignin decomposition because lignin is an abundant plant polymer that limits the decomposition of organic material (Wickland & Neff, 2007). We focused on oxidative enzyme activities as they control the oxidative depolymerization of lignin and other compounds, and are produced largely by soil fungi, particularly basidiomycete fungi (Gramss *et al.*, 1999). Although fungi produce the great majority of these enzymes, we have limited information regarding the controls on decomposition at the molecular scale, where interactions between microbial communities, their genetic potential for decomposition, functional genes, enzyme synthesis, and organic matter transformations occur (Zak *et al.*, 2006). With modern molecular techniques, these connections at the molecular scale can be quantified, but what form do they take, and do the connections affect carbon dynamics measured at the plot scale? We tested the hypothesis that there is a microbial limitation to decomposition in postfire soils. Therefore, we should observe reductions in soil fungi, oxidative enzyme

activities, and lower rates of lignin decomposition in post fire soils. We further hypothesize that the reductions in microbial populations and activities would be more extreme in drier, permafrost-free soils.

Materials and methods

Field sites and sampling

Our field sites were located in interior Alaska in the Donnelly Flats area (63°N, 145°W), located approximately 80 miles southeast of Fairbanks near Delta Junction (Manies *et al.*, 2004). During the summer of 1999, a wildfire burned over 18000 acres of mature black spruce (*P. mariana*) on moderately to somewhat poorly drained permafrost and well-drained nonpermafrost soils (Harden *et al.*, 2001). The permafrost soils (Creek site) had ~40 cm thick active layers before burning and were underlain by 1–2 m thick silt deposits. The non-permafrost soils (Tower site) were underlain by <1 m thick silt overlying sand and gravel deposits. Mean annual precipitation in this region is 30 cm, and the growing season (frost-free) is approximately 114 days.

Eight replicate 1 m × 1 m plots were established along transects at 10 m intervals in burned and unburned (control) forests on permafrost and nonpermafrost soils. Burned and unburned forests were adjacent to each other and separated by several hundred meters. Permafrost and nonpermafrost sites were separated by a few miles. The sites have been intensively studied for soil characteristics, ground cover (% coverage of different

moss species and lichen), tree density, woody debris, and temperature and moisture regimes (Marries *et al.*, 2004). Surface temperature and mineral soil moisture were characterized over the growing season of 2003 for replicate plots within each stand (Harden *et al.*, 2006).

In August 2004, we collected soils from the eight locations within each of the four sites for microbial community characterization and functional assays. We collected less than eight samples from the no permafrost burn site because the organic horizon was very thin. We randomly selected one core location 5 m away from each plot, in the direction of least disturbance. We did not sample within the plot because they were used for moss inventories. At each randomly selected core location, we sampled using a sharpened metal core barrel (4.7 cm diameter) attached to a power drill. Each core was gently pushed from the core barrel intact, described in terms of depth of each horizon, root colonization, and the presence of mycorrhizal mats, charcoal, and finally separated into live and dead moss (L/D), organic (O) horizon, and mineral (A) horizon. The O horizon was composed of slightly decomposed organic material (mostly moss) and roots. The mineral (A) horizon was typically a brown silt loam, sometimes grading into a sandy loam or loam, occasionally with charcoal and/or rocks (Table 1). Samples were shipped on dry ice to the USGS in Menlo Park, CA, USA, within 48 h of collection. Samples were frozen at -80°C for DNA analysis or at -20°C for all other assays. Moisture content was determined by drying organic soils at 65°C for 48 h and mineral soils at 105°C for 48 h. Carbon and

Table 1 Field descriptions of well-drained nonpermafrost soils and moderately well-drained to somewhat poorly drained permafrost soils with control and burned paired comparisons

Site	Treatment	Horizon	Depth (cm)	Description
No permafrost	Control	L/D	0–3.5	<i>Hylocomium splendens</i> , <i>Picea mariana</i> litter, <i>Vaccinium vitis-idaea</i> , and lichen present
		O	3.5–8.9	Fungal mat generally present, some identifiable plant parts
		A	8.9–13.9	Tan to brown mineral soil
No permafrost	Burn	O	0–2.6	Highly variable from thick organic friable soil to burnt organic matter, and a sparse, mostly dead moss layer. Moss consists of <i>Polytrichum</i> sp. and <i>Ceratodon purpureus</i>
		A	2.6–7.6	Silt loam, rocky, reddish soil
Permafrost	Control	L/D	0–5.3	Live and dead <i>Pleurozium schreberi</i> , <i>Polytrichum</i> sp., <i>H. splendens</i> , <i>Aulacomnium</i> spp., <i>V. vitis-idaea</i> , and lichen
		O	5.3–14.9	Roots, moss, fungal mycelia
		M	14.9–20.1	Amorphous unrecognizable plant parts and roots
		A	20.1–25.4	Darkened mineral soil
Permafrost	Burn	L/D	0–1.3	Some <i>Polytrichum</i> sp., <i>C. purpureus</i> , lichen, and <i>V. vitis-idaea</i> over burned organics and charcoal. <i>Epilobium angustifolium</i> present
		O	1.3–4.5	Highly decomposed almost unrecognizable plant parts. Burned wood, dead roots
		A	4.5–9.4	Brown mineral soil

Table 2 Chemical and biological attributes of control and burned soils in permafrost and nonpermafrost forests

	Horizon	Permafrost		No permafrost		Significance*
		Control	Burn	Control	Burn	
Moisture content (%)	O	77 ± 4.0	26 ± 9.0	51 ± 3.0	35 ± 6.0	Fire $P = 0.001$
	A	69 ± 7.0	66 ± 4.0	42 ± 4.0	38 ± 1.0	Perm $P < 0.001$
C (%)	O	40.3 ± 1.3	36.0 ± 1.2	36.2 ± 1.7	32.2 ± 3.2	Perm $P = 0.053$ Fire $P = 0.043$
N (%)	O	0.97 ± 0.1	0.98 ± 0.1	1.03 ± 0.1	0.97 ± 0.1	ns
Soil C/N	O	42.5 ± 3.0	36.9 ± 1.2	35.4 ± 1.8	32.9 ± 2.0	Perm $P = 0.011$ Fire $P = 0.053$
Microbial respiration (Lab) ($\mu\text{g CO}_2 \text{ g}^{-1} \text{ soil day}^{-1}$)	O	2252 ± 407	360 ± 76	1514 ± 866	250 ± 110	Fire $P = 0.001$
Microbial biomass C/Soil C ($\mu\text{g C g}^{-1} \text{ soil C}$)	O	9900 ± 1480	2980 ± 817	6140 ± 1570	2670 ± 360	Fire $P < 0.0001$
Volumetric soil moisture [from Harden <i>et al.</i> (2006)]	A	58.82 ± 3.5	37.55 ± 3.9	30.37 ± 8.5	17.3 ± 4.1	Fire × perm $P < 0.05$

$n = 8$ except for no permafrost burn where $n = 6$.

Values are means ± 1 SE.

*Significant 'Perm' is a permafrost effect and 'Fire' is a wildfire effect from two-way ANOVA.

nitrogen concentrations of organic soils were measured on a C/N analyzer (CE Elantech, Lakewood, NJ, USA) (Table 2). Field respiration was measured on five plots in each site using a TPS-2 PP meter to record CO_2 flux from an opaque plastic chamber (~15 cm diameter, 15 cm height). We used soil collars to create a tight seal between the chamber and soil. Light and dark respiration were measured over the course of a few minutes, and replicate flux rates were averaged for each chamber (PP Systems, Amesbury, MA, USA). We simultaneously logged soil temperature, chamber temperature, Photosynthetically active radiation (PAR), and soil moisture (using an ECH_2O logger; Decagon Devices, Pullman, WA, USA). Four replicate measurements were made each week during a 4-week period in September and October of 2003.

Microbial biomass

Microbial biomass carbon was extracted from O horizon soils using the chloroform fumigation direct extraction (CFDE) technique (Vance *et al.*, 1987). One subsample (2–5 g) was extracted with 50 mL 0.5 M K_2SO_4 through a Whatman #1 filter after shaking for 1 h. This was considered the dissolved organic carbon (DOC) sample. The second sample was fumigated with chloroform for 48 h, the chloroform was purged five times by opening the chamber to the atmosphere, closing it, and then pumping the gas out of the chamber. Fumigated samples were extracted in a similar manner as unfumigated samples. Filtrates were acidified with 50 μL of 85% phosphoric acid. Microbial biomass carbon was calcu-

lated as the difference between the fumigated samples minus the unfumigated 'DOC' sample. Extracted carbon was converted to microbial biomass carbon by dividing the concentration of extracted carbon by 0.45 and nitrogen by 0.54 (Vance *et al.*, 1987). It is important to note that our microbial biomass technique (CFDE) underestimates microbial biomass in fungal mats (Ingham *et al.*, 1991), which were commonly present in the control sites and less abundant or nonexistent in the burned stands. Therefore, reported differences between control and burned stands are likely underestimates.

For C and N analysis of microbial biomass extracts, 1 μL of filtrate was dried onto a watch glass placed on a benchtop heat source. The watch glasses had been pre cleaned in a muffle furnace. The samples were slowly heated on the bench top heat source to minimize combustion of the dissolved carbon. Dried crystals were scraped into a pressed tin capsule (5 mm × 9 mm, Costech, Valencia, CA, USA), and combusted in a C/N autoanalyzer (CE Elantech). Dry weight conversions were made on all samples by oven-drying moist samples at 65°C for 48 h.

DNA extraction and quantification

DNA was extracted on all O horizon and A horizon soils using the Powersoil DNA extraction kit (Mo Bio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. DNA was extracted from 0.15 g of O horizon material and 0.25 g A horizon material, owing to differences in density. We confirmed DNA extractions on a 1% agarose gel. DNA was quantified

using Pico Green by adding 1–5 μL DNA to 95–99 μL , Quant-IT pico green standard solution (Invitrogen, Carlsbad, CA, USA). DNA standards were created from lambda DNA at concentrations from 1 to 50 $\text{ng } \mu\text{L}^{-1}$. The excitation wavelength was set at 492 nm and emission was measured at 516 nm.

Phenol oxidase assay

Phenol oxidase was measured on 0.15 g subsamples of O horizon soils and 0.50 g subsamples of A horizon soils. Samples for enzyme assays were stored as freeze-dried material and then homogenized by vortexing. To each sample was added 13 mL of 2 mM L-DOPA in acetate buffer (pH 5.0). Sample controls were made by adding 13 mL of acetate buffer only. Samples and controls were placed on a Glas-Col Rugged Rotator (Terre Haute, IN, USA) and rotated for 17 h at 25°C. Samples and controls were then centrifuged at 2000 rpm for 10 min and the absorbance of the supernatant was measured at 450 nm on a Cary 50 Bio UV-vis spectrophotometer (Varian, Palo Alto, CA, USA). Standards were made by creating 2 mM and 1 mM L-DOPA solutions and a blank, and oxidizing the L-DOPA with 1 mL of 0.2 mg mL^{-1} horseradish peroxidase (HRP). Generally, the maximum absorbance reading of the standards would be reached in 24 h. The maximum absorbance values measured were used for the standard curve.

Lignin decomposition and respiration

^{13}C -labeled lignin (Isolife BV, Wageningen, the Netherlands) was extracted from ^{13}C -labeled chicory (*Cichorium intybus*) plants, and extracted following published procedures (Gosselink *et al.*, 2004). The ^{13}C -lignin (12.0 mg) was solubilized in 50 mL methylene chloride whereupon 1 mL was added to a cellulose filter (Millipore # AP30034PO), and allowed to dry on aluminum foil in a hood. To determine the rate of lignin decomposition, 5 g of soil was moistened to approximately 0.33 $\text{g H}_2\text{O g}^{-1}$ soil, and preincubated for 3 days in 500 mL jars with airtight lids containing a gas-sampling port. One ^{13}C -lignin disk was then placed within the 5 g soil sample, the jars were sealed, and the soils were incubated at 5°C. After 4 days, the headspace gas was sampled with a syringe after first ensuring that the gas in the jars was well mixed by pumping the syringe 10 times. A 1 mL aliquot of headspace gas was injected directly into NA 1500 NCS autoanalyzer (Carlo Erba Instruments, Milan, Italy) with a GC port interfaced onto a VG Optima isotope ratio mass spectrophotometer (Fisons Instruments, Inc., Beverly, MA, USA). The CC column was a 2 m Hayesep column (1/4 in. diameter), preceded by a P_2O_5 water trap. Standards for

CO_2 concentrations were obtained from Scott Specialty gases (Airgas, San Mateo, CA, USA). ^{13}C - CO_2 standards were made by comparing the reference gas with a known laboratory isotope standard. Microbial respiration was measured as the rate of total CO_2 evolution while the decomposition of lignin was measured as the rate of ^{13}C - CO_2 evolution.

Quantitative PCR (QPCR)

The QPCR of fungal-specific DNA was completed using a MX3005P quantitative PCR machine (Stratagene, La Jolla, CA, USA) (Landeweert *et al.*, 2003; Fierer *et al.*, 2005). We chose to amplify the intergenic transcribed spacer (ITS) region for QPCR because of its ease of amplification from virtually all fungi and the ability to use it in conjunction with denaturing gradient gel electrophoresis (DGGE) or other fingerprinting techniques for community analysis (Bruns & Shefferson, 2004). Before the QPCR reactions, DNA from all samples was quantified using the picogreen assay (see 'Materials and methods'), and diluted to 6 $\text{ng } \mu\text{L}^{-1}$ using DNase and RNase free water. Standards were created by amplifying DNA from three samples, mixing the products, quantifying the concentration of DNA using our picogreen assay, and making serial dilutions. Each PCR reaction consisted of 1 μL of DNA, 1.0 μL of 1.0 $\text{pmol } \mu\text{L}^{-1}$ ITS1F (5'-CTT GGT CAT TTA GAG GAA GTA A-3') and 10 $\text{pmol } \mu\text{L}^{-1}$ ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') primers, 12.5 μL of Sybr Green master mix (Stratagene), 0.4 μL Rox, and 9.1 μL DNase free H_2O . The run was 95°C for 5 min, then 40 cycles of 95°C for 1 min, 57°C for 30 s, and 72°C for 1 min. R^2 -values for the standard curve were between 0.85 and 0.99. The relative abundance of fungi was represented as the quantity of the ITS amplicon per unit DNA extracted from soil. Total abundance of fungi was represented as the quantity of the ITS amplicon per unit soil.

DGGE

DNA was amplified using fungal-specific ITS1F and ITS4 primers. A 40-mer GC clamp was attached to the ITS1F primer (5'-GCG CCG CCG CGC CCC GCG CCC GGC CCG CCG CCG CGG CCG C-3') for DGGE analysis. The PCR reactions consisted of 1 μL DNA, 0.5 μL DNTPs (10 mM), 1 μL of forward and reverse primers (10 $\text{pmol } \mu\text{L}^{-1}$), 3 μL of 10 $\times$ buffer, 0.5 μL Taq and 18.5 μL of DNase free water. The PCR reaction was 35 cycles of 95°C (1 min), 53.3°C (30 s), 73.5°C (1 min), and a final 5 min extension step.

We ran a 7.5% acrylamide DGGE gel consisting of a denaturing gradient from 40% to 60%. The 40%

denaturant consisted of 4 mL formamide, 4.25 g urea, 4.71 mL acrylamide, 1.25 mL of 20 × TAE buffer, and milli-Q water to 25 mL. The 60% denaturant consisted of 6 mL formamide, 6.375 g urea, 4.71 mL acrylamide, 1.25 mL of 20 × TAE buffer, and milli-Q water to 25 mL. Then, 130 µL of fresh ammonium persulfate (APS) and 8 µL of TEMED were added to 11.5 mL of each denaturant solution. The solution was added to the gradient mixer before being poured into the gel casting. Gels were run for 10 min at 200 V and then 100 V for 22 h at 60°C in an electrophoresis tank. To each well was added 10 µL DNA and 2 µL loading dye. A 100 bp ladder was added to three lanes to act as a standard. The gels were stained with Sybr Green (15 µL, into 150 µL, TAE) for 20 min. Bands were detected and grouped digitally using Kodak ID 3.5 imaging software (Kodak, Rochester, NY, USA) and manual identification (Kowalchuk & Smit, 2004).

Statistics

Values were log-transformed in order to meet the assumptions of normality before analysis of variance (ANOVA). We used ANOVA with wildfire and permafrost as dependent variables to determine the treatment effects on microbial community composition and function. Except for statistical analysis of DGGE banding patterns, analyses were split by horizon, because many analyses (C, N, lignin decomposition) were not performed on A horizon soils. Significant ANOVAs were followed by Fisher LSD *post hoc* tests. Correlations and stepwise multiple regression were performed in STATISTICA (Statsoft Inc., Tulsa, OK, USA). Statistical analysis of DGGE patterns were compared using Non-metric Multi-Dimensional Scaling (NMDS) (McCune & Grace, 2002). For NMDS, we used the Jaccard distance measure for binary data (presence/absence), and, after using a random starting configuration, we settled upon a 3D run for the final solution. The run had 111 iterations, final stress was 17.3, and the final instability was 0.00001. The run became stable at iteration 30.

Results

Five years after wildfire, burned organic soils had lower microbial biomass than control soils, an effect which reflected decreases in labile carbon sources. Microbial biomass was significantly decreased in burned soils compared with control soils (Fig. 1a; fire effect: $F = 6.68$, $P = 0.019$), and was not significantly affected by the presence of permafrost ($P = 0.24$). The same was true for microbial biomass normalized to units of soil carbon (Table 2, fire effect, $P < 0.05$). Soil DOC concentrations were also reduced in the burned soils (Fig. 1a;

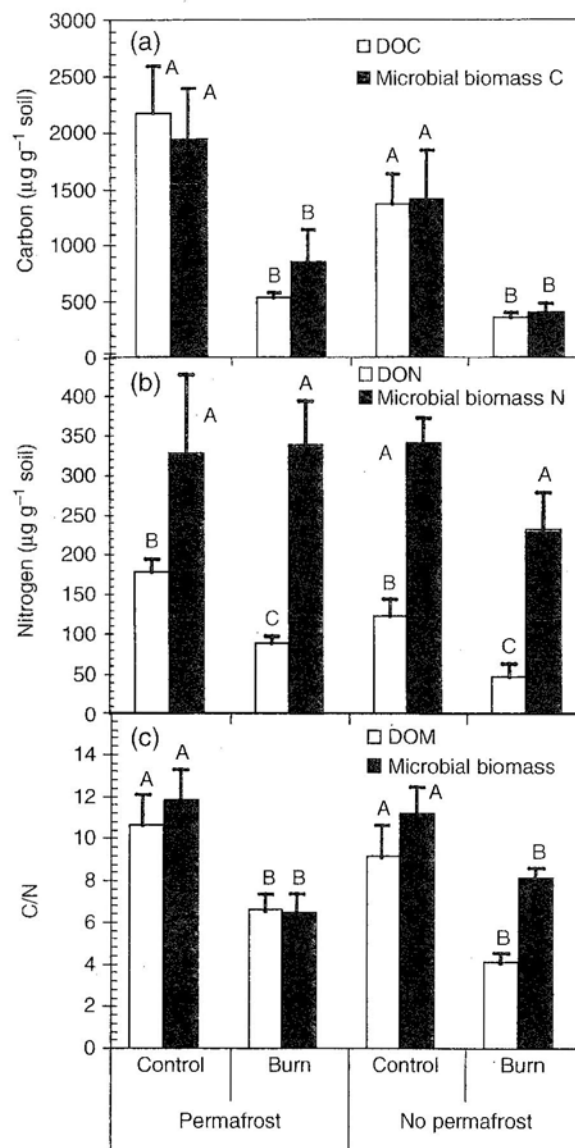


Fig. 1 Carbon (a), nitrogen (b), and C/N ratios (c) of extracted dissolved organic matter (DOM) and microbial biomass from O horizon soils. Values are means ($n = 8$, except for no permafrost burn where $n = 4$, error bars are 1 SE). Different letters indicate means that are significantly different from each other.

fire effect: $F = 12.25$, $P = 0.002$), but unaffected by permafrost presence. Microbial biomass carbon and DOC were strongly correlated ($r = 0.71$) indicating that either extractable carbon was limiting microbial growth and metabolism or that microbial activity was limiting the production of DOC.

Nitrogen in microbial biomass was not significantly affected by the permafrost or wildfire treatments (Fig. 1b). However, dissolved organic nitrogen (DON) was lower in nonpermafrost soils compared with permafrost soils (Fig. 1b, permafrost effect, $F = 9.29$, $P = 0.006$).

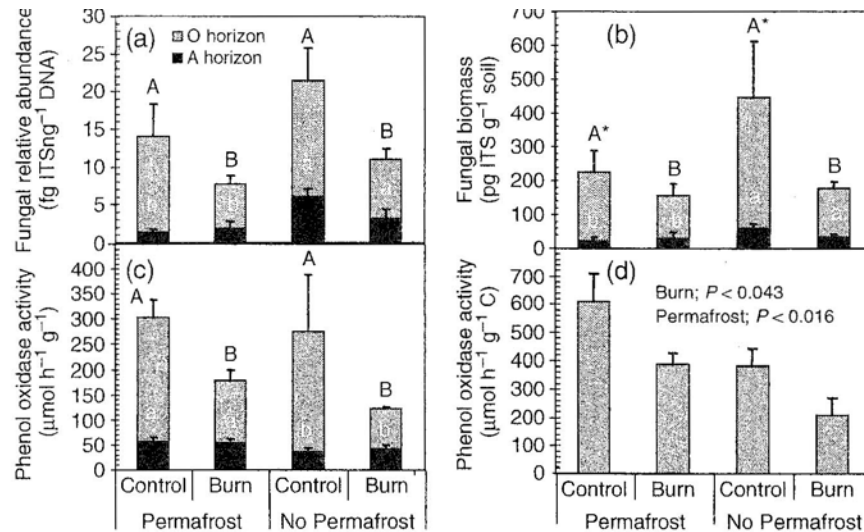


Fig. 2 Relative fungal abundance (a), total fungal abundance (b), phenol oxidase activity (c), and normalized phenol oxidase activity (d) in control and burned sites of permafrost and nonpermafrost soils. Different letters indicate significant differences by ANOVA. Differences are either due to a significant wildfire effect or a permafrost effect. There was no permafrost $\times$ wildfire interaction. The symbol asterisk (*) indicates significance at $P = 0.056$. Uppercase letters represent O horizons and lowercase letters represent A horizons ($n = 8$, no permafrost burn, $n = 6$; error bars are ± 1 SE).

and was reduced by wildfire (fire effect: $F = 25.6$, $P < 0.001$). The C/N ratios of microbial biomass and DOC were lower in the burned soils compared with the control soils (Fig. 1c, fire effect: $F = 8.55$, $P = 0.009$), and there was no effect of permafrost presence on either of these values. There was no interactive effect of wildfire and permafrost on microbial biomass C, N, DOC, or C/N ratio.

Wildfire tended to reduce total fungal biomass (fungal DNA/ g soil) in the O horizon but the effect was only marginally significant (fire effect: $F = 4.103$, $P = 0.056$) (Fig. 2b). Total fungal biomass was unaffected by the presence of permafrost. The relative abundance of fungi (fungal DNA/g total DNA) in the O horizon was lower in burned compared with control soils (fire effect: $F = 7.38$, $P = 0.013$) and was unaffected by the presence of permafrost, an effect that mirrored changes in total microbial biomass and DOC concentrations.

In the A horizon soils, permafrost was a more important factor influencing the fungal community compared with wildfire. Fungal relative abundance in the A horizon was 14% of the biomass in the O horizon (Fig. 2B, $F = 8.16$, $P = 0.007$). In the A horizon, the relative abundance and total fungal biomass were higher in the nonpermafrost soils compared with the permafrost soils (Fig. 2b, $F = 8.85$, $P = 0.008$ for relative abundance: $F = 6.33$, $P = 0.022$ for total abundance), but there was no significant effect of wildfire or wildfire by permafrost interaction on these variables.

Phenol oxidase enzyme activity displayed a similar pattern to fungal biomass: activity was reduced by wildfire in the O horizon soil, but unaffected by permafrost presence (Fig. 2c; $F = 8.43$, $P = 0.008$). In the A horizon, there was a different pattern; phenol oxidase activity was higher in the permafrost soils than the nonpermafrost soils ($F = 5.59$, $P = 0.026$), and unaffected by wildfire (Fig. 2c). Normalized phenol oxidase enzyme activity (activity per unit soil carbon) in the O horizon was higher in the control plots compared with the burned plots (Fig. 2d, $F = 4.681$, $P = 0.043$), and higher in the permafrost soil compared with the nonpermafrost soil (Fig. 2d, $F = 6.98$, $P = 0.016$). There was no interaction of fire and permafrost on enzyme activity. Phenol oxidase activity per unit microbial biomass was unaffected by wildfire or drainage class (data not shown), indicating that reductions in microbial biomass in wildfire affected soils were reducing the rates of enzyme production. DON and phenol oxidase activity were tightly correlated, whether enzyme activity was expressed on a per g soil or per g C basis ($r = 0.79$ – 0.81). Finally, the rate of lignin decomposition in O horizon soils reflected the differences in soil microbial biomass and enzyme activity: rates were highest in the control plots and were reduced by 83% in the burned soils (Fig. 3, fire effect: $F = 18.05$, $P = 0.002$). Yet, there was no effect of permafrost presence or interaction between wildfire and permafrost on lignin decomposition.

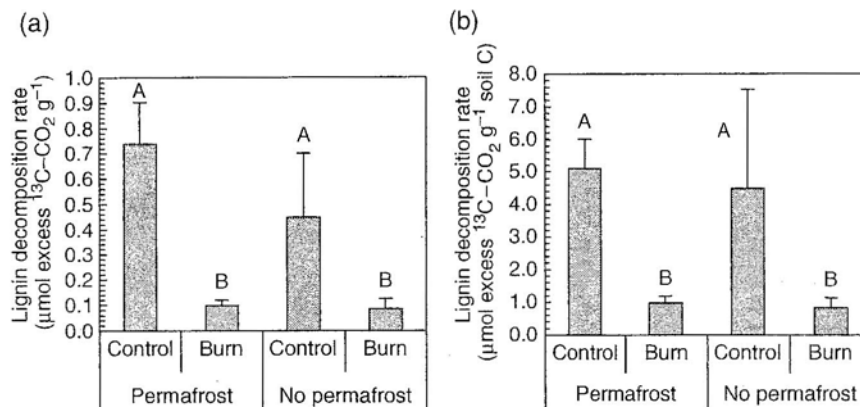


Fig. 3 Lignin decomposition rates in organic horizons of control and burned sites of permafrost and nonpermafrost soils. Lignin decomposition was quantified by measuring respired ¹³C-CO₂ after ¹³C-lignin addition to soil ($n = 4$, error bars are ± 1 SE). Different letters indicate means that are significantly different from each other.

Microbial respiration measured in the lab was reduced by fire in the O horizon (Table 2, fire effect: $F = 18.56$, $P = 0.0(2)$, which is consistent with a reduction in microbial biomass, fungal DNA abundance, and phenol oxidase enzyme activity (Figs 1 and 2). The absence of permafrost did not alter microbial respiration, consistent with the lack of an effect of permafrost presence on microbial biomass and enzyme activity. To estimate microbial respiration from field respiration rates, we assumed that microbial respiration was 60% of the total soil flux in the control sites (Schoor & Trumbore, 2006), and in the burned soils, microbial respiration was assumed to be 90–100% of the field flux because plots were mostly bare. In the field, heterotrophic respiration was reduced by wildfire ($2.20 \pm 0.13 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the control site and $1.53 \pm 0.15 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the burn site; $F = 11.64$, $P = 0.001$). Additionally, heterotrophic respiration in the field was lower in the permafrost site ($1.45 \pm 0.14 \mu\text{mol m}^{-2} \text{s}^{-1}$) as compared with the no permafrost site ($2.27 \pm 0.13 \mu\text{mol m}^{-2} \text{s}^{-1}$, $F = 16.75$, $P < 0.001$), and there was no significant burn by permafrost interaction.

Fungal abundance and the influence of permafrost on soil moisture affected the activity of soil enzymes that, in turn, controlled rates of decomposition (Fig. 4a). Stepwise multiple regression indicated that the relative abundance of fungi and soil moisture created the best model fit for soil phenol oxidase activity ($R^2 = 0.44$; fungal relative abundance $F = 6.0$, $P = 0.02$; moisture content $F = 11.8$, $P = 0.0015$). The relationship between fungal biomass and enzyme activity was modified by the presence of permafrost; where wetter permafrost soils had higher phenol oxidase activity for each unit of fungal biomass. Given that phenol oxidase activity was linearly related to the rate of lignin decomposition (Fig. 4b, $R^2 = 0.64$,

$P = 0.0006$) changes in the relative abundance of soil fungi interacts with soil moisture to control the degradation rate of recalcitrant lignin compounds.

Fungal community composition, as measured by DGGE, differed between soil horizons, burned and control stands, but these effects were mediated by the presence of permafrost (Fig. 5). NMDS was used to create fingerprints of fungal community composition. Along Factor 1, there was a significant burn $\times$ horizon interaction ($F = 25.63$, $P < 0.0001$) in which only A horizon communities were affected by burning. Also, along Factor 1, there was a significant permafrost $\times$ burn interaction ($F = 9.75$, $P = 0.004$) in which communities were significantly affected by burning only when permafrost was absent. Along Factor 2, there was a significant fire effect ($F = 5.73$, $P = 0.0242$) and a significant permafrost $\times$ horizon interaction ($F = 6.16$, $P = 0.02$) in which the presence of permafrost-affected soil communities in A horizon soils, and not in O horizon soils (Fig. 5). We did not determine the genetic composition of the DGGE bands, and therefore cannot ascribe changes along principal component axes to individual populations of fungi.

Discussion

Our findings show that in wildfire-affected soils, reductions in fungal abundance limits enzyme production, decomposition, and heterotrophic respiration from soils. Five years' following fire, wild fire-affected soils had reduced microbial biomass, microbial biomass per unit soil carbon, and total and relative fungal abundance. Wildfire and postwildfire soil conditions have been shown to reduce microbial population sizes, in particular soil fungi, through direct heating, reductions in labile substrates

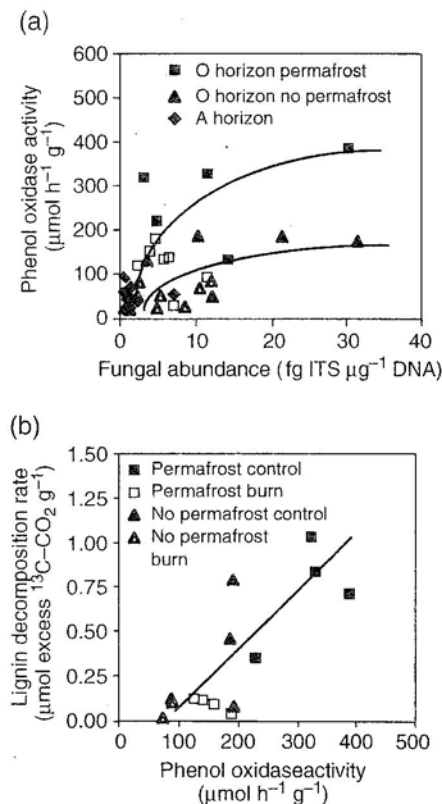


Fig. 4 Relationship between fungal relative abundance and phenol oxidase activity (a) and phenol oxidase and lignin decomposition (b). Closed symbols are control soils, open symbols are burned soils. The best fit for phenol oxidase activity was the relative abundance of fungi split by permafrost ($R^2 = 0.47$, $P = 0.0008$) and no permafrost treatments ($R^2 = 0.35$, $P = 0.006$). The equations are $(\text{phenol oxidase})^{0.5} = 4400 + 4100 (\text{relative abundance})$ for the permafrost soil and $(\text{phenol oxidase})^{0.5} = 2000 + 1000 (\text{relative abundance})$ for the nonpermafrost soil. Phenol oxidase activity was linearly related to lignin decomposition (b) by the equation $y = 0.003 (\text{phenol oxidase activity}) - 0.19$ ($R^2 = 0.63$).

from plant litter and exudates, and reductions in symbiotic relationships with plants (Ahlgren, 1974; Crigal & McColl, 1977; Choromanska & Deluca, 2001, 2002; Dahlberg, 2002; Ryan, 2002; Bergner *et al.*, 2004; Certini, 2005; Hart *et al.*, 2005; Wickland & Neff, 2007). Our results go one step further and show that reductions in fungal biomass that occurred postfire were the likely cause for reductions in enzyme activity (Figs 2 and 4), and the decomposition of lignin. Soil fungi produce the largest quantities of phenol oxidases and are the primary agents of lignin degradation (Hammel, 1997). Therefore, any reductions in the biomass of fungi should reduce the rate of degradation of lignin-type compounds. Why is microbial biomass reduced in postfire soils? It is likely that the reduction in plant roots and mosses that are sources of labile organic matter, producing the tight correlation

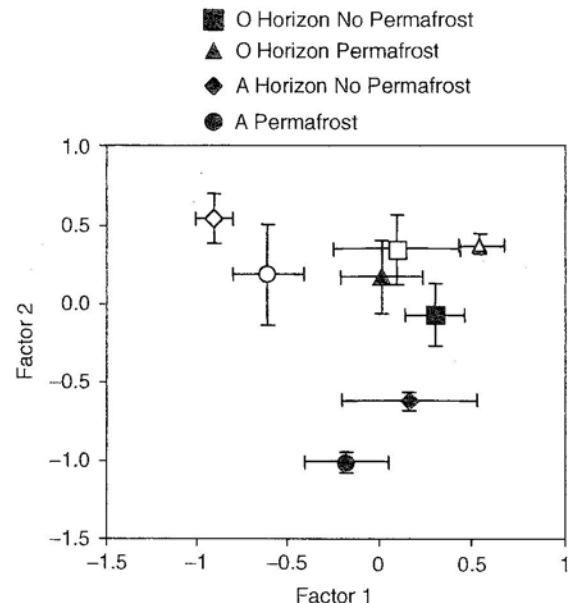


Fig. 5 Denaturing gradient gel electrophoresis (DGGE) 'fingerprints' of fungal community composition in control, burned, permafrost, and nonpermafrost O and A horizon soils ($n = 6$ for O horizon and $n = 3$ for A horizon, error bars are ± 1 SE). Nonmetric Multi-Dimensional Scaling (NMDS) was used to create fingerprints of fungal community composition from the multi-component DGGE dataset. Solid symbols are control soils; open symbols are burned soils.

between soil DOC and microbial biomass. Additionally, DON and enzyme activities were tightly correlated. This may indicate that enzyme concentrations are being reflected in the DON pool. On the other hand, enzymes are N-rich, and therefore the tight correlation between DON and enzyme activities could indicate a nutrient limitation to enzyme production.

Fungal biomass was directly related to phenol oxidase activity but the relationship was logarithmic in form. That is, there is a maximum threshold beyond which any increase in fungal biomass does not increase the production of soil enzymes. This is significant because recently developed enzyme-based decomposition models (Schimel & Weintraub, 2003) predict that there must be some mechanism that keeps enzyme activity in soil from increasing without limit. Such a nonlinear relationship is required so that micro-organisms do not waste energy-producing enzymes that do not give them a return (soluble carbon) on their investment (exoenzymes). Other hypotheses, such as the limited number of binding sites for exoenzymes on organic matter and mineral surfaces in soil, have been proposed to explain the nonlinear relationship (Schimel & Weintraub, 2003). Our work suggests that the mechanism producing the nonlinearity may be biotic; at a certain point more enzyme production does not result in increased resource

acquisition (Aro *et al.*, 2005). This can be caused by several mechanisms including diffusion limitation of enzymes and substrates, end-product inhibition of transcription or perhaps through a buildup of signaling molecules (quorum sensing), both of which are currently not well understood or described but may have tremendous importance for understanding the microbial ecology of decomposition (Aro *et al.*, 2005; Hogan, 2006).

Despite reductions in fungal biomass and enzyme activity in postfire soils, heterotrophic respiration can be elevated for several years, because reduced shading and decreased albedo increases soil temperatures (Bergner *et al.*, 2004). Our incubation and community data demonstrate that the *potential* for microbial decomposition is reduced even though the environment for decomposition is enhanced by elevated soil temperatures. From a larger scale perspective, the postfire reduction in fungal biomass and activity may be key to the persistence of the organic mat during the significant warming period that occurs initially after wildfire (Carrasco *et al.*, 2006). For example, in 2004, 5 years after the fire, heterotrophic respiration was lower in burned soils compared with control soils, despite the fact that soil temperatures were warmer in burned plots. Published data from these sites show that summer soil temperatures were elevated by 3.1 °C in the burned nonpermafrost soil and 1.6 °C in the burned permafrost soil compared with controls (Harden *et al.*, 2006). Although wildfire-affected soils also had lower soil moisture contents which would reduce rates of substrate diffusion to living cells, multiple regression analysis indicated that both moisture and fungal abundance interacted to explain soil process rates.

In the A horizon, which lies 2–20 cm below the burned surface, fungal biomass and activity were largely unaffected by burning (Fig. 2), indicating that the effect of wildfire was reduced at depth. However, the *composition* of the fungal community in mineral soils was affected by wildfire, and it was more strongly affected when permafrost was absent and soils were drier. Thus, wildfire effects on microbial communities are more severe when permafrost has been thawed. Future changes in the fire regime resulting from climate warming, specifically permafrost thaw and an increase in fire intensity, could alter the microbial potential for root recolonization, particularly for mycorrhizal fungi. If this reduces the potential for Black Spruce to reoccupy intensely burned soils, this could alter the trajectory of plant succession in the boreal forest (Johnstone & Kasischke, 2005).

Although the effect of permafrost on microbial communities is less dramatic than the effect of wildfire, changes in the permafrost regime could have long-lasting effect on microbial communities and biogeochemical cycling. Permafrost thaw can have a strong effect on soil carbon storage, for example, by changing

the hydrologic regime and decomposition rates at depth (Trumbore & Harden, 1997), by increasing the depth of the active layer, by altering the thermal conductivity of the soil, or by increasing the intensity of wildfire. In our study, permafrost soils, particularly mineral soils, were wetter than nonpermafrost soils in both burned and unburned environments owing to permafrost acting as a barrier to vertical water movement. We found that the presence of permafrost decreased fungal abundance and altered composition, particularly in mineral soils (Fig. 2). This may have occurred because nonpermafrost soils are warmer and, in this case, more oxygenated at depth in comparison with permafrost soils. Additionally, phenol oxidase enzyme activity was higher in the wetter permafrost soils compared with drier nonpermafrost soils, likely due to the fact that moisture strongly limits the activity of soil enzymes (Waldrop & Firestone, 2006). It is reasonable to conclude that the presence of permafrost influences microbial community composition and function, inasmuch as soil moisture is affected.

At the landscape scale, temperature, substrate quality, and soil drainage have been shown to control decomposition and turnover on annual and decadal timescales (Trumbore & Harden, 1997). A more mechanistic understanding of the decomposition processes and spatial and temporal variation in those processes can help decipher biotic from abiotic effects of wildfire on carbon cycling. Our analysis shows that direct linkages can be made between variation in microbial communities, enzyme synthesis, and decomposition of specific fractions of organic matter. Over the short term, wildfire reduces fungal biomass, enzyme activities, lignin decomposition, and the rate of heterotrophic respiration from soil. Over the long term, as permafrost thaws, changes in the moisture regime can affect microbial community composition and function. If permafrost thaw results in more intense wildfires, this could reduce the rate at which microbial communities recover from fire, potentially affecting nutrient availability and vegetation succession (Dahlberg, 2002; Johnstone & Kasischke, 2005).

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