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Low levels of nitrogen addition stimulate decomposition by boreal forest fungi

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ABSTRACT

Climate warming and associated increases in nutrient mineralization may increase the availability of soil nitrogen (N) in high latitude ecosystems, such as boreal forests. These changes in N availability could feed back to affect the decomposition of litter and organic matter by soil microbes. Since fungi are important decomposers in boreal forest ecosystems, we conducted a 69-day incubation study to examine N constraints on fungal decomposition of organic substrates common in boreal ecosystems, including cellulose, lignin, spruce wood, spruce needle litter, and moss litter. We added 0, 20, or 200 μg N to vials containing 200 mg substrate in factorial combination with five fungal species isolated from boreal soil, including an Ascomycete, a Zygomycete, and three Basidiomycetes. We hypothesized that N addition would increase CO_2 mineralization from the substrates, particularly those with low N concentrations. In addition we predicted that Basidiomycetes would be more effective decomposers than the other fungi, but would respond weakly or negatively to N additions. In support of the first hypothesis, cumulative CO_2 mineralization increased from 635 ± 117 to $806 + 108$ μg C across all fungal species and substrates in response to 20 μg added N; however, there was no significant increase at the highest level of N addition. The positive effect of N addition was only significant on cellulose and wood substrates which contained very little N. We also observed clear differences in the substrate preferences of the fungal species. The Zygomycete mineralized little CO_2 from any of the substrates, while the Basidiomycetes mineralized all of the substrates except spruce needles. However, the Ascomycete (*Penicillium*) was surprisingly efficient at mineralizing spruce wood and was the only species that substantially mineralized spruce litter. The activities of β-glucosidase and *N*-acetyl-glucosaminidase were strongly correlated with cumulative respiration ($r = 0.78$ and 0.74, respectively), and *Penicillium* was particularly effective at producing these enzymes. On moss litter, the different fungal species produced enzymes that targeted different chemical components. Overall, our results suggest that fungal species specialize on different organic substrates, and only respond to N addition on low N substrates, such as wood. Furthermore, the response to N addition is non-linear, with the greatest substrate mineralization at intermediate N levels.

1. Introduction

Ecosystems in high northern latitudes store ~30% of global terrestrial carbon (C) (Gorham, 1991; Jobbagy and Jackson, 2000; Kasischke, 2000) and are expected to warm by 4–7 °C during this century due to anthropogenic climate change (ACIA, 2004). Temperatures in these regions have already increased by ~1.5 °C (Moritz et al., 2002). As climate warming intensifies, the decomposition of soil organic matter will likely increase, resulting in soil C losses and greater levels of soil nutrient mineralization and availability (Rustad et al., 2001; Hobbie et al., 2002). Such changes in nutrient availability could then feed back to have additional effects on plant and microbial communities in boreal ecosystems (Strömgren and Linder, 2002; Mack et al., 2004; Olsson et al., 2005).

Fungi are believed to play a dominant role in the mineralization of soil organic matter in boreal ecosystems because they tolerate low soil pH and are capable of degrading the recalcitrant litter produced by boreal plants (Högberg et al., 2007; Lindahl et al., 2007). Many species of fungi produce extracellular enzymes that degrade complex forms of organic material and release nutrients from soil organic matter (Kjøller and Struwe, 2002). For example, some Basidiomycete fungi produce oxidative enzymes required for the breakdown of lignin and humic materials (Kirk and Farrell, 1987; Blanchette, 1991). Other types of fungi may produce enzymes that target different C substrates in litter and soil, such as cellulose and chitin (Lynd et al., 2002; Lindahl and Finlay, 2006). Thus fungi may specialize on the decomposition of different C compounds.

Not only do decomposer fungi likely differ in their C substrate preferences, but they may also differ in their responses to N

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availability. Basidiomycetes, or certain taxa within the Basidiomycetes (i.e. white rot fungi) are often predicted to respond negatively to N addition based on microcosm and culture studies (Fog, 1988). More recent studies show that saprotrophic fungal biomass and activity in microcosms may increase (Boberg et al., 2008) or decline with nitrogen addition (Entry, 2000). Thus, the response of fungal abundance to N addition is highly variable, and this variation appears to extend to the N response of extracellular enzymes and soil respiration (Frey et al., 2004; Waldrop et al., 2004; Blackwood et al., 2007; Hofmockel et al., 2007; Demoling et al., 2008). Variation in fungal substrate preferences and responses to added N makes it difficult to predict how ecosystem processes will respond to climate warming and increased N availability.

To address this uncertainty, we have been examining feedbacks between warming, nutrients, and C cycling in a boreal forest near Delta Junction, Alaska. We have found that soil warming increases N mineralization but not soil respiration (Allison and Treseder, 2008). Consistent with this finding, direct fertilization of the soil with 100 kg ha^{-1} yr^{-1} N has no effect on soil respiration (Allison et al., 2008). These results were surprising, given that N is generally thought to be a limiting nutrient for plant and microbial growth in boreal ecosystems (Hobbie et al., 2002). Using clone libraries, we then looked for changes in fungal community composition that could explain the lack of response to N. The community composition changed dramatically with N addition, but most of the taxa we identified were likely ectomycorrhizal, so their relevance for soil C cycling is unclear (Allison et al., 2007, 2008).

Based on these field studies, it was not possible to evaluate how N addition affects C cycling by saprotrophic fungi because the clone libraries contained relatively few known saprotrophs. Therefore, the objective of the current study was to isolate fungi from our field site and examine their individual responses to added N on different C substrates. In this way, we could determine specifically how non-mycorrhizal members of the fungal community respond to N addition. For instance, the observed lack of change in soil respiration under N fertilization could reflect a decrease in C respiration by mycorrhizal fungi and roots offset by an increase in respiration by saprotrophic fungi under N addition.

Using a laboratory microcosm approach, we aimed to test four main hypotheses. First, N addition should increase CO_2 mineralization by saprotrophic fungi, since decomposition of most plant and soil substrates requires the production of N-rich extracellular enzymes (Sinsabaugh, 1994). However, decomposition driven by Basidiomycete fungi should show a weaker response to N and may decline at high levels of N addition, as observed in culture studies. We also predicted that decomposition of substrates with lower N concentrations would respond more strongly to N addition. Finally, we hypothesized that Basidiomycete fungi would produce more C-degrading enzymes, and would therefore catalyze greater substrate mineralization, than would Zygomycete and Ascomycete fungi. We tested these hypotheses with five fungal species and five common C substrates from our boreal forest field site.

2. Materials and methods

2.1. Fungal isolation

We isolated *Mortierella* spp. (Zygomycete) and *Penicillium* spp. (Ascomycete) strains from the O-horizon of unfertilized soils underlying the mature black spruce forest site described in Treseder et al. (2007). A 1 g subsample of frozen soil was diluted 1:1000 (fresh wt:vol) in sterile 0.9% NaCl solution to create an inoculum. Inoculum (500 μl) was spread on agar plates containing (per liter) 500 mg KH_2PO_4, 150 mg $MgSO_4 \cdot 7H_2O$, 50 mg $CaCl_2 \cdot 2H_2O$, 25 mg NaCl, 20 mg ferric EDTA, 0.1 μg thiamine HCl, 12 g agar, 130 μg streptomycin, and 10 mg chlortetracycline. Sterile-filtered antibiotic solutions were added to media after autoclaving 40 min (121 °C) and cooling to ~40 °C. Glucose medium used for isolation of *Mortierella* additionally contained 250 mg $(NH_4)_3PO_4$, 3 g malt extract, and 10 g glucose. Media used for isolation of *Penicillium* additionally contained 1 mg malt extract, 1 g bovine serum albumin, and 10 g tannic acid. The fungal orders Mortierellales and Eurotiales, which include *Mortierella* and *Penicillium*, represented 0.2–1.0% and 0.4–2.4%, respectively, of the sequences present in fungal clone libraries from the field site (Allison et al., 2007, 2008; Allison and Treseder, 2008).

The Basidiomycetes *Pholiota carbonaria* and *Agrocybe praecox* were isolated from sporocarps collected from an early successional black spruce forest that burned in 1999 (Treseder et al., 2007), and *Polyporus varius* (also a Basidiomycete) was cultured from a decaying spruce stump at the same site. *Pholiota* and *Agrocybe* belong to the order Agaricales, which represents 39–59% of the sequences in fungal clone libraries from the field site. *Polyporus* belongs to the Polyporales, which have relative abundances of 0.7–4.9% in clone libraries. Basidiomycetes were isolated by excising a small piece of sporocarp or stump tissue that was placed on agar containing (per liter) 20 g agar, 5 g malt extract, 5 g yeast extract, 8 mg benomyl, and 6 mg 2-phenyl phenol. All 5 fungal isolates were transferred to malt–yeast agar containing (per liter) 20 g agar, 5 g malt extract, and 5 g yeast extract prior to inoculation of microcosms.

Fungal identifications were made to at least genus level based on morphology, ecological characteristics, and sequences of the 18S and internal transcribed spacer (ITS) regions of the fungal rRNA gene complex (Allison et al., 2008). Using the BLAST tool, DNA sequences were matched to known fungal taxa in the GenBank database (Table 1). 18S and ITS regions were BLAST searched separately, and the returned hits were used in combination with morphological and ecological characters to identify taxa to the genus or species level.

Table 1
Top two BLAST hits for fungal identifications used in microcosms

Fungal ID	ITS hit	Percent identity	Match length (bp)	18S hit	Percent identity	Match length (bp)
Mortierella spp.	Zygomycete spp.	99	498	*Mortierella parvispora*	99	667
	Uncultured	98	524	*Mortierella alpina*	99	666
Penicillium spp.	*Penicillium soppii*	98	534	*Penicillium* spp.	99	715
	Penicillium kojigenum	96	535	*Eladia saccula*	99	715
Pholiota carbonaria	*Pholiota carbonaria*	94	483	*Nivatogastrium nubigenum*	99	687
	Uncultured *Pholiota*	91	504	*Pholiota squarrosa*	99	687
Agrocybe praecox	Uncultured	99	641	*Pholiota squarrosa*	99	688
	Agrocybe praecox	99	639	*Agrocybe smithii*	99	688
Polyporus varius	*Polyporus varius*	89	146	*Polyporus squamosus*	90	271
	Polyporus varius	94	97	*Polyporus squamosus*	90	271

2.2. Substrates

Substrates were obtained from Sigma–Aldrich or from the mature forest field site (Treseder et al., 2007). Powdered cellulose from spruce (cat. no. 22182) and hydrolytic lignin (cat. no. 371076) were obtained from Sigma. The black spruce wood substrate was derived from downed branches (~3 cm diameter) that were slightly weathered but had not decayed substantially; bark was removed before use. Senesced spruce needles were collected directly from living trees. Moss litter was collected from just below the living moss layer. Wood, needle, and moss litters were dried at 65 °C and ground in a Wiley mill to pass a #20 mesh screen. All substrates were sterilized with 2.5–3.0 Mrad gamma radiation. Based on elemental analysis, moss litter contained 1.2% N, lignin contained 0.6% N, spruce needle litter contained 0.4% N, and the wood and cellulose substrates contained N concentrations below detection.

2.3. Microcosms

Microcosms were established in sterile 40 ml vials with gas-tight septum caps containing 2 g sterile sand, 200 mg substrate, and 800 μl fungal inoculum. The fungal inoculum consisted of three ~5 mm cubes of mycelium-rich agar that were mashed, combined, and suspended in 75 ml sterile growth medium. The growth medium contained (per liter) 500 mg KH_2PO_4, 150 mg $MgSO_4 \cdot 7H_2O$, 50 mg $CaCl_2 \cdot 2H_2O$, 20 mg ferric EDTA, 0.1 μg thiamine HCl, and either 0, 71.4, or 714 mg NH_4NO_3. Thus, each microcosm received 0, 20, or 200 μg added N as NH_4NO_3 (plus 1.2 μg N as EDTA, 0.06 μg N as malt–yeast agar, and 90 μg P as KH_2PO_4 from the growth medium). We chose these N levels based on the assumption that fungal biomass C could reach 2% of added substrate C, or ~2 mg if substrates contain ~50% C. At a fungal C:N ratio of 10:1, this biomass would require 200 μg added N. We therefore used 200 μg added N as the maximum level because some of our substrates already contained N, and the fungal biomass added in the initial inoculum was much less than 2% of substrate C. A set of "no-fungus" control vials received growth medium (0 μg added N) without mycelial suspension.

2.4. Cumulative CO_2 measurements

CO_2 concentrations in the microcosm headspace were measured every 3–7 days and used to calculate cumulative CO_2 respiration over a 69-day incubation period at 21 °C. For each measurement, an 8 ml subsample of headspace gas was withdrawn by syringe and injected into an infrared gas analyzer (PP-Systems EGM-4). After measurement, vials were opened under sterile conditions, equilibrated with ambient air for ~5 min, and then closed. The CO_2 concentrations of blank vials were subtracted from sample vials to calculate cumulative respiration of substrate C.

2.5. Enzyme assays

We measured the activities of extracellular enzymes involved in C and nutrient cycling using fluorimetric and colorimetric assays. At the end of the 69-day experiment, microcosms were frozen at −20 °C until analysis of enzyme activities. Four enzymes were assayed using the fluorimetric procedures of Saiya-Cork et al. (2002): β-glucosidase hydrolyzes glucose monomers from cellulose, *N*-acetyl-glucosaminidase releases amino sugars from chitin, leucine aminopeptidase hydrolyzes amino acids from polypeptides, and acid phosphatase cleaves phosphate groups from organophosphates. We also measured polyphenol oxidase activity using the method of Sinsabaugh et al. (1992) to assess fungal potential for lignin degradation. The contents of each microcosm were homogenized with a magnetic stirrer in 50 ml sodium acetate buffer, pH 5.0. Resulting slurry (200 μl) was combined with 50 μl substrate solution and incubated in microplates (Table 2). For the fluorimetric assays, fluorescence was measured at 360 nm excitation and 460 nm emission after adding 10 μl 1.0 M NaOH. We ran 8 analytical replicates, and controls were included to account for fluorescence quenching and background fluorescence of the sample slurry and substrate solutions. We converted fluorescence to activity in $\mu mol\ g^{-1}$ substrate h^{-1} based on the fluorescence of 4-methylumbelliferone or 7-amino-4-methylcoumarin standards. Polyphenol oxidase activity was determined at 460 nm absorbance on 16 analytical replicates. After accounting for the absorbance of slurry and substrate controls, activity was expressed in $\mu mol\ g^{-1}$ substrate h^{-1} based on the absorbance of a known concentration of L-DOPA oxidized by tyrosinase.

2.6. Statistical analyses

Cumulative CO_2 respiration and enzyme activities were initially analyzed by three-way analysis of variance (ANOVA) using SAS PROC MIXED (SAS Institute, 2004) and fungal species, substrate, and N level as fixed effects. To facilitate interpretation, we ran two-way ANOVA on each substrate with fungal species and N level as fixed effects. We chose this approach because substrates are often spatially separated in the field, and we were interested in the effects of fungal species and N on each substrate individually. For post hoc mean separation, we used Tukey tests adjusted for multiple comparisons. We also tested for significant linear correlations among enzymes and cumulative respiration using PROC CORR. No-fungus control vials were not included in statistical analyses. To improve normality, all data were log-transformed prior to statistical analyses, and 3 wood-containing microcosms were removed from the analyses due to visually apparent contamination by molds.

3. Results

3.1. Overall patterns in cumulative respiration and enzyme activities

Fungal species, substrate, and N level each explained significant amounts of variation in cumulative respiration and activities of β-glucosidase, *N*-acetyl-glucosaminidase, and acid phosphatase (Table 3). These four response variables were also highly correlated with one another (Table 4). Activities of leucine aminopeptidase and polyphenol oxidase were generally very low (overall means of 0.45 ± 0.12 nmol $h^{-1}\ g^{-1}$ and 0.53 ± 0.05 μmol $h^{-1}\ g^{-1}$, respectively) and only weakly correlated with cumulative respiration and the other enzyme activities. When pooled across different species and substrates, N only had a significant positive effect on cumulative respiration at 20 μg N addition and not 200 μg (Fig. 1A).

Table 2
Substrates and incubation times used in extracellular enzyme assays

Enzyme	Substrate	Incubation time (h)
β-Glucosidase	200 μM 4-methylumbelliferyl β-D-glucopyranoside	0.5–1.0
N-acetyl-glucosaminidase	200 μM 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide	0.5–1.0
Acid phosphatase	200 μM 4-methylumbelliferyl phosphate	0.5–1.0
Leucine aminopeptidase	200 μM L-leucine-7-amido-4-methylcoumarin hydrochloride	18–24
Polyphenol oxidase	25 mM L-dihydroxyphenylalanine (DOPA)	18–24

Table 3
Analysis of variance *P*-values for fungal species, substrate, and nitrogen addition effects on cumulative respiration (CO_2) and β-glucosidase (BG), *N*-acetyl-glucosaminidase (NAG), acid phosphatase (AP), leucine aminopeptidase (LAP), and polyphenol oxidase (PPO) activities in microcosms

	CO_2	BG	NAG	AP	LAP	PPO
Species	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.014**	0.674
Substrate	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Species × substrate	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.102	**<0.001**
Nitrogen	**<0.001**	**<0.001**	**0.006**	**0.033**	0.125	0.105
Species × nitrogen	**0.011**	0.330	**0.002**	**0.046**	0.693	0.621
Substrate × nitrogen	**0.028**	**0.003**	**0.019**	0.667	0.692	0.971
Species × substrate × nitrogen	0.720	**<0.001**	**0.001**	**0.010**	0.658	0.457

Significant *P*-values ($P < 0.05$) are shown in bold text.

However, the two-way interaction terms were also significant in the ANOVA. For example, the effect of N on cumulative respiration was only strongly positive for *Agrocybe* (Fig. 1F). There was also a significant substrate × N effect, so we present results separately for each substrate in the following sections.

3.2. Wood litter

Penicillium mineralized the most CO_2 from wood litter, with cumulative respiration 10-fold higher than the Basidiomycete species (Fig. 2A). Cumulative respiration by *Penicillium* increased threefold with addition of N, to >1100 μg C. Across all species, there was a positive effect of N on cumulative respiration that was-significant for the 20 μg N addition level ($P = 0.033$). Patterns in β-glucosidase and *N*-acetyl-glucosaminidase activities paralleled CO_2 responses, although the N effect was only significant for β-glucosidase (Fig. 2B, C). Polyphenol oxidase activities were low and variable on wood, but there was a significant fungal species × N interaction ($P = 0.029$), with *Penicillium* polyphenol oxidase activities relatively high at the 200 μg N addition level (Fig. 2D).

3.3. Cellulose

Cellulose is a major component of wood, and showed a pattern similar to wood in cumulative respiration, which peaked at 20 μg N addition across all fungal species (Fig. 3A; $P < 0.001$ and $P = 0.014$ for comparison to 0 and 200 μg N, respectively). *Pholiota* respired the most CO_2 from cellulose at all N levels, although *Agrocybe* also respired >1500 μg C with 20 μg N addition ($P < 0.001$ for species effect).

Responses of β-glucosidase and *N*-acetyl-glucosaminidase activity were somewhat similar to respiration responses on cellulose, but there were significant fungal species × N interactions (Fig. 3B-C). The interactions were due to high enzyme activities in the 200 μg N treatment with *Polyporus*, which contrasted with high enzyme activities at the 20 μg N level for the other strong cellulose decomposers. However, across all species, there was a significant peak in β-glucosidase activity at 20 μg N addition ($P = 0.026$ and $P = 0.016$ for comparison to 0 and 200 μg N, respectively). Across all species, *N*-acetyl-glucosaminidase activity at the highest N addition level was significantly lower than activity at 0 μg added N ($P < 0.001$).

Table 4
Pearson correlation coefficients for relationships between cumulative respiration (CO_2) and β-glucosidase (BG), *N*-acetyl-glucosaminidase (NAG), acid phosphatase (AP), leucine aminopeptidase (LAP), and polyphenol oxidase (PPO) activities in microcosms

	BG	NAG	AP	LAP	PPO
CO_2	**0.78**	**0.74**	**0.51**	**0.33**	0.04
BG		**0.78**	**0.55**	**0.29**	0.06
NAG			**0.76**	**0.26**	−0.02
PHOS				0.12	−0.04
LAP					0.08

Significant correlations ($P < 0.05$) are shown in bold text.

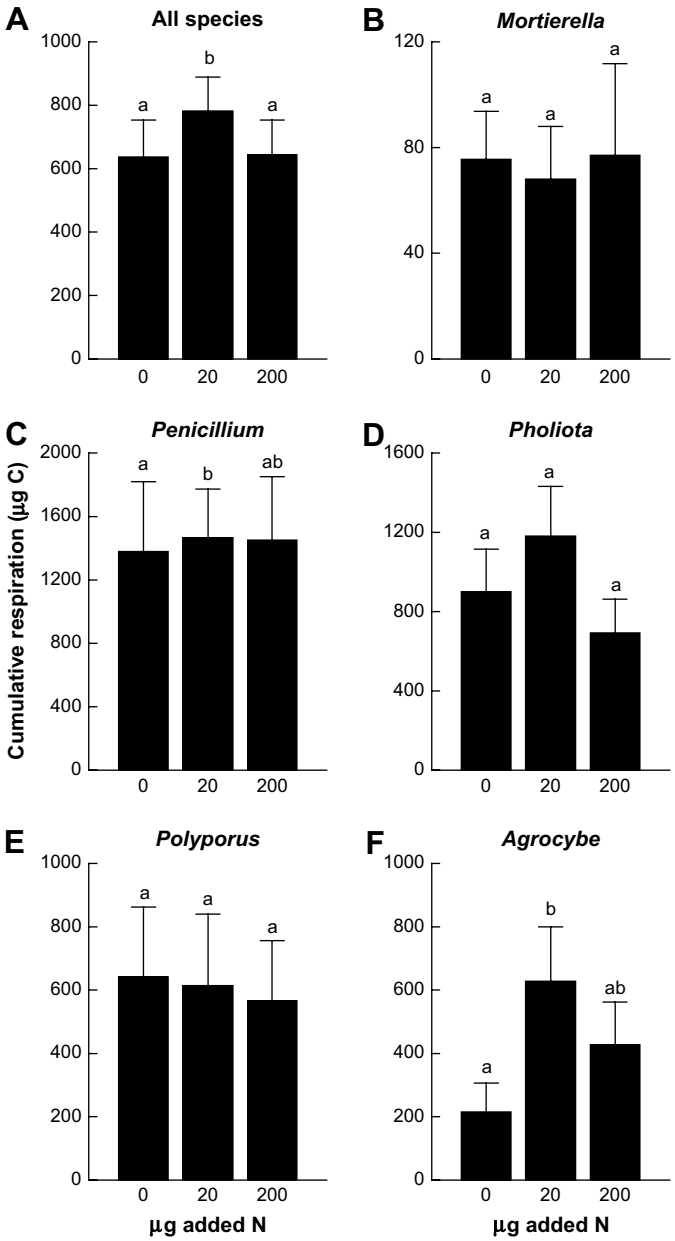

Fig. 1. Cumulative CO_2 respiration averaged across substrates in response to N addition for all fungal species (A) and each individual species (B–F). Bars represent means (±SE), and means with the same letter are not significantly different (ANOVA on log-transformed data).

3.4. Lignin

Like cellulose, lignin is also a major wood constituent, but respiration and enzyme patterns for lignin were distinct from wood and cellulose. There was no significant N effect on lignin mineralization, and all fungal species respired CO_2 from the lignin substrate (Fig. 4). Only this substrate was substantially decomposed by *Mortierella*, although the other fungal species produced more cumulative CO_2 (Fig. 4). The activities of β-glucosidase, *N*-acetyl-glucosaminidase, and acid phosphatase tended to correspond with

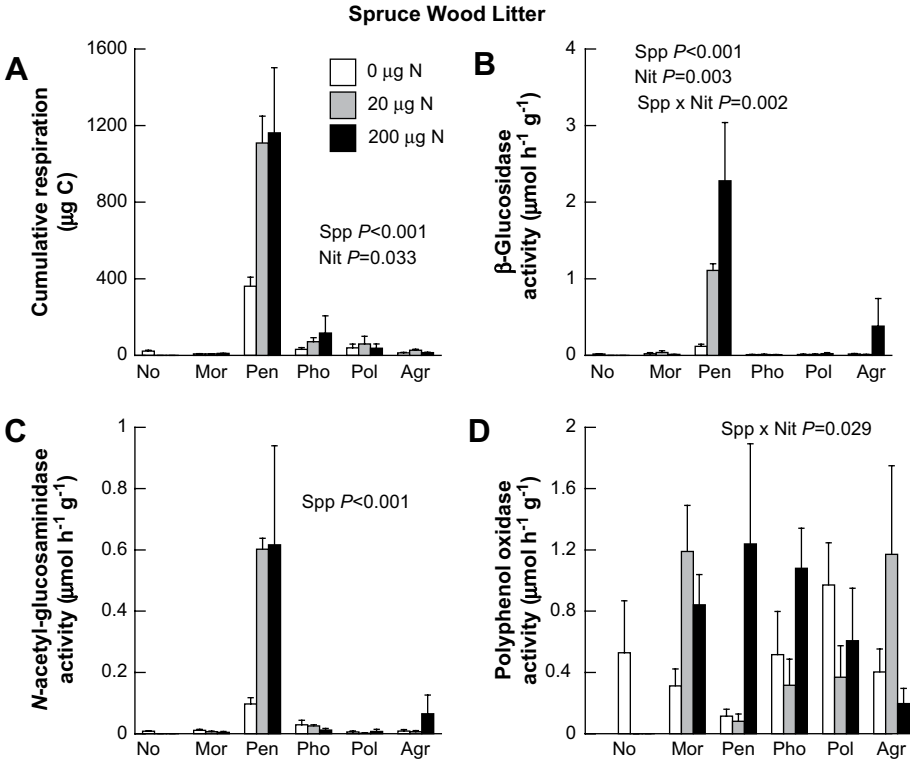

Fig. 2. Cumulative CO_2 respiration (A) and activities of β-glucosidase (B), *N*-acetyl-glucosaminidase (C), and polyphenol oxidase (D) on spruce wood substrate. Bars represent means (±SE), and significant effects from ANOVA are shown on each panel. No = no fungus control; Mor = *Mortierella* spp.; Pen = *Penicillium* spp.; Pho = *Pholiota carbonaria*; Pol = *Polyporus varius*; Agr = *Agrocybe praecox*. Nitrogen addition and fungal species did not significantly affect leucine aminopeptidase or acid phosphatase activities.

cumulative lignin respiration, but there was no apparent relationship between the lignin-degrading enzyme polyphenol oxidase and lignin mineralization (data not shown).

3.5. Moss litter

All species except *Mortierella* produced relatively large amounts of CO_2 from moss litter, but there was no effect of added N on cumulative respiration (Fig. 5A). *Pholiota* and *Polyporus* each respired >2000 µg C from the moss substrate, while *Penicillium* and *Agrocybe* respired half of this amount ($P < 0.001$ for species effect).

Activities of extracellular enzymes produced on moss litter also varied significantly with fungal species ($P < 0.001$), but not with N addition (Fig. 5B–E). *Penicillium* produced large amounts of β-glucosidase (5.3 ± 0.4 µmol h^{-1} g^{-1}; Fig. 5B), and both *Penicillium* and *Pholiota* showed high levels of *N*-acetyl-glucosaminidase activity exceeding 7.0 µmol h^{-1} g^{-1} (Fig. 5C). *Polyporus* growing on moss litter produced the highest values of polyphenol oxidase activity recorded in the experiment (3.4 ± 0.2 µmol h^{-1} g^{-1}; Fig. 5D), and also produced a large quantity of acid phosphatase (Fig. 5E).

3.6. Needle litter

The spruce needle results were distinctive because only *Penicillium* was able to substantially mineralize CO_2 from this substrate (Fig. 6A). Furthermore, the cumulative mineralization value of 4162 ± 575 µg C for *Penicillium* was the highest recorded for any species–substrate combination in the experiment. Although there was no significant N effect on CO_2 respiration, N addition did have a strong effect on β-glucosidase and *N*-acetyl-glucosaminidase production by *Penicillium*; activities of these enzymes increased to 5.6 ± 1.9 µmol h^{-1} g^{-1} and 3.4 ± 1.1 µmol h^{-1} g^{-1}, respectively, under the 200 µg N addition level (Fig. 6B, C).

4. Discussion

4.1. Nitrogen effects

Across all fungal species and substrates, we found support for the hypothesis that N addition stimulates the decomposition of C substrates common in boreal forest ecosystems. Importantly, though, the effect was only positive at the lower level of N addition (20 µg N per 200 mg substrate), and was only significant for wood and cellulose substrates that had extremely low initial N concentrations. A study with decomposing pine litter also found that fungal growth peaked at intermediate levels of N availability (Hogervorst et al., 2003). Even with 0 µg added N, our fungal isolates were able to mineralize a substantial amount of C from all of the substrates. The only external N sources would have been the trace amounts available from EDTA and residual malt–yeast agar from the fungal inoculum.

Contrary to our expectations based on other laboratory studies (Fog, 1988), we did not find that Basidiomycete fungi were inhibited by N addition. *Pholiota*, *Polyporus*, and *Agrocybe* increased or maintained CO_2 mineralization rates under N addition (Fig. 1D–F), suggesting that not all Basidiomycetes respond negatively to N. It is possible that our incubation was too short to detect a negative N effect on Basidiomycete decomposition. However, our incubation time was similar to other studies that have observed substantial C mineralization (Osono et al., 2003) and studies that have observed negative N effects (Fog, 1988). Instead, the relatively low (but ecologically relevant) levels of N that we added may explain why decomposition rates did not decline. Many of the pure culture studies with Basidiomycetes referenced by Fog (1988) also found increased decomposition at low levels of inorganic N addition. Suppression of decomposition only occurred at N levels much higher than those used in our study (e.g. Findlay, 1934; Schmitz

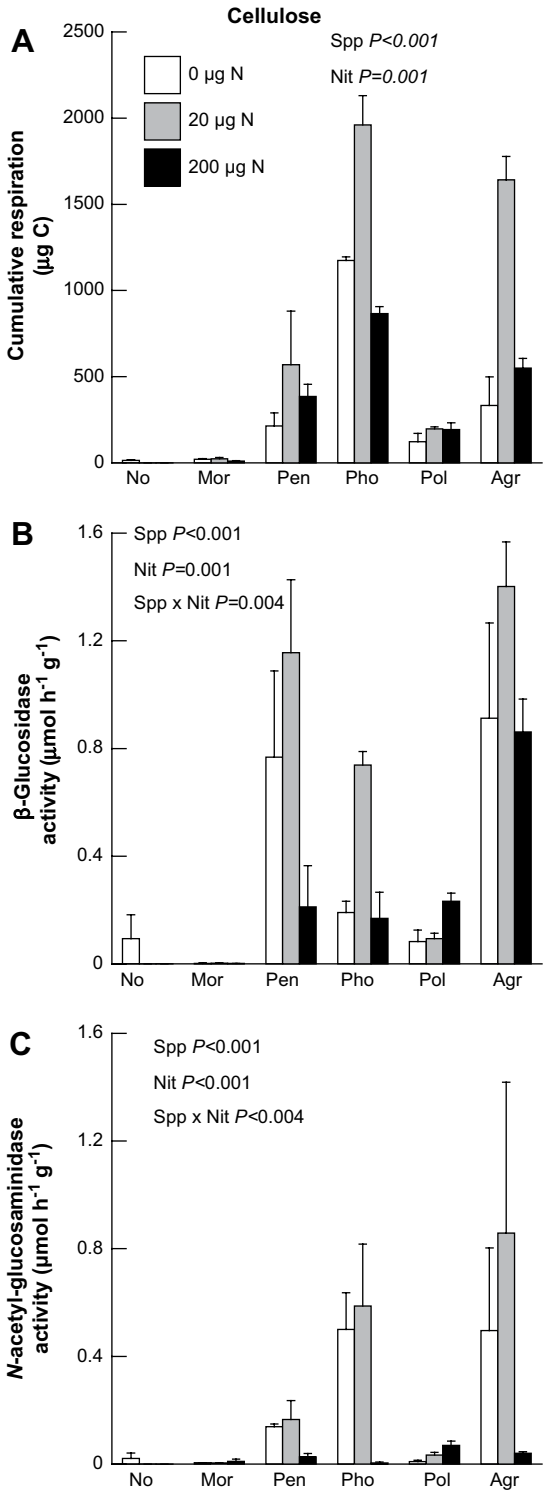

Fig. 3. Cumulative CO_2 respiration (A), β-glucosidase activity (B), and *N*-acetyl-glucosaminidase activity (C) on cellulose substrate. Bars represent means (±SE), and significant effects from ANOVA are shown on each panel. X-axis symbols as in Fig. 2. Other enzymes are not shown because they did not show a clear relationship with cellulose mineralization.

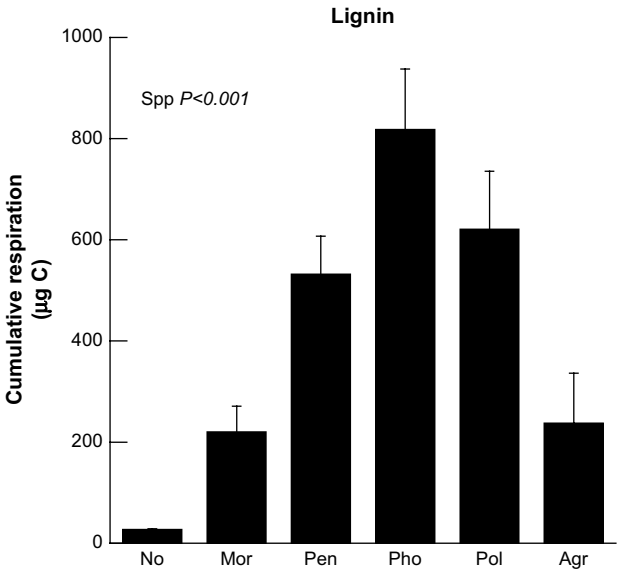

Fig. 4. Cumulative CO_2 respiration on lignin substrate by fungal species ($P < 0.001$, ANOVA). Bars represent means (±SE). X-axis symbols as in Fig. 2. Enzyme activities were not strongly related to lignin mineralization and are not shown.

and Kaufert, 1936; Zadražil and Brunnert, 1980). Clearly all fungi require some amount of N for growth, and it should not be surprising that the optimal amount varies across fungal species and substrates. Indeed, some of the ongoing debate about positive versus negative fungal responses to N addition (DeForest et al., 2004; Frey et al., 2004; Clemmensen et al., 2006) might be resolved by assessing the N requirements of fungi in N-fertilized ecosystems.

A major goal of our study was to determine how increased N availability affects the fungi that cycle C in Alaskan boreal ecosystems. To accomplish this goal, we used substrates that comprise the major C inputs to mature black spruce forests (Treseder et al., 2004) and isolated fungi that are representative of the taxa present in clone libraries from field soils. However, we recognize that we cannot predict the exact contribution of these species to soil CO_2 respiration because we have not quantified the absolute abundances of our fungal isolates in the field. Nonetheless, our results are consistent with the field-level respiration responses of Alaskan boreal soils to added N. In a recent study, we found that N addition had no effect on soil respiration over a 3-year period (Allison et al., 2008). Although roots and mycorrhizal fungi also contribute to soil CO_2 efflux, the N response of the saprotrophic fungi in our microcosms may partly explain why there is no N response in the field. Except for new inputs of coarse woody debris, most of the litter and soil organic matter substrates in the field may contain enough N to prevent N limitation of decomposition by fungi. Furthermore, the fertilization rate of 100 kg NH_4NO_3-N ha^{-1} yr^{-1} in the field may be high enough to transform the fungal N response from positive to neutral, as occurred at the 200 µg N addition level in the microcosms. Assuming a total soil C pool of 3600 ± 600 g m^{-2} (King et al., 2002; Treseder et al., 2004), the field fertilization rate corresponds to a C:fertilizer N ratio of ~360, which is comparable to our 200 µg N microcosm treatment (~100 mg C/0.2 mg fertilizer N = C:N of 500). Our field level of N addition is typical of many other field studies that add N in the range of 24–600 kg ha^{-1} yr^{-1} (Knorr et al., 2005). Thus many fertilization studies may be adding N at levels above optimal for fungal decomposition, especially since these systems probably have smaller soil C pools than our boreal ecosystem.

4.2. Enzyme relationships

As expected, cumulative respiration was positively correlated with the activity of some extracellular enzymes (Table 4). β-glucosidase and *N*-acetyl-glucosaminidase were strongly correlated with CO_2 production, while the correlations with acid

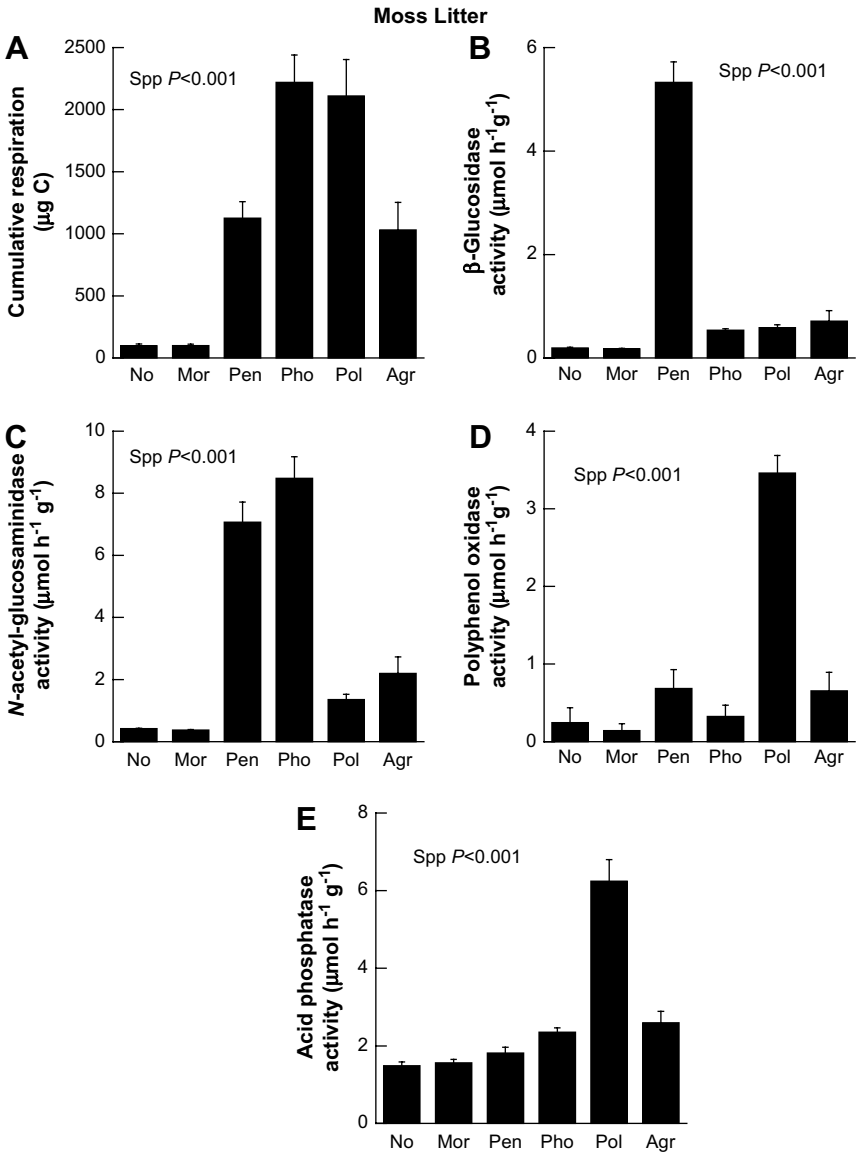

Fig. 5. Cumulative CO_2 respiration (A) and activities of β-glucosidase (B), *N*-acetyl-glucosaminidase (C), polyphenol oxidase (D), and acid phosphatase (E) on moss litter substrate. Bars represent means (±SE), and significant effects from ANOVA are shown on each panel. X-axis symbols as in Fig. 2. Nitrogen addition and fungal species did not significantly affect leucine aminopeptidase activity.

phosphatase and leucine aminopeptidase were weaker, and polyphenol oxidase showed no correlation. These results are consistent with the functions of the different enzymes. All of the substrates except lignin contained cellulose, so β-glucosidase is probably essential for decomposition of these substrates. *N*-acetyl-glucosaminidase degrades chitin in fungal cell walls, and is probably also involved in the metabolism of living fungal biomass (Lindahl and Finlay, 2006). Thus, this enzyme should be tightly correlated with fungal growth and activity, as measured by cumulative CO_2 respiration.

Acid phosphatase activity was positively correlated with CO_2 respiration and C-degrading enzymes, although the correlation was not particularly strong. Although there was probably some constitutive production of phosphatase by fungal biomass, phosphorus was provided in the inoculum and may have prevented induction of phosphatase enzyme production (Chróst, 1991). Only *Polyporus* on moss litter showed high phosphatase activity, potentially indicating phosphorus limitation on this substrate (Sinsabaugh et al., 1993).

Relative to the other enzymes, leucine aminopeptidase and polyphenol oxidase activities were very low. Given that the fungi were not strongly N limited on most of the substrates, there may have been little induction of aminopeptidase activity to acquire N (Chróst, 1991). There is also evidence that peptidase activities are suppressed in the presence of high mineral N concentrations (Saiya-Cork et al., 2002; Allison and Vitousek, 2005). Alternatively, mycorrhizal fungi or bacteria may play a larger role than saprotrophic fungi in polypeptide breakdown (Lilleskov et al., 2002; Romaní et al., 2006). Polyphenol oxidase activity was also low with the notable exception of *Polyporus* growing on moss litter. Even on the lignin substrate, polyphenol oxidase activities were <1 µmol h^{-1} g^{-1} and were not closely linked to CO_2 mineralization, suggesting that this enzyme may not always be a good indicator for lignin degradation. These results are consistent with broad patterns in polyphenol oxidase activity, which varies from 0 to 1000 µmol h^{-1} g^{-1} in field studies (Sinsabaugh et al., 2008) and is probably only expressed by certain groups of fungi (Luis et al., 2004).

Particularly on moss, our data suggest that different fungal species use enzymes to target different C components in litter substrates with high chemical complexity (Fig. 5). *Penicillium*

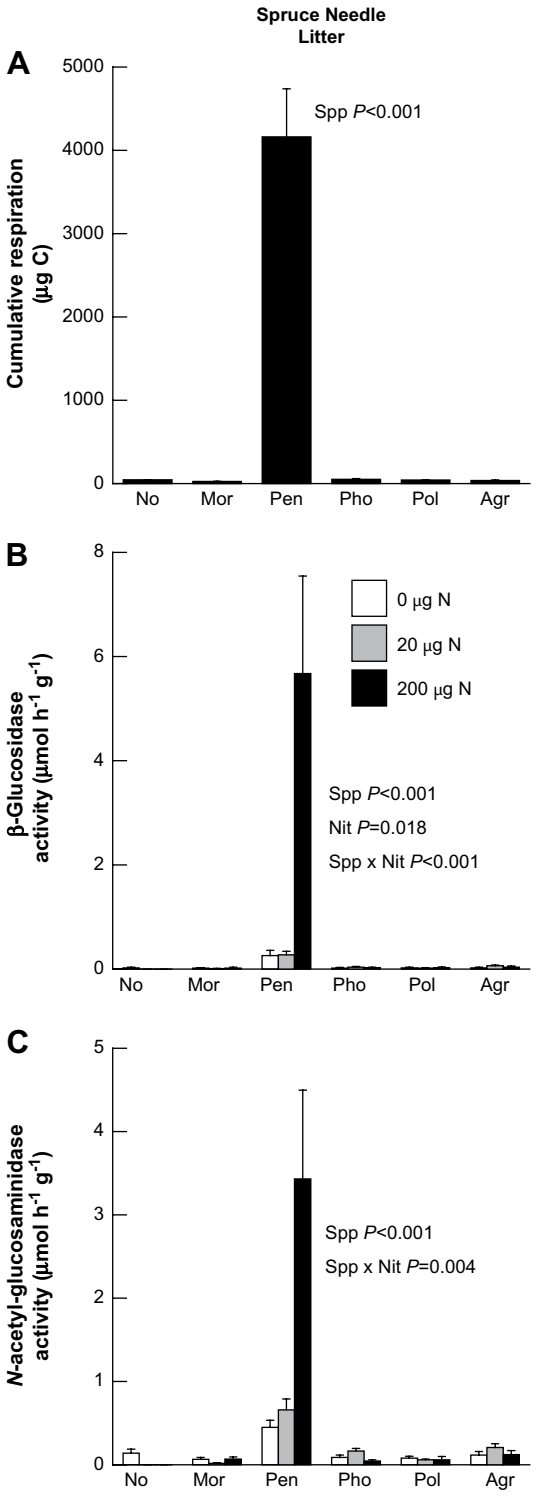

Fig. 6. Cumulative CO_2 respiration (A), β-glucosidase activity (B), and *N*-acetyl-glucosaminidase activity (C) on spruce needle litter substrate. Bars represent means (±SE), and significant effects from ANOVA are shown on each panel. X-axis symbols as in Fig. 2. Other enzymes are not shown because they did not show a clear relationship with spruce needle mineralization.

appeared to target more labile litter components such as cellulose and chitin with β-glucosidase and *N*-acetyl-glucosaminidase enzymes. In addition, *Pholiota* likely attacked amino sugar compounds with *N*-acetyl-glucosaminidase, while *Polyporus* produced a large amount of polyphenol oxidase, potentially to degrade the recalcitrant phenolic components of the moss (Erickson and Miksche, 1974). These results are consistent with a recent DNA labeling study which showed that different fungi target different C substrates in Harvard Forest soils (Hanson et al., 2008).

4.3. Fungal species effects

In contrast to our third hypothesis, we did not find that the Basidiomycete fungi were necessarily the best decomposers or enzyme producers. It is possible that Basidiomycete growth rates were slow and the 69-day incubation did not allow enough time for these fungi to colonize the substrates. However, *Pholiota* and *Polyporus* produced the most CO_2 from lignin and moss litter, suggesting that these isolates did not have inherently slow growth rates. Rather, these fungi may act as "miners" and preferentially colonize litter that has already been invaded by early successional fungi (Moorhead and Sinsabaugh, 2006).

Most surprising was the versatility of the Ascomycete, *Penicillium*. The decomposer abilities of *Penicillium* were probably due to both enzyme production and the capacity to use soluble components of the substrates. On several of the substrates, especially wood and moss litter, this fungus produced very large amounts of β-glucosidase and *N*-acetyl-glucosaminidase activity. Thus, some of the respiration potential was probably due to degradation of cellulose and amino sugar polymers. However, on the needle litter, the link between enzyme activity and respiration was weak; enzyme activities increased dramatically with N addition but CO_2 respiration did not change. It is therefore likely that *Penicillium* has degrading soluble compounds, such as tannins and phenolics, which are known to be abundant in spruce litter (Kanerva et al., 2006). We speculate that some of these compounds may have inhibited the growth of the other fungi through toxic effects (Selvakumar et al., 2007). Our *Penicillium* strain was isolated on media containing tannic acid as a carbon source, suggesting that it can tolerate and metabolize phenolic compounds. Other studies have also observed wide variation in conifer needle decomposition across fungal species, but *Penicillium* was not a particularly effective needle litter decomposer in these studies (Osono et al., 2003; Osono and Takeda, 2006).

Consistent with our initial hypothesis, *Mortierella* was a poor decomposer on all the substrates. This strain was isolated on media containing glucose as a C source, and may therefore target relatively labile C compounds. *Mortierella* mineralized C from the lignin substrate, probably because the lignin preparation we used contains a soluble fraction (J. Talbot, unpublished data). In the study with Harvard Forest fungi, the abundance of *Mortierella* strains increased in response to the same lignin substrate (Hanson et al., 2008). If *Mortierella* is targeting labile contaminants of the commercially obtained lignin, our results would be consistent with the classification of Zygomycetes as *r*-strategists that grow primarily on simple sugars (Kjøller and Struwe, 2002).

5. Conclusions

If N availability in boreal soils increases due to N deposition or increased N mineralization with climate warming, the effects on decomposition rates will likely depend on the quantity of N available and the type of decomposing substrate. The fungi we studied were highly N conservative and did not require large amounts of N to reach maximal decomposition rates. For the relatively slow rates of N release associated with climate warming, there may be positive effects on the decomposition of wood and other high C:N substrates. However, fungal decomposition of leaf litter and soil organic matter may not increase substantially. Even with N-poor substrates, fungal decomposition may be unresponsive if N availability increases above a certain threshold, as observed at the 200 µg N addition level.

Fungal species had a strong effect on mineralization of the common substrates tested in our study. Consistent with other work (Hanson et al., 2008), we found that different fungal taxa and their associated enzymes specialize on the breakdown of different C substrates. Contrary to our initial expectations, *Penicillium* produced large quantities of cellulose- and chitin-degrading enzymes, and mineralized all substrates used in the experiment. Additional research will be required to determine the quantitative contribution of these fungal taxa to organic matter decomposition in boreal forest soils. Combined with our new knowledge of responses to N availability, this information would be useful for predicting rates of soil C cycling under global change.

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