Microbial Ecology

Microbial Community Structure and Oxidative Enzyme Activity in Nitrogen-Amended North Temperate Forest Soils

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Abstract

Large regions of temperate forest are subject to elevated atmospheric nitrogen (N) deposition which can affect soil organic matter dynamics by altering mass loss rates, soil respiration, and dissolved organic matter production. At present there is no general model that links these responses to changes in the organization and operation of microbial decomposer communities. Toward that end, we studied the response of litter and soil microbial communities to high levels of N amendment (30 and 80 kg ha^{-1} yr^{-1}) in three types of northern temperate forest: sugar maple/basswood (SMBW), sugar maple/red oak (SMRO), and white oak/black oak (WOBO). We measured the activity of extracellular enzymes (EEA) involved directly in the oxidation of lignin and humus (phenol oxidase, peroxidase), and indirectly, through the production of hydrogen peroxide (glucose oxidase, glyoxal oxidase). Community composition was analyzed by extracting and quantifying phospholipid fatty acids (PLFA) from soils. Litter EEA responses at SMBW sites diverged from those at oak-bearing sites (SMRO, BOWO), but the changes were not statistically significant. For soil, EEA responses were consistent across forests types: phenol oxidase and peroxidase activities declined as a function of N dose (33–73% and 5–41%, respectively, depending on forest type); glucose oxidase and glyoxal oxidase activities increased (200–400% and 150–300%, respectively, depending on forest type). Principal component analysis (PCA) ordinated forest types and treatment responses along two axes; factor 1 (44% of variance) was associated with phenol oxidase and peroxidase activities, factor 2 (31%) with glucose oxidase. Microbial biomass did not respond to N treatment, but nine of the 23 PLFA that formed >1 mol% of total biomass showed statistically significant treatment responses. PCA ordinated forest types and treatment responses along three axes (36%, 26%, 12% of variance). EEA factors 1 and 2 correlated negatively with PLFA factor 1 ($r = -0.20$ and -0.35, respectively, $n = 108$) and positively with PLFA factor 3 ($r = +0.36$ and $+0.20$, respectively, $n = 108$). In general, EEA responses were more strongly tied to changes in bacterial PLFA than to changes in fungal PLFA. Collectively, our data suggests that N inhibition of oxidative activity involves more than the repression of ligninase expression by white-rot basidiomycetes.

Introduction

Production in many terrestrial and marine ecosystems is regulated by the supply of bioavailable N. In the past century, human activities have more than doubled the global flux of this limiting nutrient [28], and deposition rates as high as 60 kg N ha^{-1} y^{-1} have been measured near major industrial and urban areas, in particular the eastern US and western Europe [9, 24].

One effect of elevated N deposition on forest ecosystems is enhanced plant productivity and biomass accumulation [28] which may contribute to the role of temperate forests as sinks for atmospheric carbon. However, tracer studies conducted by Nadelhoffer et al. [24] in several types of temperate forest indicated that soil, rather than tree biomass, was the primary sink for depositional N inputs. The effects of N deposition and N accumulation on soil microbial communities and the processes they catalyze remain poorly understood despite extensive study. Recent reviews have concluded that N amendment increases the degradation of labile cellulosic litter and retards degradation of lignified or humified organic matter [5, 8]. A leading explanation for the N inhibition effect extends from the observation that lig-

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ninase expression by at least some white-rot basidiomycetes, considered the principal degraders of lignin, is repressed by N [10, 18].

Carreiro et al. [6] studied changes in mass loss and litter composition for three types of deciduous leaf litter at three levels of N amendment. Cellulolytic activity increased with N amendment for all three litter types, but mass loss responses to N addition correlated with changes in extracellular phenol oxidase activity which in turn was linked to the acid insoluble fiber content (lignin and humus) of the litter. Decomposition rates increased for labile dogwood (*Cornus florida*) leaves, declined for lignified red oak (*Quercus borealis*) leaves, and varied with N dose and decomposition stage for intermediate red maple leaves (*Acer rubrum*) [6, 27].

If the inhibitory effect of N on oxidative enzyme activity is restricted to white-rot basidiomycetes, then the burden of decomposition will pass from litter fungi to less efficient soil bacteria. However, a study by Saiya-Cork et al. [26] showed the potential for a wider N effect. Litter and soil from a sugar maple (*Acer saccharum*) stand under long-term N amendment showed losses in oxidative enzyme activity of 20% and 40%, respectively, compared to control plots. The large decline of oxidative activity in the soil suggests that N-induced repression may extend beyond white-rot basidiomycetes.

The degradation of lignin is mediated by two types of enzymes, peroxidases and phenol oxidases. Some fungi have both types, others have one or the other [21]. Phenol oxidases act on phenolic substrates which are oxidized to phenoxy radicals that may participate in further reactions [3, 10, 18]. Lignin-degrading peroxidases can act on the abundant β-O-4 ether linkage of lignin by cleaving the Cα–Cβ bond [3, 4, 10, 11, 18].

Peroxidases requires a source of H_2O_2; therefore within the ligninolytic system there are enzymes that catalyze its formation. One enzyme is glyoxal oxidase, which uses aldehydes such as glyoxal, glyoxalate, and formaldehyde as electron donors [2, 16]. One substrate, glycolaldehyde, is a cleavage product of the oxidation of β-O-4 lignin linkage by peroxidase [10, 11]. Another H_2O_2-producing enzyme is glucose oxidase, which is produced intra- and extracellularly [10, 19]. Glucose oxidase catalyzes the formation of gluconolactone from glucose with concurrent reduction of oxygen [22].

The studies by Carreiro et al. [6] and Saiya-Cork et al. [26] showed that phenol oxidase, an enzyme produced by many groups of fungi and bacteria, responds more strongly to N addition than peroxidase, suggesting that repression of fungal ligninase may not be the principal mechanism for inhibition of decomposition. To further resolve the relationships among oxidative enzyme activity, organic matter composition, and microbial community structure, we conducted a N addition experiment across three types of northern hardwood forest, representative of the Midwest region. We measured the responses of phenol oxidase, peroxidase, glucose oxidase, and glyoxal oxidase activities in litter and soil and correlated them with microbial community structure as indicated by phospholipid fatty acid composition.

Table 1. Stand identification numbers and locations for our nine experimental sites[a]

Stand	*Forest type*	*GPS coordinates*
3	BOWO	N 44° 15.855′, W 86° 10.637′
6	SMBW	N 44° 13.206′, W 85° 40.327′
7	SMRO	N 44° 11.525′, W 85° 40.410′
22	SMBW	N 44° 22.279′, W 85° 42.422′
24	SMBW	N 44° 13.402′, W 85° 45.309′
41	SMRO	N 44° 20.830′, W 85° 28.905′
56	SMRO	N 44° 16.588′, W 85° 42.611′
58A	BOWO	N 44° 18.599′, W 85° 53.829′
58B	BOWO	N 44° 18.579′, W 85° 53.713′

[a]All sites are located within the Manistee National Forest in northern lower Michigan. BOWO: black oak, white oak; SMBW: sugar maple, basswood; SMRO: sugar maple, red oak.

Methods

Site Description. The study sites are located within the Manistee National Forest in Manistee and Wexford counties in northern Lower Michigan (Table 1). The sites represent three types of northern lake states forest: sugar maple-basswood (SMBW), sugar maple-red oak (SMRO), and black oak-white oak (BOWO) [31, 33]. Mean annual precipitation for the study area is 81 cm; mean annual temperature is 7.2°C [1]. The growing season varies from 100 to 150 days depending on distance from Lake Michigan.

The sites are located on sandy till-derived soils: typic haplorthods of the Kalkaska series for SMBW and SMRO and entic haplorthods of the Rubicon series for BOWO [15]. Although similar in overstory age, the three forest types differ in microbial community composition, N cycling rates, overstory composition, and litter chemistry (Table 2).

In April 2001, nine sites, three per forest type, were selected for study. These sites are a subset of those examined by Zak et al. [31] and have been used in previous studies [14, 23, 32]. The minimum distance between sites of the same forest type was 6 km. Within each site, three 10 × 30 m plots, separated by at least 20 m, were delineated. One plot (Low N Treatment) was amended with $NaNO_3$ equivalent to 30 kg N $ha^{-1}yr^{-1}$; one plot (High N Treatment) received the equivalent of 80 kg N $ha^{-1}yr^{-1}$; the remaining plot (Ambient Treatment) received no supplemental N. N amendments were applied in the form of sodium nitrate pellets, which were broadcast across the forest floor in monthly applications from April through September.

Table 2. Soil and overstory properties of three hardwood forests in northern Lower Michigan[a]

	BOWO	*SMRO*	*SMBW*
Overstory			
Age (yr)	88	81	80
Biomass (Mg/ha)	151	178	209
Leaf litter (Mg ha^{-1} yr^{-1})	1.8	3.2	2.6
Leaf litter N (kg N/yr)	13.1	30.4	32.5
Leaf litter C:N	133	104	80
Percent oak	100	30	0
Mineral soil (0–4 cm)			
Texture (% sand)	72	56	55
Bulk density (Mg/m^3)	0.69	0.71	0.7
pH	3.9	4.1	5.6
Organic-C (g/kg)	44	39	55
Total N (mg/kg)	1913	1835	3040
C:N	23	21	18
Microbial Biomass N (g/m^2)	1.4	1.7	1.6
Bacterial PLFA/Fungal PLFA	0.2	0.9	1.2
Net N mineralization (mg N g^{-1} yr^{-1})	313	382	426
Net nitrification (mg N g^{-1} yr^{-1})	18	43	364

[a]The values represent the mean of three sites of each forest type. (After [14, 23, 31, 32]).

Sample Collection and Preparation. Soil and litter samples were collected on 30 May, 27 June, 27 August, and 3 October 2001. The plots were visually divided into quadrats, and two samples were randomly removed from each section. The litter samples were taken as grab samples and the soil samples were removed with a 2 cm diameter × 20 cm deep soil corer. The eight litter grab samples and the eight soil cores were combined to form a composite litter and a composite soil sample for each plot. The samples were transported to the nearby field laboratory (North Central Forest Experimental Station, Wellston, MI) for analysis. At the lab the samples were stored at 4°C. Processing and analysis occurred within a few hours of collection.

Litter samples were cut into smaller pieces using scissors, placed into a dry food blender, and chopped into particles 1–2 mm in size. The composite soil samples were passed through a 2–mm mesh sieve. Subsamples of soil and litter were dried at 90°C to determine moisture content.

Enzyme Assays. Each soil and litter sample was assayed for phenol oxidase (EC 1.10.3.2), peroxidase (EC 1.11.1.14), glucose oxidase (EC 1.1.3.4), and glyoxal oxidase (no EC number) activities using a protocol modified from Saiya-Cork et al. [26]. Sample suspensions were prepared by placing 0.5 g litter or 1.0 g soil in a 125-mL Nalgene bottle. Bicarbonate buffer (10 mM, pH 8) was added to the bottle and the resulting suspension was homogenized using a Brinkmann Polytron for approximately 1 min. Additional buffer was added to the bottle to bring the final suspension volume to 125 mL.

The assays were conducted in 96-well microtiter plates using L-3,4-dihydroxyphenylalanine (L-DOPA, 5 mM) as the substrate. Sixteen replicate wells were used for each assay; eight additional wells were used as negative substrate controls and another eight wells served as negative sample controls. The assay wells received 200-μL aliquots of sample suspension and 50 μL of substrate. The negative sample control wells contained 200-μL aliquots of sample suspension and 50 μL of bicarbonate buffer. The negative substrate control wells received 200-μL aliquots of bicarbonate buffer and 50 μL substrate. The peroxidase, glucose oxidase, and glyoxal oxidase assay and negative control wells received additional 10-μL aliquots of H_2O_2 (0.3%), glucose (25 mM), and glycolaldehyde (25 mM), respectively. The plates were placed in an Echotherm incubator at 20°C, for up to 24 h depending on activity. Activity was measured spectrophotometrically at 460 nm using a Molecular Devices VERSAmax plate reader.

Activity was expressed in μmol h^{-1} g^{-1}. The final activities reported for peroxidase, glucose oxidase, and glyoxal oxidase were calculated as the incremental response relative to phenol oxidase. For example, the peroxidase activity is the increment in L-DOPA oxidation rate that results from the addition of hydrogen peroxide to the assay mixture, and glucose oxidase activity is the increment in the rate of L-DOPA oxidation associated with glucose addition.

Phospholipid Fatty Acid Analysis. The effect of N treatment on soil microbial community structure was assessed using phospholipid fatty acid (PLFA) analysis. Five-g soil subsamples were lyophilized and extracted by a single-phase, phosphate-buffered $CHC1_3$-CH_3OH solvent system [29]. Additional chloroform and water were added to separate aqueous and organic phases. The PLFAs in the organic phase were fractionated on silicic acid columns and transesterified in an alkaline system to convert the PLFAs into fatty acid methyl esters (FAMEs). The abundance and identification of the PLFAs was determined by gas chromatography and mass spectrometry [30]. Relative abundance was expressed as mol%. Total PLFA recovered was used as a measure of microbial biomass.

Data Analysis. The experiment was laid out in a random block design: three forest types, three sites for each forest type, three treatment plots for each site. A three-factor (forest type, sampling date, treatment), fixed effects, analysis of variance (ANOVA) was used to assess differences in enzymatic activity and PLFA abundance; least significant difference (LSD) post hoc tests were performed on significant results. Only the most common PLFAs, those having a mol% >1, were analyzed. The PLFA mol% data were arcsine transformed and the en-

Table 3. ANOVA results for soil and litter enzyme activities[a]

	Treatment		*Forest type*		*Date*		*Date* × type		*Trt* × type	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Soil:										
Phenox	**9.65**	**0.000**	**20.75**	**0.000**	**37.04**	**0.000**	**2.61**	**0.024**	**3.32**	**0.015**
Perox	0.67	0.515	**4.36**	**0.016**	**18.46**	**0.000**	1.78	0.115	0.27	0.899
Glyox	0.79	0.458	**10.48**	**0.000**	**6.236**	**0.001**	1.66	0.143	0.50	0.735
Glucox	**10.30**	**0.000**	**8.94**	**0.000**	**21.88**	**0.000**	1.84	0.104	1.79	0.141
Litter:										
Phenox	0.21	0.815	**30.72**	**0.000**	**23.1**	**0.000**	1.65	0.147	1.42	0.236
Perox	0.20	0.818	**3.61**	**0.032**	**8.87**	**0.000**	**3.32**	**0.006**	1.87	0.126
Glyox	0.07	0.934	**2.95**	**0.059**	1.86	0.144	0.46	0.835	0.44	0.778
Glucox	0.63	0.534	1.45	0.240	**13.93**	**0.000**	1.67	0.140	0.82	0.517

[a]Treatment, date and forest were fixed effects (n = 108).

zyme data were log transformed prior to ANOVA. Patterns in enzyme activity and PLFA data across sites and treatments were ordinated using principal component analysis (PCA) with varimax rotation.

Results

Extracellular Enzyme Activity (EEA). In soil, EEA varied significantly by forest type and sampling date (Table 3). Phenol oxidase (phenox), peroxidase (perox), glucose oxidase (glucox) and glyoxal oxidase (glyox) activities in SMBW stands were significantly higher than those in SMRO and BOWO stands, and perox activity in BOWO soil was significantly higher than that of SMRO soil (Table 4).

In litter, phenox and perox activities varied significantly by forest type and date. Glucox showed a significant date effect; glyox showed a significant forest type effect (Table 3).

In general, soil and litter EEA showed divergent responses to N amendment. In soil, enzyme responses were consistent across forest types: phenox and perox activities tended to decline with N addition while glucox increased (Table 4). Only the phenox and glucox responses were statistically significant (Table 3). Litter EEA responses to N amendment were generally smaller than soil responses and varied in magnitude and direction across forest types. None of the litter enzyme responses to N addition were statistically significant.

Table 4. Mean enzyme activity (SD) for soil and litter by forest type and treatment (n = 12)[a]

		Soil			
		Phenox	*Perox*	*Glucox*	*Glyox*
SMBW	Amb	7.1 (6.2)	9.4 (10.8)	0.7 (1.1)	0.8 (0.9)
	Lo N	7.2 (7.1)	6.1 (4.6)	2.7 (3.2)	0.9 (1.0)
	Hi N	1.9 (2.6)	6.2 (3.1)	2.8 (2.3)	1.4 (1.8)
SMRO	Amb	2.1 (2.4)	4.4 (2.1)	0.6 (1.1)	0.2 (0.4)
	Lo N	3.6 (8.6)	4.9 (4.4)	0.8 (1.1)	0.1 (0.2)
	Hi N	1.4 (2.7)	4.2 (3.0)	1.1 (1.2)	0.6 (1.3)
BOWO	Amb	2.0 (2.6)	9.1 (16.9)	0.6 (1.2)	0.2 (0.3)
	Lo N	0.7 (0.8)	5.8 (4.0)	0.7 (0.6)	0.3 (0.4)
	Hi N	0.9 (1.1)	5.4 (3.0)	1.2 (1.0)	0.3 (0.6)
		Litter			
SMBW	Amb	26 (13)	31 (14)	3.8 (5.8)	5.7 (9.0)
	Lo N	28 (19)	28 (21)	2.5 (6.6)	1.7 (2.9)
	Hi N	33 (15)	27 (24)	1.3 (2.7)	1.5 (2.8)
SMRO	Amb	57 (33)	30 (32)	8.2 (14.8)	7.4 (13.3)
	Lo N	49 (26)	47 (27)	9.2 (12.1)	5.7 (7.2)
	Hi N	56 (29)	48 (38)	9.2 (11.3)	6.8 (13.1)
BOWO	Amb	65 (28)	46 (32)	5.2 (14.5)	3.6 (7.6)
	Lo N	80 (27)	54 (31)	3.7 (7.3)	2.0 (4.5)
	Hi N	62 (29)	54 (35)	8.5 (16.2)	4.3 (6.1)

[a]Units of activity are μmol h^{-1} g^{-1}. SMBW: Sugar maple, basswood; SMRO: sugar maple, red oak; BOWO: black oak, white oak. Phenox: phenol oxidase; perox: peroxidase; glucox: glucose oxidase; glyox: glyoxal oxidase. Amb: ambient, no N added; Lo N = 30 kg N m^{-2} yr^{-1} added; Hi N = 80 kg N m^{-2} yr^{-1} added.

Table 5. PCA factor loadings for each soil and litter enzyme activities

	Soil activity		*Litter activity*	
	Factor 1	*Factor 2*	*Factor 1*	*Factor 2*
Phenox	–0.849	0.301	0.354	–0.819
Perox	–0.836	–0.164	–0.353	–0.816
Glyox	–0.593	–0.540	–0.745	–0.063
Glucox	0.077	–0.917	–0.786	0.066
Variance	44.4%	31.3%	35.6%	33.6%

PCA was performed independently on soil and litter EEA data to ordinate the functional responses to N amendment by forest type (Table 4). The soil PCA accounted for 76% of the variance, 44.4% explained by factor 1 and 31.3% by factor 2. Factor 1 correlated with phenox and perox activity; factor 2 correlated with glucox (Table 5). Except for the SMRO low-N treatment, the three forest types showed a consistent pattern of lower phenox and perox activity and higher glucox with increasing N amendment (Fig. 1). The response by SMBW forest was much larger than that of the two oak-containing forests.

The litter PCA accounted for 69% of the variance (Table 5). Factor 1, which correlated with glucox activity, explained 36% of the variance and factor 2, correlated with phenox and perox activities, explained 34%. The forest types separated along both axes (Fig. 2), but N application tended to displace plots along factor 2 (phenox and perox activity). Oxidative activity increased for SMBW litter and decreased for SMRO litter. BOWO responses varied in direction with N dose. Overall, phenox and perox activity tended to decrease in oak containing stands and increase in no-oak stands at high N dose.

Phospholipid Fatty Acids (PLFA). GC-MS analysis of soil samples detected 64 PLFAs; 5 were not identified, and the remaining 59 were classified into indicator groups. Only the 23 PLFAs whose abundance was >1 mol% were included in statistical analyses (Fig. 3).

Total biomass was similar across forest types (40 ± 5 nmol/g), but 22 of the 23 most abundant PLFAs varied significantly among forest types, and 14 of 23 varied by sampling date (Table 6). The SMBW soils had the most Gram-negative PLFAs, while BOWO soil was dominated by fungal PLFAs (Fig. 3). Actinomycete

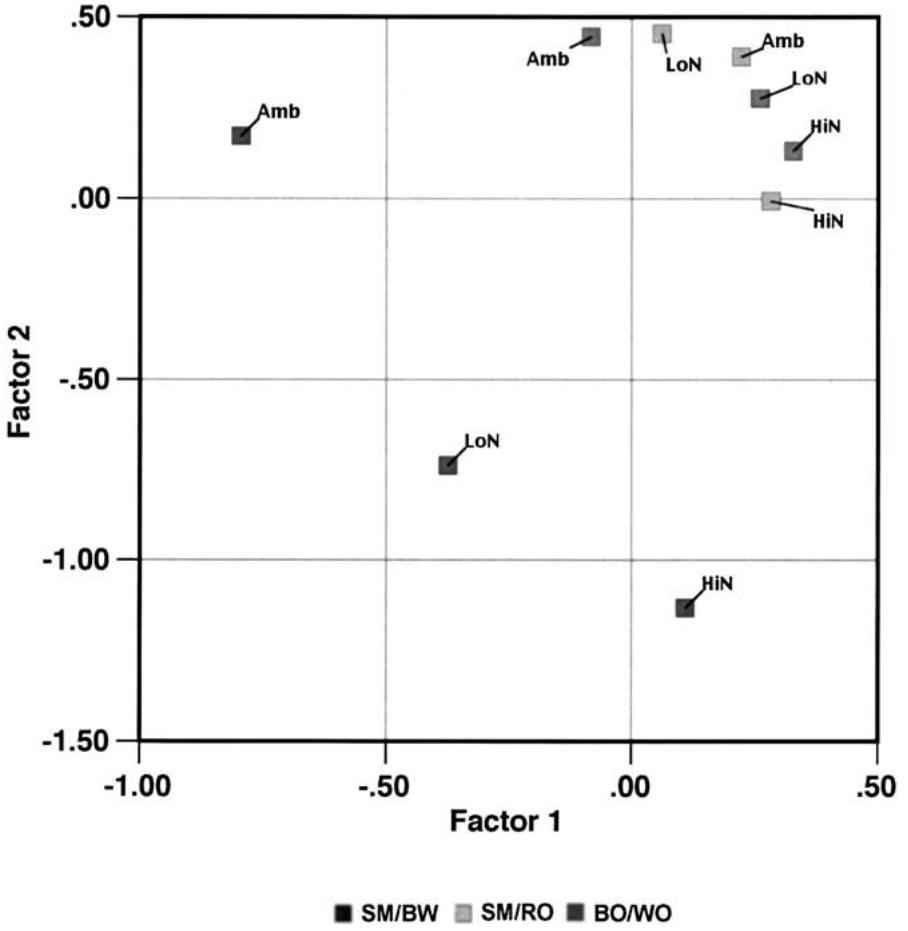

Figure 1. PCA for soil enzyme activities. Factor 1 (44% of variance) correlated with phenox and perox activities; factor 2 (31% of variance) is weighted toward glucox. With N addition, all treatments, except the low N SM/RO, shift toward less phenox and perox activity and more glucox. SM/BW: sugar maple, basswood; SM/RO: sugar maple, red oak; BO/WO: black oak, white oak.

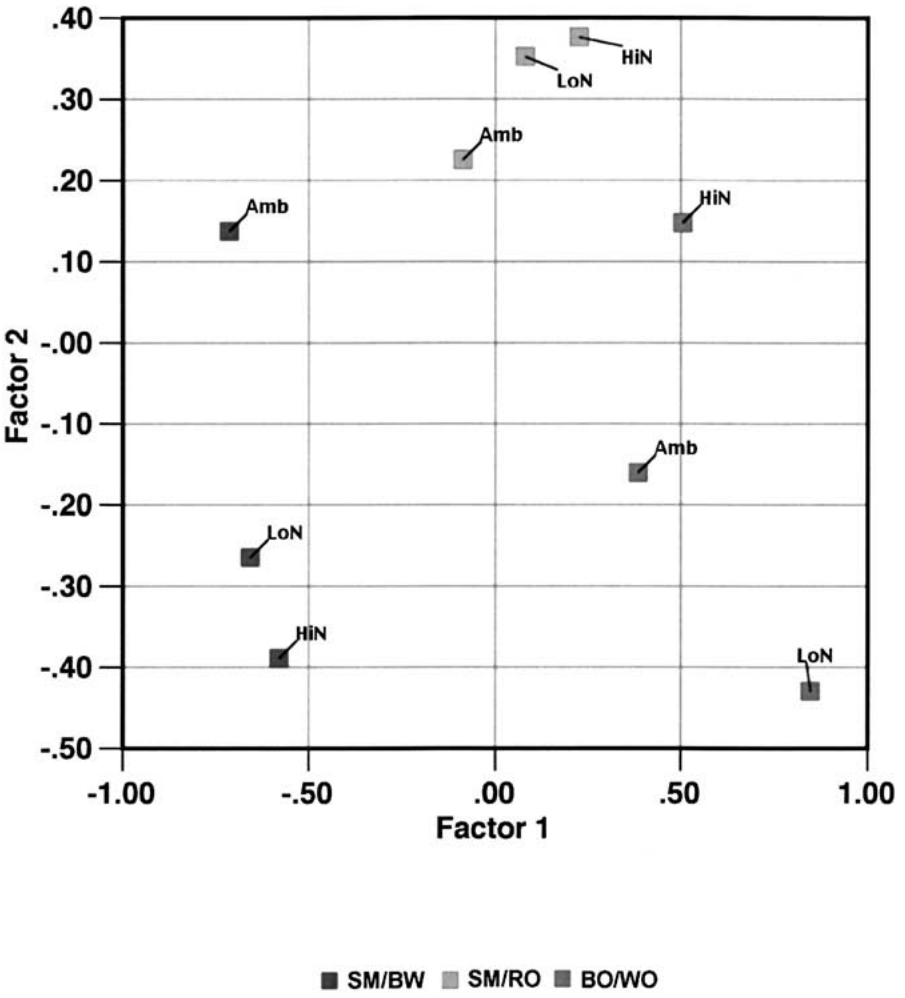

Figure 2. PCA for litter enzyme activities. Factor 1 (36% of variance) correlated with glucox activity; factor 2 (34% of variance) correlated with phenox and perox activities. Most treatment response was along factor 2 with each forest type showing a distinct pattern. SM/BW: sugar maple, basswood; SM/RO: sugar maple, red oak; BO/WO: black oak, white oak.

PLFAs were the highest in BOWO and the lowest in SMRO. Bacterial to fungal PLFA ratios for SMBW and SMRO were the same as those observed in a previous study (Table 2), but for the BOWO ratio, which was higher (0.6 vs 0.2).

Community structure was modestly affected by N amendment: 5 of 23 PLFAs (i16:0, 16:1ω7c, br16:0, 10Me16:0, 18:1ω9c) showed a statistically significant treatment effect (Table 6). The abundance of 16:1ω7c (Gram-negative bacteria, mean abundance in ambient plots = 5.0 mol%), br16:0 (Gram-positive bacteria, mean abundance = 0.6 mol%), and 10Me16:0 (Gram-positive bacteria, mean abundance = 4.6 mol%), decreased with N amendment (by –9%, –23%, –9% respectively, in high-N plots) while i16:0 (Gram-positive bacteria, mean abundance = 3.0 mol%) and 18:1ω9c (fungi, mean abundance 7.4%) increased (by 6% and 5%, respectively, in high-N plots). Four other PLFAs showed significant interactions between N treatment and forest type. Three of these were associated with Gram-negative bacteria (cy19:0, abundance = 11.8 mol%, br16:1, abundance = 2.0 mol%; 16:1ω9c, abundance = 1.0 mol%) and one with Gram-positive bacteria (a17:0, abundance = 1.7 mol%). The abundance of cy19:0 increased 20% with N addition in the SMBW plots; the other three declined by –14%, –15%, –13%, respectively, in the SMBW plots. Collectively, the nine PLFAs that showed statistically significant responses to N addition represented, on average, 37 mol% of the biomass. If all PLFA responses are included, ratios of bacterial to fungal mass tended to decrease slightly in response to N addition in all forest types: SMBW 1.23 ⇒ 1.18 ⇒ 1.07, SMRO 0.94 ⇒ 0.98 ⇒ 0.91, BOWO 0.64 ⇒ 0.62 ⇒ 0.62.

PCA was performed to compare community structure across forest types in relation to N amendment responses. PCA produced a three-factor solution that accounted for 71% (factor 1 = 33%, factor 2 = 26%, factor 3 = 12%) of the variance in the PLFA data (Table 7). Factor 1 was positively correlated with eight PLFA, a mix of Gram-positive and Gram-negative indicators, accounting for 19 mol% of the PLFA mass on average, and negatively correlated with two PLFA, representing. 12.7 mol% mean abundance, including the second most abundant Gram-negative bacterial indicator (cy19:0,

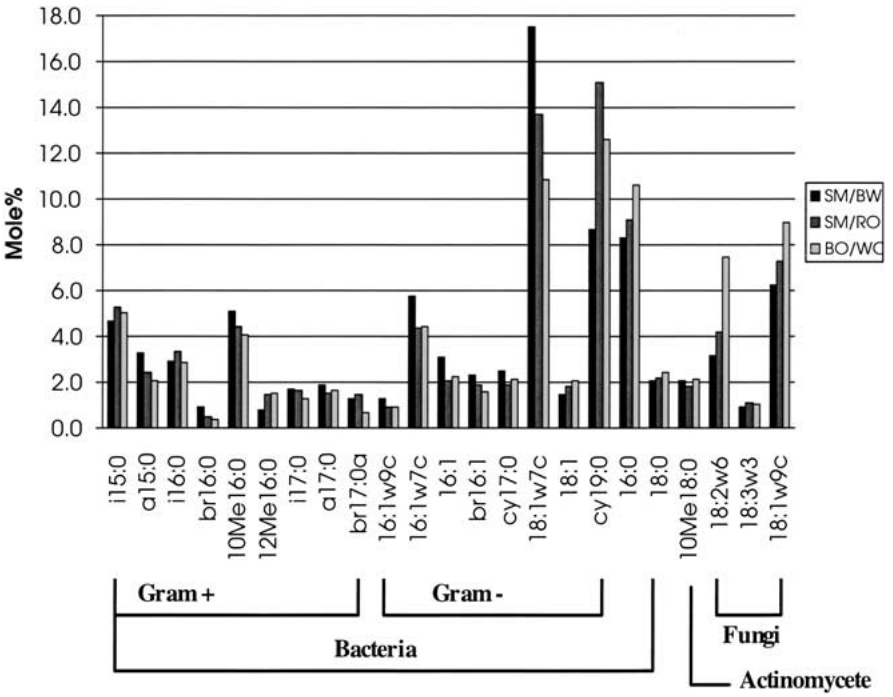

Figure 3. Microbial community structure by forest type as represented by phospholipid fatty acids. The PLFAs are grouped by the taxa they characterize. The values are means across sites, dates and treatments (n = 36). SM/BW: sugar maple, basswood; SM/RO: sugar maple, red oak; BO/WO: black oak, white oak.

average abundance = 11.8 mol%). Factor 2 was positively correlated with five PLFA (total of 22.0 mol% average abundance); these included the most abundant fungal marker (18:1ω9c, mean abundance 7.4 mol%) and saturated PLFAs indicative of bacteria in general. Factor 2 was negatively correlated with two PLFA (total of 14.7% mean abundance), including the most abundant Gram-negative bacterial marker (18:1ω7c, 14.0 mol% mean abundance). Factor 3 was positively correlated with three Gram-positive bacterial markers (total 5.6 mol% mean abundance) and negatively correlated with one fungal marker (4.8 mol% mean abundance).

Table 6. ANOVA summary for the 23 most abundant soil PLFAs (n = 108)

	Treatment		*Date*		*Forest type*		*Trt* × Type	
PLFA	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
i15:0	0.92	0.402	1.40	0.250	**14.6**	**0.000**	1.13	0.241
a15:0	0.05	0.954	0.41	0.746	**147**	**0.000**	1.41	0.221
i16:0	**4.25**	**0.018**	**6.28**	**0.001**	**28.0**	**0.000**	1.66	0.170
16:1ω9c	0.34	0.712	**15.1**	**0.000**	**97.4**	**0.000**	**3.35**	**0.014**
16:1ω7c	**3.16**	**0.048**	**6.26**	**0.001**	**39.3**	**0.000**	**5.20**	**0.001**
16:1	0.73	0.486	**5.11**	**0.003**	**72.2**	**0.000**	2.12	0.088
16:0	0.64	0.533	**4.10**	**0.001**	**38.0**	**0.000**	1.68	0.164
br16:0	**3.19**	**0.047**	2.26	0.882	**51.3**	**0.000**	**3.75**	**0.008**
br16:1	1.12	0.333	**10.7**	**0.000**	**159**	**0.000**	**3.32**	**0.015**
10Me16:0	**5.66**	**0.005**	0.87	0.463	**19.8**	**0.000**	**3.25**	**0.016**
12Me15:0	1.08	0.345	**8.95**	**0.000**	**209**	**0.000**	1.86	0.126
i17:0	0.11	0.895	**8.62**	**0.000**	**116**	**0.000**	0.57	0.687
a17:0	1.59	0.211	**6.42**	**0.001**	**115**	**0.000**	**4.35**	**0.003**
cy17:0	1.26	0.289	2.53	0.064	**49.2**	**0.000**	1.33	0.266
br17:0a	0.05	0.949	1.18	0.324	**80.8**	**0.000**	0.88	0.478
18:2ω6	0.56	0.572	**18.1**	**0.000**	**129**	**0.000**	0.04	0.997
18:3ω3	1.82	0.170	**4.35**	**0.007**	1.86	0.163	1.70	0.159
18:1ω9c	**3.22**	**0.046**	**4.56**	**0.006**	**99.7**	**0.000**	2.22	0.075
18:1ω7c	0.40	0.673	2.04	0.116	**175**	**0.000**	1.45	0.225
18:1	0.90	0.410	**20.5**	**0.000**	**177**	**0.000**	0.26	0.905
18:0	0.28	0.754	15.5	**0.000**	**32.6**	**0.000**	2.22	0.075
10Me18:0	1.64	0.200	1.42	0.243	**5.26**	**0.007**	0.56	0.689
cy19:0	1.59	0.210	2.36	0.079	**140**	**0.000**	**2.75**	**0.035**

Table 7. PCA factor loadings for the 23 most abundant PLFAs extracted from the 27 experimental plots

PLFA	*Factor 1*	*Factor 2*	*Factor 3*
i15:0	−0.039	0.554	0.383
a15:0	0.722	−0.460	0.222
i16:0	−0.411	−0.037	0.712
16:1ω9c	0.849	−0.356	−0.142
16:1ω7c	0.720	−0.335	−0.247
16:1	0.875	−0.235	−0.186
16:0	−0.129	0.790	−0.154
br16:0	0.488	−0.599	−0.018
br16:1	0.720	−0.552	0.182
10Me16:0	0.710	−0.257	0.215
12Me16:0	−0.603	0.606	0.196
i17:0	0.230	−0.642	0.644
a17:0	0.855	−0.203	−0.118
cy17:0	0.868	−0.047	−0.228
br17:0a	−0.156	−0.635	0.684
18:2ω6	−0.433	0.477	−0.633
18:3ω3	−0.685	−0.248	−0.211
18:1ω9c	−0.477	0.652	−0.374
18:1ω7c	0.293	−0.856	0.181
18:1	−0.357	0.714	0.018
18:0	−0.079	0.754	0.026
10Me18:0	0.052	−0.042	−0.309
cy19:0	−0.773	0.284	0.437
Variance	33.1%	26.1%	12.2%

The SMBW forest segregated from the oak forests along factors 1 and 2, indicating higher relative abundance of Gram-negative bacteria and lower relative abundance for fungi (Fig. 4). The oak containing SMRO and BOWO forests separated along factor 3, indicating relatively higher abundance for Gram-positive bacteria in SMRO and relatively higher abundance for fungi in BOWO. As with their functional responses, the SMBW and BOWO communities showed a consistent dose response trajectory in relation to N addition. For SMRO communities, the structural response varied with N dose, like the functional responses. For SMBW, factor 1 was the major response axis, indicating a shift in bacterial community composition with little effect on fungi. For SMRO and BOWO, responses involved both bacterial and fungal components.

We cannot resolve specific structure–function relationships within the soil microbial communities with our data, but correlation analysis of the EEA and PLFA PCA results indicates some correspondence. Both EEA factors were negatively correlated with PLFA factor 1, positively correlated with PLFA factor 3, and showed no relationship to PLFA factor 2 (Table 8). Six of the nine PLFAs that showed statistically significant responses to N treatment load strongly on PLFA factor 1 (Tables 6 and 7) and one of the nine loads strongly on PLFA factor 3; the other two were most strongly weighted toward PLFA factor 2, but also showed moderate loading on the other factors. The general interpretation is that oxidative enzyme increased as community composition shifted positively along PLFA factor 1 and negatively along PLFA factor 3 (Fig. 5). For PLFA factor 3 a negative shift means a reduction in Gram-positive bacteria and an increase in fungi that contain 18:2ω6. This shift is more closely tied to increased phenox and perox activity than to higher glucox. For PLFA factor 1 a positive shift is associated with a decline in the abundance of Gram-negative bacteria containing cy19:0 and an increase in a group of several Gram-negative and Gram-positive PLFAs. These changes are more strongly associated with glucox than with phenox and perox (Fig. 5).

Discussion

A number of studies have addressed the direct effects of nitrogen additions on decomposition. These experiments have produced a wide range of results: some have shown increases in decomposition rates [6, 13, 25, 27], others have found no or little effect [12, 13], and some have shown negative effects [5, 6, 8, 20, 25, 27]. The mixed effects of N additions have been explained several ways. Some explanations are extensions of the ecosystem concept of nutrient limitation: Nitrogen may or may not be a limiting nutrient for decomposition at certain sites; or the N content of particular substrates (low C:N) may mitigate N limitation. Other explanations for varied effects are based on changes in biochemical reaction rates: N addition may inhibit lignin degradation by suppressing the expression of ligninolytic enzymes by basidiomycetes; or nitrogen in various forms may react with litter components to produce recalcitrant humic compounds that slow decomposition [5, 8, 12, 17].

The purpose of this study was to examine at higher resolution the functional and structural response of microbial decomposer communities to N amendment across a range of forest types. We focused specifically on the ability of these communities to degrade lignin and humic materials. Our prior studies showed that mass loss responses for decomposing deciduous leaf litter were related to changes in the activity of oxidative enzymes, and secondarily to increases in cellulolytic enzyme activity [6, 27]. These studies included assays for phenol oxidase (phenox) and peroxidase (perox). In this study, we included these along with assays for glucose oxidase (glucox) and glyoxal oxidase (glyox), enzymes that produce hydrogen peroxide as a product of glucose or glycolaldehyde oxidation. To our knowledge, these activities have not been measured in conjunction with litter and SOM decomposition. Our assumption was that glucox activity would be linked to cellulose degradation because cellulose is the largest proximate source of glucose, and gluconolactone, a reaction product, is an inducer for cellulase expression. We assumed that glyox activity would be related to lignin degradation which generates glycoaldehyde.

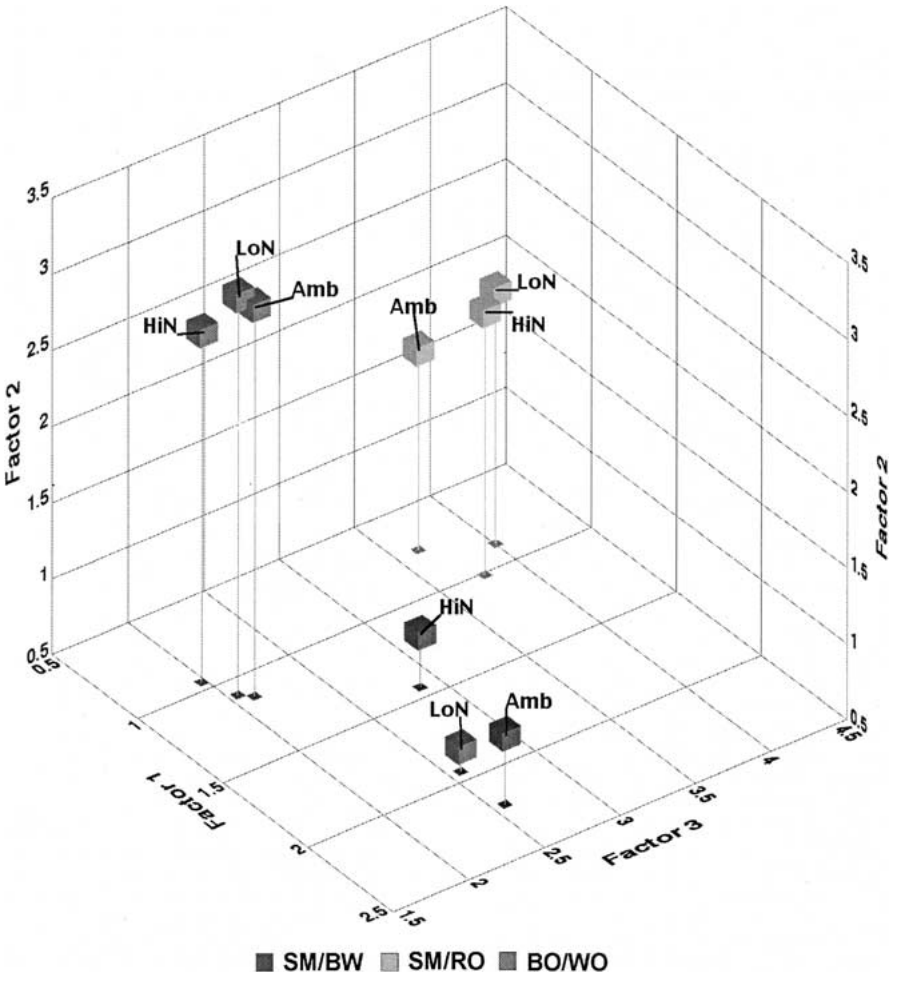

Figure 4. PCA for PLFAs. Factor 1 (33% of variance) is negatively correlated with Gram-negative bacteria and positively correlated with a mix of bacterial PLFA. Factor 2 (26% of variance) is positively correlated with fungal and saturated PLFA and negatively correlated with Gram-negative bacterial PLFA. Factor 3 (12% of variance) is positively correlated with Gram-positive bacterial PLFA and negatively correlated with fungal PLFA. SM/BW: sugar maple, basswood; SM/RO: sugar maple, red oak; BO/WO: black oak, white oak.

The treatment responses of the peroxide-producing enzymes varied by forest type and horizon. In litter, glyox and glucox activities had similar patterns of response under high N treatment across forest types (except for glyox in SMRO); activities decreased in SMBW and increased in SMRO and BOWO. The responses paralleled those for perox, suggesting that their activity in the litter was linked to hydrogen peroxide demand. For all three forest types, the ratio of glucox to glyox activity in litter was circa 1:1 (range 0.7–2.0) and the sum of the two potential activities totaled 10–50% of potential perox activity. In soil, glyox and glucox activities were consistently enhanced by N amendment for all forest types. Glucox:glyox activity ratios in soil averaged about 3:1 (range 1–8) and the sum of the two potential activities accounted for 10–60% of potential perox activity. Although we did not measure cellulolytic activity in this study, the distribution of glucox activity and its treatment response were consistent with predictions based on our prior observations of cellulase response to N amendment [27]. We had predicted that glyox activity would be correlated to lignin distribution, i.e., higher in oak plots, and that activity would decline with N addition along with phenox and perox. The data did not support either of these predictions. Glyox activity tended to parallel that of glucox, but the correlation was weak.

Activity levels for litter phenox and perox were higher than those reported by Carreiro et al. [6] and Saiya-Cork et al. [26] because we assayed activity at pH 8 to optimize enzyme response, rather than at pH 5, which was used in the other studies to approximate the pH of

Table 8. Correlation matrix relating soil community function to soil community structure across sites, dates, and N treatments using factors generated from principal components analysis (n = 108)[a]

		PLFA[c]		
		Factor 1 (33%)	*Factor 2 (26%)*	*Factor 3 (12%)*
EEA	Factor 1 (44%)	–0.21[b]	–0.02	0.36[c]
	Factor 2 (31%)	–0.35[c]	0.11	0.20[b]

[a]PLFA: Phospholipid fatty acid; EEA: extracellular enzyme activity. Numbers in parentheses indicate proportion of total variance explained by each factor.
[b]$P < 0.03$.
[c]$P < 0.001$.

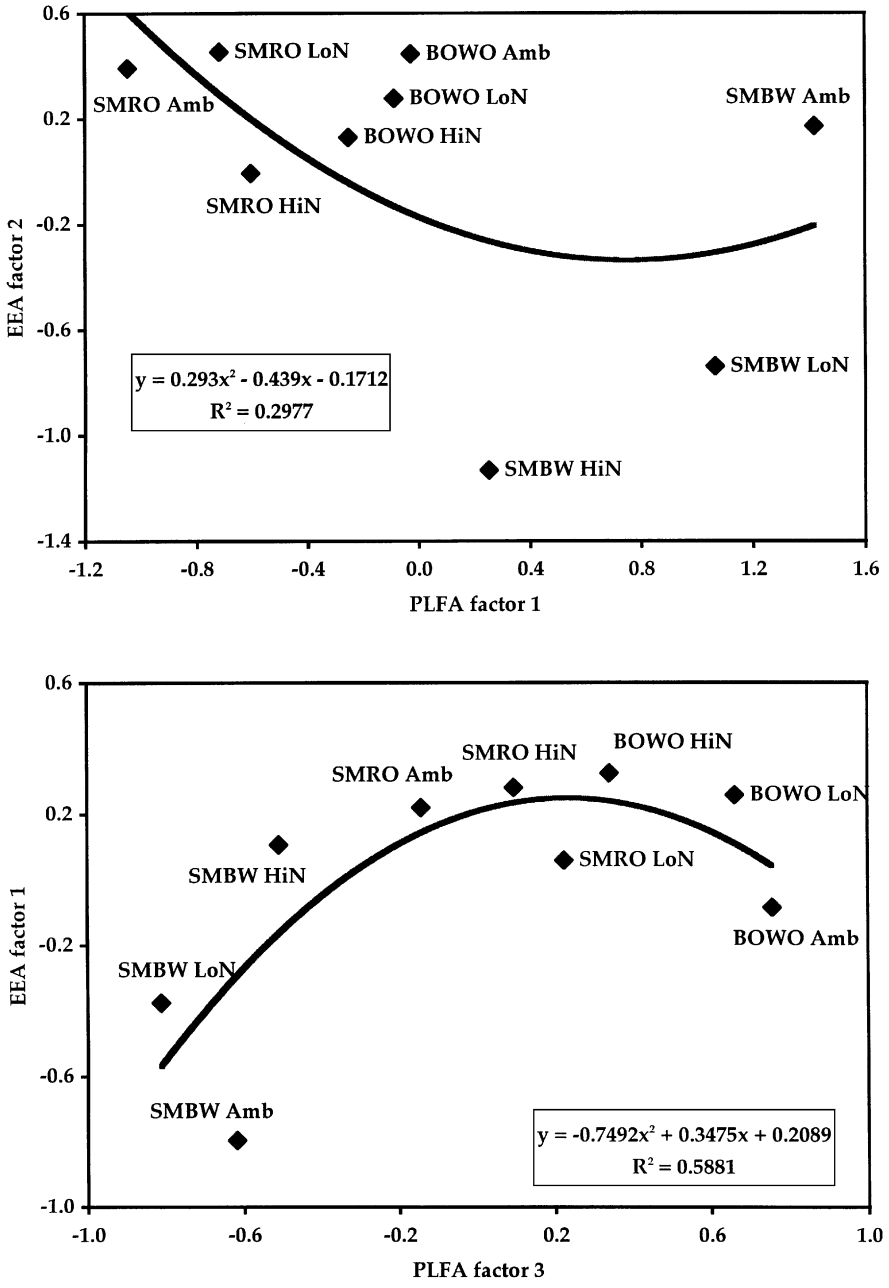

Figure 5. Community structure–function relationships across three types of north temperate forest in response to N addition. EEA factor 1 describes changes in phenol oxidase and peroxidase activity. In general, these activities increase with the abundance of a subset of fungi and decrease with the abundance of a subset of Gram-positive bacteria. EEA factor 2 is linked to glucose oxidase activity. Activity tends to increase as community compositions shift away from a subset of Gram-negative bacteria and toward a mixed assemblage of other Gram-negative and Gram-positive bacteria. SMBW: sugar maple, basswood; SMRO: sugar maple, red oak; BOWO: black oak, white oak.

the environment. The study by Carreiro et al. [6] and Sinsabaugh et al. [27] examined community functional responses for three litter types; the results, like ours, showed varied effects with N dose and litter quality. They observed repression of phenox activity in red oak litter (high lignin) in low–and high–N treated plots, enhanced activity in low-lignin litter (dogwood), and little response for litter of intermediate quality (red maple) at N doses similar to those we used. Perox responses were inverted relative to phenox. Our results for litter were generally consistent, but weaker than those found by Carreiro et al. [6]. One reason may be that we assayed partially decomposed material containing a mix of litter cohorts, rather than following a defined cohort of newly senescent litter through time as was done by Carreiro et al. [6]. Perhaps more important was the difference in the method of N application. The use of sodium nitrate pellets may have mitigated short-term litter responses. To some extent, the pellets settled through the litter layer, concentrating application in the soil. Also, the release of the nitrate from the pellets was tied to precipitation. The fertilizations in the Carreiro et al. study [6] were applied directly to the litter in dissolved form.

We can compare phenox and perox responses from our low-N treated SMBW sites to those from similar forest sites that are part of a long term study gradient

extending from central to northern Michigan [7, 26]. Treatment plots at the four gradient sites receive the same N supplement (30 kg N ha^{-1} y^{-1}), applied in the same manner, as our low N treatment; treatment at these sites began in 1996. Saiya-Cork et al. [26] examined litter and soil EEA responses at one of the gradient sites (near Pellston, MI) over a 2-year period. They found increased phenox activity for litter and a small decline in perox activity; neither response was statistically significant. A more extensive study by DeForest et al. [7], which included all four gradient sites, showed large declines in both oxidative activities, but only the phenox response was statistically significant. The data from our low-N SMBW sites showed a decline in perox activity that was consistent with the other studies, and a small positive effect for phenox activity; neither was statistically significant. The core problem for all these studies is that phenox and perox activities are highly dynamic. While hydrolytic enzyme activities typically have coefficients of variation of <100%, the CV for oxidative enzymes tend to be twice as large and activities show very marked seasonal trends (Table 4). A statistical resolution to the question of whether low N doses affect oxidative activity in the litter horizon of these forests will require a meta-analysis or long-term sampling.

Soil responses, in contrast, were relatively clear with statistically significant trends that were consistent across forest types. In general, phenox activity declined and glucox activity increased as N dose increased. Perox and glyox showed parallel trends but the changes were not statistically significant. Saiya-Cork et al. [26] found that phenox activity in soil was 40% lower in N addition plots at the Pellston, MI gradient site; perox activity showed little change. The data from our Manistee SMRO low N plots seem anomalous in that these were the only sites that diverged from the general patterns observed in both EEA and PLFA data (Figs. 1, 5); phenol oxidase activity at these sites declined only in the high-N treatment. DeForest et al. [7] found lower, but not significant, soil phenol oxidase and peroxidase activities across the four SMBW sites making up the gradient study. As was the case for litter, statistical resolution of EEA responses at low N application rates will probably require a meta-analysis or long-term sampling.

At the high N dose, soil phenox and perox activities were strongly repressed in all forest types. In the study by Carreiro et al. [6, 27], EEA in litter also showed a dose-response effect. Although individual studies appear to lack sufficient statistical power to resolve questions about individual enzyme responses, the consensus view seems consistent with the framework proposed by Sinsabaugh et al. [27]: specifically, that cellulase and phosphatase activities tend to increase and oxidative activities tend to decrease in response to N application and that, depending on litter chemistry, these changes may increase or decrease mass loss rates. Because the characteristics of residual organic matter tend to converge as decomposition proceeds, effects on soil decomposer communities appear to be more consistent across systems than those for litter, which improves the chances for connecting EEA responses to particular microbial groups.

Myers et al. [23] examined the composition of the soil microbial communities at the same sites used in our study. They found higher total PLFAs than we did, but both studies indicated that each forest type had a distinct microbial community. The SMBW and SMRO soils were dominated by bacterial PLFAs, while the BOWO forest was dominated by fungal PLFAs.

N amendment had only modest effects on PLFA abundance: 9 of the 23 most abundant PLFAs varied significantly with treatment or showed a treatment × forest type interaction. Biomass as well as the relative abundance of indicator groups (fungi, actinomycetes, Gram-positive bacteria, Gram-negative bacteria) remained relatively constant. DeForest et al. [7] conducted similar analysis of the four Michigan nitrogen gradient sites. They found a 20% decline in biomass but no evidence of a relative shift among the four groups. Despite the absence of gross changes in community composition, PCA showed dose-response patterns in the PLFA data (Fig. 4), and some of these shifts (those associated with PLFA factors 1 and 3) were correlated with the EEA responses (Table 8, Fig. 5).

Taken collectively, available evidence from EEA and PLFA studies suggests that N inhibition of oxidative activity is not a phenomenon narrowly limited to white-rot basidiomycetes. Loss of oxidative activity does not correlate with fungal abundance, and phenol oxidase activity is affected to a much greater extent than peroxidase activity. The mechanisms responsible for this wider effect are unclear. Direct mechanisms include physiological effects on extant populations of bacteria and fungi that alter extracellular enzyme expression. The role of N in the expression of ligninases is well understood for some basidiomycetes, but most soil microorganisms have never been cultured, so it is conceivable that N repression pathways are more common than is currently thought. Indirect N effects that result in population substitutions are also likely; mitigation of mineral N limitation may stimulate populations involved in the degradation of complex polysaccharides.

It is clear that N amendment alters the function and structure of microbial decomposer communities. It is also clear that the effects are comparatively subtle and lie near the boundaries of statistical and methodological resolution. However subtle, these small-scale effects are linked to comparatively large changes in the decomposition process, which in turn affect carbon dynamics at the ecosystem scale. Reported effects include reduced soil respiration, slower decomposition rates for lignified or

humified organic matterial, and increased production of dissolved organic matter, all of which influence where carbon accumulates and where it decomposes across the landscape.

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