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Soil microbial communities and extracellular enzyme activity in the New Jersey Pinelands

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Abstract

We have much to learn about the roles of various groups of soil microorganisms in the decomposition of soil organic matter. Any changes in the type or amount of organic matter entering the soil, due to increasing atmospheric nitrogen (N) deposition and elevated carbon dioxide, could directly affect soil microbial community structure or the decompositional functions performed by the various microbial groups. We experimentally altered soil microbial communities using a factorial combination of trenching and in-growth bags crossed with fertilization treatments consisting of two forms of inorganic N and three N-containing organic molecules of increasing molecular weight and complexity. We tested three hypotheses: (1) Different components of soil microbial communities change in different ways following the application of fertilization treatments; (2) soil fungi decrease with increased inorganic N but increase following the application of organic molecules; and (3) activity of the extracellular enzymes peroxidase and phenol oxidase, which are important in lignin degradation, decrease following the addition of inorganic N. We found that the abundance of soil microbes and their composition (measured by lipid analysis) was significantly altered following the addition of glutamic acid, but not with inorganic N or more complex N-containing organic molecules. Lipids indicative of ectomycorrhizal fungi experienced the greatest increase in abundance. Extracellular enzyme activity, in contrast, changed very little and did not parallel changes in the structure of the soil microbial community that resulted from the isolation treatments. We conclude that small additions of N-containing organic compounds can cause changes in the structure of the soil microbial community but that community changes do not necessarily have an impact on extracellular enzyme activity.
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1. Introduction

Soil microbial communities are important contributors to the decomposition of organic matter, but there is much yet to learn about the roles of the various microbial groups in breaking down organic molecules. Saprophytic fungi play a major role in decomposition because they must rely on dead organic matter as their source of carbon and energy. Mycorrhizal fungi, which obtain carbon primarily from their host plants (Smith and Read, 2002), contribute to decomposition as they can access organic sources of nitrogen (Bending and Read, 1996). Some species of bacteria are also important in the decomposition process, contributing particularly to the mineralization of nutrients (Ley and Schmidt, 2002).

The chemical composition of soil organic matter is an important factor controlling the activity of soil microbes and decomposition processes (Waldrop et al., 2006). The decomposition of lignin is of particular interest because it is a major constituent of plant litter (Taiz and Zeiger, 1991) and represents an important means of global C storage.

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The decomposition of lignin may be affected by the abundance of inorganic N (DeForest et al., 2004), and this control may become increasingly important as atmospheric N deposition increases as a consequence of anthropogenic activities (Driscoll et al., 2003). Lignin is an energy-rich, recalcitrant polyphenolic macromolecule and its decomposition is accomplished in large part by the activity of extracellular enzymes that catalyze the oxidation of phenylpropane alcohols from the lignin polymer (Kirk and Farrell, 1987). Lab studies have found saprotrophic fungi and ectomycorrhizal fungi (Colpaert and van Laere, 1996; Courty et al., 2006) possess the physiological capacity to produce phenol oxidase and peroxidase, two enzymes important in lignin depolymerization (Kirk and Farrell, 1987). Field studies show that the activity of these enzymes decreases with an increase in atmospheric N deposition (DeForest et al., 2004; Gallo et al., 2004).

We know very little about what groups of soil microbes are involved in the decomposition of lignin in nature and why the activity of lignin-degrading enzymes are reduced by increasing levels of inorganic N (Fog, 1988; Berg and Matzner, 1997; Sinsabaugh et al., 2004). The inhibition of lignin degradation may be due to changes in the composition or abundance of the soil fungal community, or it may be due to repressed fungal enzyme production or activity (Kirk and Farrell, 1987; Fog, 1988). The rate of lignin decomposition may be further affected because increased atmospheric N deposition and elevated atmospheric carbon dioxide have the potential to alter ecosystem productivity (Lichter et al., 2005), which could change the organic inputs into the pool of soil organic matter (Waldrop et al., 2006). Whether N-containing organic molecules similarly affect the activity of phenol oxidase and peroxidase is not known. Understanding the factors that alter lignin degradation is important to understanding the controls on soil carbon storage and the carbon cycle.

This study uses a series of manipulative treatments to isolate different functional groups of soil microbes (i.e. saprotrophic fungi, ectomycorrhizal fungi, Gram+ bacteria, and Gram− bacteria) in order to better elucidate their functional roles in lignin decomposition. The responses of the different microbial groups were examined by crossing the isolation treatments with soil amendments of inorganic N, and three N-containing organic molecules, differing in carbon content and complexity, that are common in soil organic matter. Glutamic acid is an amino acid, creatine is a labile protein common in invertebrates and higher animals, and *N*-acetylglucosamine is the monomer of chitin which is common in arthropods and fungal cell walls. We used a lipid analysis to determine how the abundance of the different microbial groups changed in response to these soil amendments under the assumption that an increase in abundance indicates utilization of that soil amendment. We also measured the activity of phenol oxidase and peroxidase in order to understand how these soil amendments and the responses of the microbial communities affect the potential for lignin degradation. The isolation treatments allow us to link responses in these two enzymes to particular components of the microbial community.

Specifically our hypotheses were (1) different components of the soil microbial communities respond differently to the application of inorganic N or N-containing organic compounds; (2) fungi, in particular, decrease with the application of inorganic N but increase following the application of N-containing compounds because of their reliance on organic molecules; and (3) peroxidase and phenol oxidase activity decrease following the application of inorganic N but will increase following fertilization with N-containing organic compounds due to the stimulation the fungal components of the microbial community receive from organic fertilizations.

2. Materials and methods

2.1. Study site

Field work was conducted in the pineland ecosystem on the coastal plain of southern New Jersey (39°44′N, 74°40′W, approximately 30 m above sea level), about 60 km southeast of Philadelphia, Pennsylvania in the Wharton State Forest. Soils in our study areas are haploorthents, an oligotrophic sandy soil (88% sand, 8% silt, and 4% clay) with a thin, typically 4–8 cm, organic horizon. Soil pH ranged from 3.4 to 3.8 (obtained from a 1:1 DI water:soil slurry). Total C and total N in the organic layer were 119.4 (6.7 se) mg g^{-1} and 9.3 (0.4 se) mg g^{-1}, respectively (Carlo-Erba NA 1500 C/N Analyzer, Fisons Instruments, Beverly, MA). The overstory of this community is about 80-years old (New Jersey Department of Environmental Protection, Division of Parks and Forestry) and is dominated by pitch pine (*Pinus rigida*) with interspersed shortleafed pine (*Pinus echinata*) and black oak (*Quercus velutina*). The understory is mostly dominated by ericaceous shrubs of huckleberry (*Gaylussacia* spp.), blueberry (*Vaccinium* spp.), and scrub oak (*Quercus ilicifolia*). The mean maximum air temperature during the summer months at nearby Hammonton, NJ is 28 °C and the mean minimum temperature during the winter months is −3 °C (NJ State weather station, Hammonton, NJ, 2006). Mean cumulative annual rainfall is 1188 mm.

2.2. Experimental design

In the spring of 2003, we established five study sites separated from each other by at least 1 km. Since most soil microbial activity takes place in the upper horizons of the soil column (Rosling et al., 2003), we decided to limit our study to the O and A horizons. Site selection was based on sites having similar: (1) basal area of dominant trees within

a 15 m radius of a permanent central stake; (2) soil type of A horizon; (3) extractable pools of nitrate (NO_3^-) and ammonium (NH_4^+) (data not shown); and (4) organic matter content of the A horizon. At each site, six 2×2 m plots were laid out at a random distance (between 1 and 10 m) and random azimuth from the central stake. Care was taken to ensure that plots were at least 3 m apart from each other. Each plot was randomly assigned one of six fertilization treatments including two inorganic N treatments ($NaNO_3$, and NH_4Cl), three N-containing organic treatments of increasing molecular weight and complexity (glutamic acid, creatine, and *N*-acetylglucosamine). Deionized water was applied as a control. The quantity of each organic molecule added was calculated so as to standardize the total amount of N across the different fertilization treatments. The rate of N application in each treatment was 5 kg N ha^{-1}. This application is low by the standards of many inorganic N addition experiments, but represents an effective doubling of the annual rate of atmospheric N deposition in this area (NADP, 2004). Fertilization treatments were dissolved in 20 l of deionized water and applied directly to the organic layer of the forest floor in two 10-l increments over a period of 10 min to avoid generating surface runoff. This application rate is equivalent to a rainfall event of 0.5 cm and is not out of the ordinary in this system (Forman, 1998). Fertilization treatments were applied as a single application in early July.

In order to experimentally manipulate the soil microbial community in the field, we employed four microbial isolation treatments (Wallander et al., 2001) nested within each fertilization treatment plot. We intended the isolation treatments to result in the alteration of the relative abundances of three different functional groups of soil microbes (i.e. saprotrophic fungi, ectomycorrhizal fungi, and bacteria). Isolation treatments were installed one month prior to fertilization so as to allow time to recover from the disturbance caused by their installation. Each isolation treatment was randomly assigned to one of four 1×1 m quadrants within each 2×2 m plot (Fig. 1).

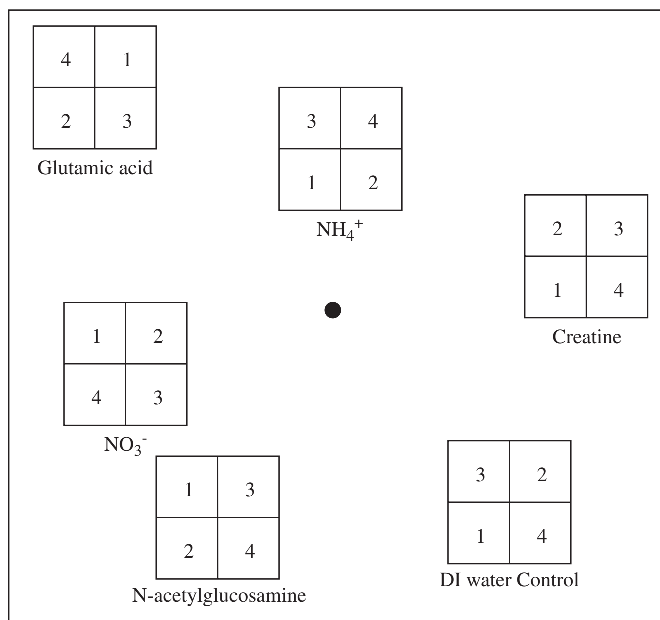

Fig. 1. Example of experimental layout at a single site. Numbers 1–4 represent soil microbial isolation treatments intended to isolate ectomycorrhizal fungi, saprotrophic fungi, bacteria, and a native soil control, respectively. Isolation treatments are nested within fertilization treatments. Solid dot in the center represents a permanent central stake.

The first isolation treatment (sapro) was designed to encourage the growth of saprotrophic fungi and eliminate ectomycorrhizal fungi from the sampling units (Wallander et al., 2001). This was accomplished by driving pieces of polyvinyl chloride (PVC) pipe (16 cm dia., 30 cm length) 25 cm into soil, which severed both plant roots and fungal hyphae. Since the growth and maintenance of ectomycorrhizal fungi depend on their association with plants for their main source of energy, ectomycorrhizas within the PVC pipe presumably died due to the removal of their main source of carbohydrates. It is possible that ectomycorrhizal fungi could have grown into the exclusion tube through the open bottom, but that is unlikely given our isolation treatments only remained in the ground for one month following the N additions.

The second isolation treatment (fungal) was designed to alter the fungal composition of the sampling units. In-growth bags ($10 \times 5 \times 2$ cm) were constructed from 50 µm nylon mesh (Sefar America, NY), a mesh size that allows for the in-growth of fungal hyphae but excludes plant roots. Each in-growth bag was filled with autoclaved soil (94% sand) collected from the mineral horizon of its respective study site. The hyphae of both saprotrophic and ectomycorrhizal fungi are able to penetrate the mesh bags. Wallander et al. (2001) used a similar design and found negligible saprotrophic colonization of the in-growth bags. In our case, we cannot be sure saprotrophic fungi were excluded from the in-growth bags given that we used field-collected soil that was not 100% acid-washed sand. We can be sure, however, that the fungal community was altered from the native soil. Bags were buried horizontally at the interface between the organic and mineral soil horizons, about 5 cm deep.

The third isolation treatment (bac) was intended to isolate only bacteria by removing both the ectomycorrhizal and saprotrophic components from the sampling units. This was achieved by burying in-growth bags horizontally at the interface between the organic and mineral soil horizons within pieces of 16×30 cm PVC pipe that had been driven 25 cm into the ground as described previously.

The final isolation treatment was our native soil treatment, which served as a control treatment for comparison with the other isolation treatments. In this treatment, soil was not disturbed. Instead, 16×5 cm rings

of PVC were placed on the soil surface to serve as a control for any shading effect generated by the PVC and to demarcate an area for sampling. Rings were secured from inadvertent movement by a single attached stake driven into the ground. All samples from these native isolation treatments were taken from within the rings.

2.3. Sample collection and processing

Two samples from each isolation treatment (8 total samples per plot) were collected immediately before the application of fertilization treatments in early July and the same number again 28 d later in early August. During both sampling events, a 5×10 cm soil core was removed from within each piece of PVC pipe or rings in the sapro and native isolation treatments. Two soil hyphal in-growth bags were retrieved in the fungal and bac isolation treatments. Samples were kept on ice and transported back to a lab at the University of Pennsylvania where a 10 g subsample of soil from each soil core or in-growth bag was immediately frozen and then lyophilized for use in the lipid analysis (described below). The remaining soil was kept refrigerated until enzyme analyses could begin (also discussed below). Enzyme analyses of samples collected during the August sampling period were begun within 24 h of collection. Enzyme analyses of samples collected during the July sampling, however, were not begun until 7 d following their collection due to an unforeseen equipment malfunction.

2.4. Lipid analysis and enzyme assays

We used microbial lipid analysis to analyze microbial community composition on freeze-dried soil. The procedure is based on the extraction of 'signature' lipid biomarkers from the cell membranes and walls of microorganisms. We extracted, purified and identified lipids in 5-g samples of lyophilized soil. Briefly, lipids were extracted from 5 g of freeze-dried soil using a chloroform–methanol extraction with a phosphate buffer (3.6 ml potassium phosphate, 8 ml methanol, and 4 ml $CHCl_3$) in 25-ml glass tubes, shaken for 1 h and centrifuged. The resulting supernatant was decanted to 30-ml tubes with additional potassium phosphate buffer and chloroform. The organic and aqueous phases were allowed to separate overnight at room temperature after which the top layer was removed by aspiration. The chloroform phase (remaining in the tube) was evaporated in a RapidVap to concentrate extracted organics. We then followed the procedure for FAME as given by Microbial ID Inc.; sodium hydroxide was added for saponification and the solution was heated in a water bath for 30 min, followed by alkaline methanolysis.

Methyl-ester derivatives of the extracted lipids were analyzed using a Hewlett-Packard 6890 Gas Chromatograph equipped with a flame ionization detector and split/splitless inlet and a $25\,mm\times0.2\,mm$ inside diameter $\times\,0.33\,\mu m$ film thickness Ultra 2 (5% phenyl, 95% methyl) capillary column (Agilent Technologies, Loveland CO) with hydrogen as the carrier gas, N_2 as the make-up gas, and air to support the flame. Gas chromatograph conditions are set by the MIDI Sherlock program (MIDI, Inc. Newark, DE). Output peaks were identified with using bacterial fatty acid standards and Sherlock peak identification software, and fatty acids were quantified by comparing peak areas from samples with those of two internal standards of known concentrations, 9:0 (nonanoic methyl ester) and 19:0 (nonadeconoic methyl ester). Internal standards were subtracted prior to statistical analysis. In all analyses, we used only fatty acids that were identifiable and present at >0.5 mol%.

The total nmol $C\cdot nmoL^{-1}$ 16:0 was used as an index of microbial abundance (DeForest et al., 2004; Gallo et al., 2004). The relative contribution of lipids to the total community profile was determined by calculating the mol% (moles of a given lipid/total moles lipid per sample). Finally, we used specific indicator lipids to quantify the abundance of different components of the soil microbial community. Fungi are indicated by 18:1ω9c, 18:2ω6c, and 18:3ω3c (Frostegård and Bååth, 1996; Ruess et al., 2002). We also used the lipid 16:1ω5c as a fungal indicator as it has been shown to be dominant in arbuscular mycorrhizal (AM) fungi (Ruess et al., 2002), which associate primarily with the herbaceous plants of our system. Gram− (Gm−) bacteria are indicated by relatively short-chained, mono-unsaturated and cyclopropyl lipids such as cy17:0, 16:1ω7c, 16:1ω9c, 18:1ω5c, and 18:1ω7c (Ratledge and Wilkinson, 1988). Finally, Gram+ (Gm+) bacteria are indicated by methyl and terminal branched lipids such as 16:0 10 methyl, 15:0 iso and anteiso, or 17:0 iso and anteiso (Ratledge and Wilkinson, 1988).

We measured the activity of phenol oxidase and peroxidase because of their importance in the degradation of lignin and the cycling of soil organic matter (Hammel, 1997). Assays were spectrophotometric and followed microplate procedures described by Saiya-Cork et al. (2002). Briefly, soil suspensions were created by adding 1.0 g wet weight soil taken from the organic layer of each soil sample and suspended in 125 ml of 50 mM sodium acetate buffer (pH 5.0). Enzyme activities were measured using clear polystyrene 96-well microtiter plates (Fisher Scientific, Pittsburg, PA) with 10 mM L-3,4-dihydroxyphenylalanine (L-DOPA) as the substrate. We used 16 analytical replicates per sample. Assays were allowed to run for 24 h using controls for both samples and substrates.

2.5. Statistical analyses and other calculations

To test the efficacy of the isolation treatments we compared total and specific abundance of the microbial community components before and after N-treatments. Lipids were assigned to guilds as described above. To

assess the impact of fertilization treatments on microbial community composition and activities we used analysis of variance (ANOVA) followed by Tukey's HSD tests. Sites were treated as a random source of variation and nested within fertilization treatment. Except for comparisons of isolation techniques and the calculations of ectomycorrhizal fungi, all other comparisons and tests were conducted on samples collected from the native isolations only, as soil in the native isolations did not receive any disturbance or artificial sterilization. The amount of ectomycorrhizal fungi present in the native isolations was estimated by subtracting the summation of all fungal lipids in the sapro isolation treatment plus the AM fungal lipid in the native isolation from the total fungal lipids in the native soil (Wallander et al., 2001). Specific enzyme activity was calculated as the total enzyme activity divided by the microbial abundance. Because different amounts of time elapsed between sample collection and enzyme analysis in the July and August sampling times, no explicit test was conducted examining the difference in enzyme activity before and after the application of the fertilization treatments. Comparisons of enzyme activity can still be made, however, among the various fertilization treatments within each sampling period and with the control treatment. Finally, we assessed differences among microbial community profiles following manipulations using ordination by principal components analysis (PCA). All lipids present in concentrations higher than 0.5 mol% of the total were arcsin transformed for use in PCA. All data were analyzed using SAS for Windows Version 8.2 (SAS, 2001).

3. Results

3.1. Efficacy of isolation treatments

The isolation treatments successfully created different communities of soil microorganisms. The bac and fungal isolations had significantly lower overall microbial abundance than the sapro and native soil isolations (Table 1), both before and after fertilization treatments were applied. The bac isolation treatment had a significantly greater proportion of Gm− bacteria present than the other isolation treatments. Following the fertilizations, the proportion of Gm− bacteria in the bac isolations significantly decreased although it remained greater than the other isolation treatments (Table 1). Lipids indicative of Gm+ bacteria were not present in either the bac or the fungal isolations. The proportion of fungi present in the fungal isolation treatments was significantly greater than that in the sapro and native isolations although it was not significantly different from that in the bac isolation.

3.2. Effect of N addition treatments

3.2.1. Soil microbial community

Based on lipid analyses, no significant differences in total microbial abundance existed among the various fertilization treatment plots prior to the application of fertilization treatments. Although microbial abundance was slightly lower in plots that were to receive the glutamic acid treatment relative to other plots, the difference was not significant (Fig. 2A). Following the application of fertilization treatments, total microbial abundance was significantly greater in the glutamic acid treatment plots compared to the control plots. Other fertilization treatments did not have a significant effect on the total microbial abundance (Fig. 2A).

Examining fungal and bacterial indicators revealed important differences in the responses to the fertilization treatments. The abundance of fungal and Gm+ bacterial components of the soil microbial community both increased significantly relative to the control treatment following amendment with glutamic acid (Fig. 2B). The increases in fungal abundance following glutamic

Table 1
Microbial abundance and relative proportions of lipids indicative of fungi, Gm−, and Gm+ bacteria in isolation treatments before and after fertilization in the New Jersey pinelands

Isolation treatment	Before				After			
	Microbial abundance nmol C nmol^{-1} 16:0 (SE)	Relative proportion (SE) of functional groups			Microbial abundance nmol C nmol^{-1} 16:0 (SE)	Relative proportion (SE) of functional groups		
		Fungi	Gm−	Gm+		Fungi	Gm−	Gm+
Bac	3.67 (0.89)a	0.31 (0.04)a	0.34 (0.02)a†	0	5.13 (2.27)a	0.25 (0.04)a	0.14 (0)a†	0
Fungal	4.09 (0.85)a	0.27 (0.03)a	0.15 (0.02)b	0	7.92 (2.19)a	0.23 (0.04)a	0.09 (0.01)b	0
Sapro	19.93 (0.89)b	0.10 (0.03)b	0.04 (0.01)c	0.04 (0.00)	21.34 (2.27)b	0.09 (0.03)b	0.04 (0.00)c	0.04 (0.00)
Native	19.61 (0.89)b	0.10 (0.03)b	0.04 (0.01)c	0.04 (0.00)	18.11 (2.27)b	0.11 (0.03)b	0.04 (0.00)c	0.04 (0.00)

nmol C nmol^{-1} 16:0 was used as an index of microbial abundance. Cells sharing the same letter within a column are not significantly different from other isolation treatments at $\alpha = 0.05$ ($n = 8$–10).
†Indicates significant difference following N addition.

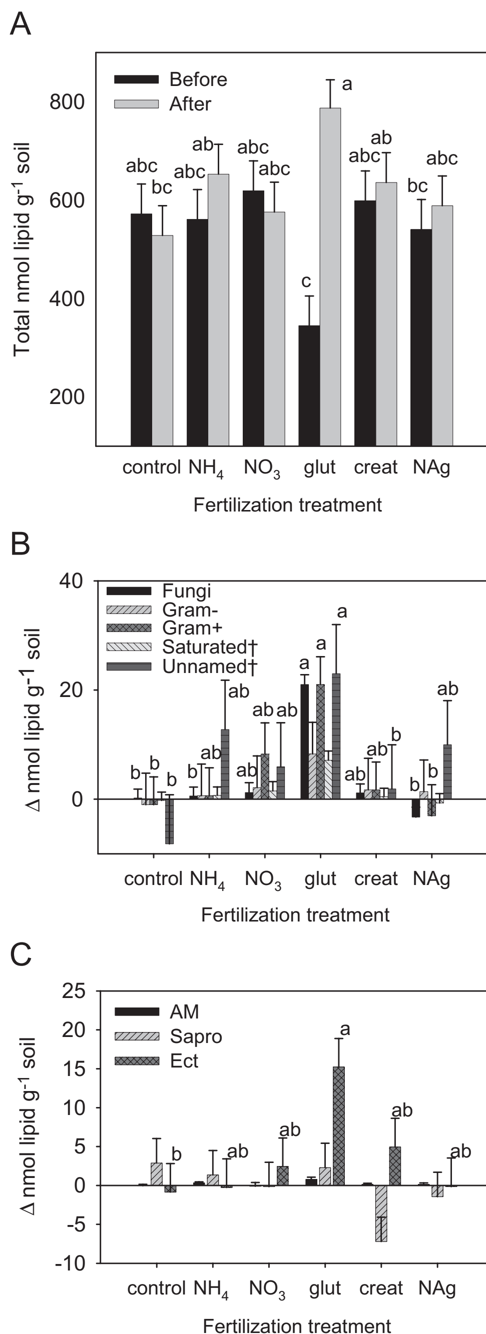

Fig. 2. Microbial abundance following inorganic and N-containing organic fertilizations in the New Jersey pinelands: (A) Total microbial abundance before and after fertilization treatments. (B) Change in microbial abundance of each guild after fertilization treatments. (C) Change in fungal abundance after fertilization treatments. Bars sharing the same letter are not significantly different from bars in other fertilization treatments at $\alpha = 0.05$. Error bars represent 1 standard error ($n = 10$). Glut = glutamic acid, creat = creatine, NAg = *N*-acetylglucosamine, AM = arbuscular mycorrhizal fungi, ECT = ectomycorrhizal fungi, and Sapro = saprotrophic fungi. †Change in nmol lipid g^{-1} soil have been multiplied by 10^{-1} so they fit on the same scale.

acid addition were significantly greater than those in plots receiving NH_4^+ and *N*-acetylglucosamine. Lipids indicative of Gm+ bacteria in plots receiving glutamic

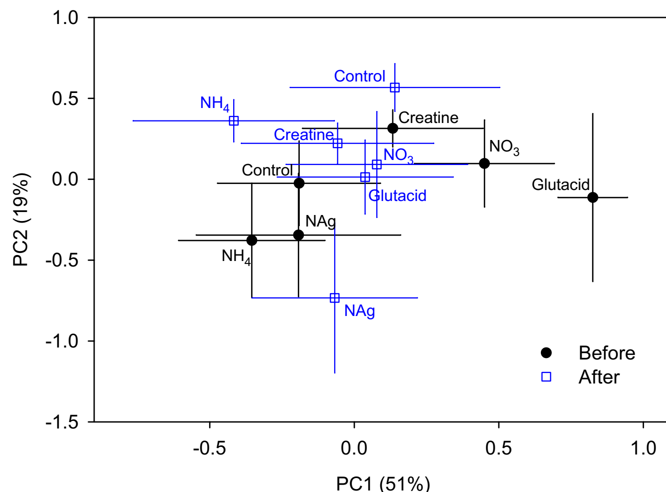

Fig. 3. Ordination of soil microbial community as determined by lipid analysis in the New Jersey pinelands by PCA showing responses to two inorganic and three N-containing organic addition treatments. Error bars represent 1 standard error ($n = 10$). Glutacid = glutamic acid and NAg = *N*-acetylglucosamine.

acid were also significantly greater than those in *N*-acetylglucosamine plots. Lipids in the unnamed category generally showed the greatest change among the fertilization treatments, increasing by one or two orders of magnitude in plots amended with any form of N. Examination of the fungal indicators, specifically, reveals significantly greater levels of ectomycorrhizal fungi following amendment with glutamic acid but no differences in other components (Fig. 2C).

The addition of glutamic acid significantly altered general soil microbial community patterns (Fig. 3). For the glutamic acid treatment only, community fingerprints before and after fertilization separated significantly along the first principal component axis. Two lipids indicative of fungi, 18:1ω9c and 18:2ω6c, and one indicative of Gm+ bacteria, 15:0 iso, were among the most important fatty acids to define differences in the soil microbial communities. The first and second principal component axes explained 51% and 19%, respectively, of the observed variation.

3.2.2. Enzyme activity

Significant differences in enzyme activity were observed following fertilization. Prior to the application of fertilization treatments, phenol oxidase and peroxidase activity were not significantly different from the activities observed in the control plots (Fig. 4A). Following the fertilization treatments, however, peroxidase activity in plots treated with *N*-acetylglucosamine was significantly lower than in the control plots (Fig. 4B). Additionally, phenol oxidase activity in *N*-acetylglucosamine treated plots was significantly greater than phenol oxidase

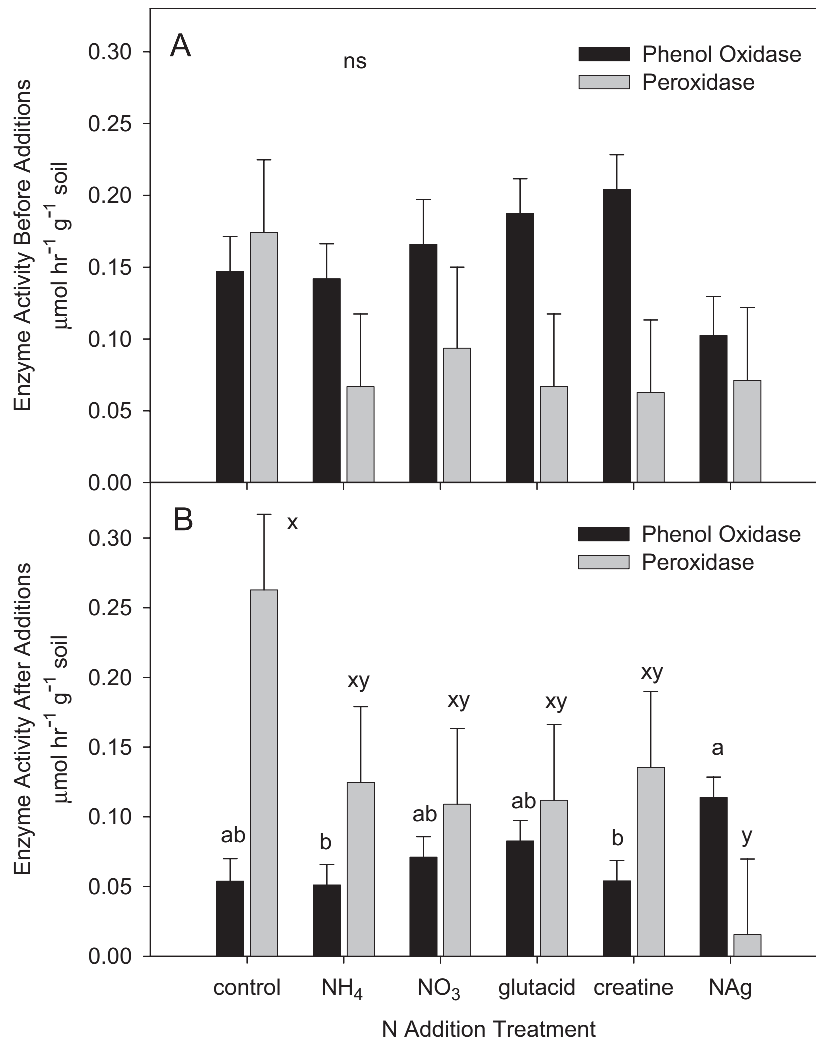

Fig. 4. Bulk enzyme activity in response to inorganic and N-containing organic fertilization treatments in the New Jersey pinelands: (A) Bulk enzyme activity before fertilizations. (B) Bulk enzyme activity after fertilizations. Bars sharing the same letter are not significantly different from bars in other fertilization treatments at $\alpha = 0.05$. Error bars represent 1 standard error ($n = 8$–10). Glutacid = glutamic acid, NAg = *N*-acetylglucosamine.

activity in NH_4^+ and creatine plots. Despite showing the greatest response in microbial community structure, phenol oxidase and peroxidase activity in the plots receiving glutamic acid fertilization did not vary in activity relative to the control or any other treatment (Fig. 4B). Enzyme activities per unit microbial abundance were also examined and yielded similar results (data not shown).

3.3. Effect of isolation treatments

Changes in bulk enzyme activity following fertilization treatments did not agree with corresponding changes observed in the microbial community. Despite large differences in the total microbial abundance of the isolation treatments (Table 1), phenol oxidase activity was not significantly different among the isolations either before of after the addition of N (Fig. 5). Significant differences were observed in peroxidase activity, but in ways counter to what was expected. Peroxidase activity was significantly greater in the fungal isolations compared to the sapro and native soil isolations prior to the addition of N. Likewise, bulk enzyme activity in the bac isolations was significantly greater than in the native soil isolations following the addition of the N (Fig. 5).

Specific enzyme activity (which expresses enzyme activity per unit microbial biomass) reveals very different results compared to bulk enzyme activity (Table 2). Specific activity of phenol oxidase and peroxidase are two to three orders of magnitude greater in the bac and fungal isolation treatments compared to the sapro and native soil isolations (Table 2). Specific activities of both enzymes were significantly less following N additions, but this is

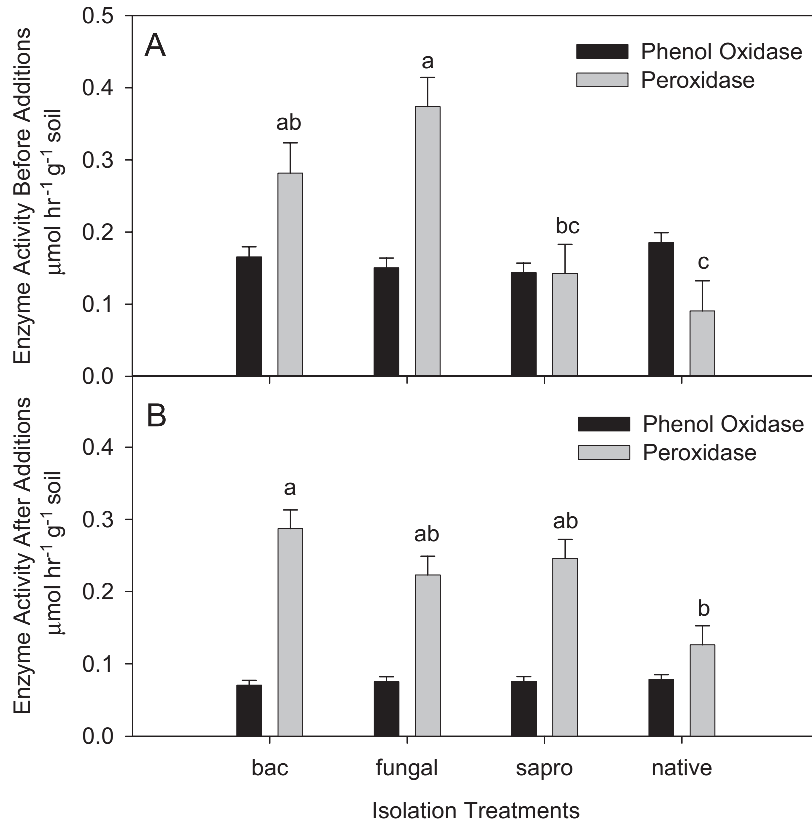

Fig. 5. Bulk enzyme activity in each isolation treatment before and after inorganic and organic N fertilization treatments in the New Jersey pinelands: (A) Bulk enzyme activity before fertilizations. (B) Bulk enzyme activity after fertilizations. Bars sharing the same letter are not significantly different from bars in other fertilization treatments at $\alpha = 0.05$. Error bars represent 1 standard error ($n = 8$–10). bac = bacterial, and sapro = saprotrophic.

Table 2
Specific enzyme activity of isolation treatments before and after fertilization in the New Jersey pinelands

	Before		After	
Isolation treatment	Specific phenol oxidase	Specific peroxidase	Specific phenol oxidase	Specific peroxidase
Bac	141.65 (32.01)a	164.08 (41.33)a	24.42 (5.47)a	91.07 (18.83)a
Fungal	99.57 (31.41)a	207.64 (40.56)a	23.47 (5.18)a	67.32 (17.82)a
Sapro	0.31 (30.80)b	0.30 (39.77)b	0.15 (5.34)b	0.48 (18.21)b
Native	0.38 (32.18)b	0.20 (40.00)b	0.15 (5.64)b	0.25 (19.11)b

Units are $\mu mol\, h^{-1}\, g^{-1}$ soil μmol^{-1} lipid (SE). Cells sharing the same letter within a column are not significantly different from other isolation treatments at $\alpha = 0.05$ ($n = 10$).

accompanied by a trend of increasing soil microbial abundance (Table 1).

4. Discussion

4.1. Effects of fertilization treatments

The response of the soil microbial community to the fertilization treatments differed among groups of microbes, lending support to our first hypothesis that different components of the soil microbial community would respond differently to the fertilization treatments. Following fertilization with glutamic acid, the abundance of two major functional components (i.e. fungi and Gm+ bacteria) of the soil microbial community increased, but the extent of the increases were group-specific (Fig. 2B). Lipids indicative of Gm+ bacteria and ectomycorrhizal fungi both exhibited large responses to glutamic acid,

but the abundance of the lipids in the unnamed category increased even more. Although unnamed lipids cannot be used to support or refute any of our hypotheses (as we are unable at this time to positively associate any of these lipids with a specific group of soil microorganisms), this result does support the conclusion that soil organisms were able to make significantly greater use of glutamic acid. Glutamic acid is the simplest organic molecule used in our study and as our data indicate, many types of soil microorganisms are able to make use of this compound because it is easily decomposed and assimilated.

Increases in fungi, and more specifically the increases we observed in ectomycorrhizal fungi (Fig. 2C), are consistent with our hypothesis that fungal abundance would increase following amendment with N-containing organic compounds. That fungi respond strongly to glutamic acid must mean that the fungi are important decomposers of this molecule and supports the hypothesis that ectomycorrhizal fungi, in addition to saprotrophic fungi, are both involved in the decomposition of organic matter (Schimel and Bennett, 2004).

Changes in fungal, or bacterial, abundance can have implications for ecosystem functions. For example, increases in ectomycorrhizal fungi could result in increased N being made available to host plants, thereby increasing annual net primary productivity (Cornelissen et al., 2001). Conversely, increased ectomycorrhizal fungi could also result in greater C being drawn from host plants if the fungi derive most of their energy from their host plants and not from the metabolism of soil organic sources. Increases in Gm+ and Gm− bacteria could lead to a decrease in the available inorganic N because bacteria are considered superior competitors for inorganic N sources (Lipson and Näsholm, 2001).

Contrary to our hypothesis, we did not observe changes in the soil microbial community following fertilization with inorganic N or more complex organic molecules. The explanation for the lack of response to fertilization treatments other than glutamic acid probably varies with the nature of the compounds used for fertilization. Inorganic N is highly labile and may have leached quickly through the sandy soil, which may explain why we did not observe significant changes in the soil microbial community following inorganic N addition despite changes in the soil microbial community being commonly observed following inorganic N fertilizations (DeForest et al., 2004; Frey et al., 2004; Gallo et al., 2004; Waldrop et al., 2004a). The complex organic molecules, creatine and *N*-acetylglucosamine, used in the experiment were not likely leached from the soil given their larger molecular size, but their structural complexity may explain why soil microbial communities did not respond as they did with glutamic acid. Creatine and *N*-acetylglucosamine take longer to break down than glutamic acid and the members of the soil microbial community that would have derived benefit from their decomposition may not have had enough time to respond. Additionally, our inorganic fertilizations were small relative to other studies (Fog, 1988; Norton et al., 1994; Kårén and Nylund, 1997). But more importantly, our inorganic N fertilizations represent an ecologically relevant increase in natural systems, effectively doubling the amount of inorganic N currently received from atmospheric N deposition in this area (NADP, 2004), and are useful for understanding changes that may be expected in the soil microbial community should atmospheric N deposition continue to increase inorganic N inputs into ecosystems (Galloway, 1998).

Changes in the microbial community due to fertilizations did not seem to have any bearing on the activity of extracellular enzymes associated with lignin degradation. It is well established that fungi play an important role in the degradation of lignin compounds through the action of oxidative enzymes (Dix and Webster, 1995; Hammel, 1997). However, significant changes to the microbial community and increases in fungal and bacterial abundance following the glutamic acid treatment (Fig. 2B) did not result in any changes in peroxidase or phenol oxidase activity (Fig. 4). Other studies have found that peroxidase and phenol oxidase activity decrease with increasing inorganic N availability (Fog, 1988; Sinsabaugh et al., 2002; Frey et al., 2004; Waldrop et al., 2004b). These studies were longer term, being from 3 to 14 years in duration, and our one time pulse addition may not have been of sufficient duration to produce changes in enzyme activity.

4.2. *Effects of isolation treatments*

Despite large community differences (Table 1), bulk enzyme activity in isolation treatments with much less microbial abundance was similar or greater than in isolation treatments with much greater microbial abundance (Fig. 5). Both the native and sapro isolations have an order of magnitude more microbial abundance than do the fungal or bac isolations (Table 1), yet the peroxidase activity in the bac isolations was greater than that in the native isolations. Similarly, both the phenol oxidase and peroxidase activity in the fungal and bac isolation treatments are statistically similar to the enzyme activities in the sapro isolation treatment (Fig. 5). The absence of corresponding changes to enzyme activity despite changes in the microbial community is not uncommon (DeForest et al., 2004; Waldrop et al., 2004a), but the degree of similarity of the enzyme activities among the very different experimentally altered microbial communities is quite surprising.

A number of explanations exist as to why bulk enzyme activity could remain relatively unchanged despite such large differences in microbial abundance. First, peroxidase and phenol oxidase activity in soil may be substrate limited instead of catalyst limited (Kirk and Farrell, 1987; Campbell and Reece, 2005). Extracellular enzyme

activity in soils is typically thought about in the context that if there is more enzyme, there will be more enzyme activity (Sinsabaugh, 1994). This is especially true in the case of lignin which is understood to be one of the most common substances in soil litter (Taiz and Zeiger, 1991). Faison and Kirk (1985) and Leatham (1986) found, however, that when lignin was added to their culture studies, increased phenol oxidase activity was observed, indicating substrate limitation. Soil microbial communities in native and sapro isolation treatments may ultimately be producing greater amounts of phenol oxidase and peroxidase than microbial communities in fungal and bac isolations. If there is a lack of suitable substrate, however, there cannot be additional activity with more enzyme.

Why might two of the enzymes that work to decompose one of the most common molecules in soil organic matter be substrate limited? It may be due to the nature of lignin itself. Lignin is a complex organic molecule, whose structure is not well understood or characterized. We know it can form multiple types of interphenylpropane linkages (Kirk and Farrell, 1987) and that it can form multiple cross-linkages between itself, cellulose, and hemicellulose (Taiz and Zeiger, 1991). We also know that polyphenols, subunits of lignin, complex with proteins which makes the complex more difficult to decompose (Hättenschwiler and Vitousek, 2000). Phenol oxidase and peroxidase may be substrate limited because under certain conditions the number of cross-linkages formed, or the number of aromatic rings exposed for oxidation may change (Kirk and Farrell, 1987). This may happen in the presence of greater amounts of inorganic N or organic N in which the amido N of peptide bonds can complex with the carbonyl oxygen on the phenol groups of lignin, thus affecting the availability of lignin to be oxidized by phenol oxidase or peroxidase. Lignin being unavailable for oxidation in high inorganic N environments may help to explain the negative relationship of peroxidase and phenol oxidase with increasing N deposition or in response to N additions (Waldrop et al., 2004b). If the chemical structure of lignin changes with increasing N, then chronic N deposition is likely having an effect on the nature of lignin, which could have important implications on long-term carbon storage and the global carbon cycle (Neff et al., 2002).

Second, peroxidase and phenol oxidase are water-soluble extracellular enzymes and migrate through the soil column by diffusion (Schimel and Weintraub, 2003). Soil microbes forage for resources by excreting extracellular enzymes directly into the soil matrix. In order to derive any benefit from the extracellular enzymes, the soil microbes must be in close proximity to the resulting products of the enzyme degradation for any absorption or active transport of nutrients into the microbe to take place (Allison, 2005). Due to this fact, extracellular enzymes are typically understood to operate on microsite scales (Kandeler et al., 1999), thereby conferring the best possible chance of reaping the benefits of an excreted enzyme on the excreting microbe. It is possible that our N additions and additional rainfall washed extracellular enzymes from the above organic horizon into our in-growth bags, which were buried at the interface of the organic and mineral soil horizons. Consequently, the enzyme activities we measured may not be representative of the microbial communities that we created, but rather of the microbial community in the near vicinity of our in-growth bags. This would not explain, however, why bac or fungal isolations would have greater enzyme activities than the native soil isolation (Fig. 5).

Additionally, microbial communities in the isolation treatments differ in many ways, but their similarities could be very important. Thirteen fatty acids are common to all isolation treatments (Appendix A) and it could be that the components of the microbial community represented by these fatty acids are the important groups in lignin degradation.

5. Conclusions

We found that N-containing organic inputs have the potential to cause changes in soil microbial communities. Changes to the microbial community, however, do not necessarily have any effect on extracellular enzyme activity. Peroxidase and phenol oxidase activity in our system may not be limited by microbial abundance or microbial community type. Instead, these enzymes may be limited by the amount of available substrate. Chronic N additions may be altering the amount of lignin stored in ecosystems and this may have profound effects on long-term carbon storage and global carbon cycling.

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Appendix A

Relative amounts of most common lipids in each isolation treatment in the New Jersey pinelands. Sapro = saprotrophic, Bac = bacterial (Fig. A1).

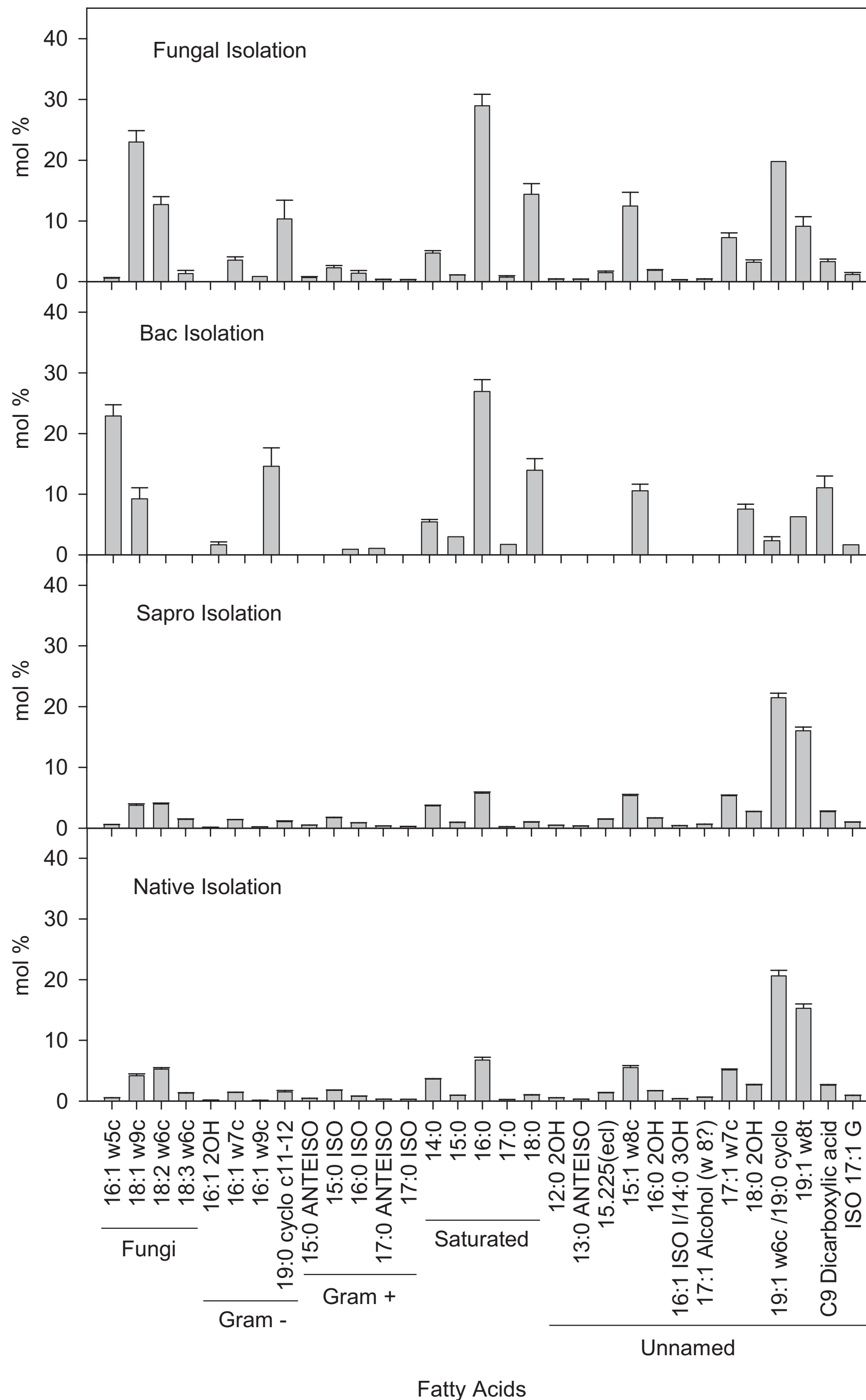

Fig. A1.

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