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Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition

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Abstract. Anthropogenic nitrogen enrichment alters decomposition processes that control the flux of carbon (C) and nitrogen (N) from soil organic matter (SOM) pools. To link N-driven changes in SOM to microbial responses, we measured the potential activity of several extracellular enzymes involved in SOM degradation at nine experimental sites located in northern Michigan. Each site has three treatment plots (ambient, +30 and +80 kg N ha^{-1} y^{-1}). Litter and soil samples were collected on five dates over the third growing season of N treatment. Phenol oxidase, peroxidase and cellobiohydrolase activities showed significant responses to N additions. In the *Acer saccharum–Tilia americana* ecosystem, oxidative activity was 38% higher in the litter horizon of high N treatment plots, relative to ambient plots, while oxidative activity in mineral soil showed little change. In the *A. saccharum–Quercus rubra* and *Q. velutina–Q. alba* ecosystems, oxidative activities declined in both litter (15 and 23%, respectively) and soil (29 and 38%, respectively) in response to high N treatment while cellobiohydrolase activity increased (6 and 39% for litter, 29 and 18% for soil, respectively). Over 3 years, SOM content in the high N plots has decreased in the *Acer–Tilia* ecosystem and increased in the two *Quercus* ecosystems, relative to ambient plots. For all three ecosystems, differences in SOM content in relation to N treatment were directly related ($r^2 = 0.92$) to an enzyme activity factor that included both oxidative and hydrolytic enzyme responses.

Introduction

The enrichment of the biosphere with anthropogenic nitrogen (N) is an expanding problem that affects nearly every aspect of ecosystem function and composition (Matson et al. 2002; Aber et al. 2003; Fenn et al. 2003). In the coming decades, the interaction of N enrichment with rising atmospheric carbon dioxide concentration and climate change may substantially alter the global carbon (C) cycle (Zavaleta et al. 2003). Terrestrial soils, which contain large reservoirs of organic C and N, have the potential to respond as both sources and sinks in the changing global environment (Neff et al. 2002).

The effects of N deposition on soil processes and properties are many, but it is often difficult to connect fine-scale responses to ecosystem-scale effects (Magill and Aber 1998; Hobbie and Vitousek 2000; Neff et al. 2002; Månsson and Falkengren-Gerup 2003). Current models predict that N saturation ultimately leads to declines in plant growth and soil respiration, and increases in soil C sequestration; however, ecosystems vary widely in their initial responses to increased N inputs and in the time required to attain N saturation (Aber et al. 1998, 2003; Michel and Matzner 2002).

The influence of N availability on organic matter decomposition has been studied extensively (Fog 1988; Berg and Matzner 1997; Hobbie 2000). High levels of inorganic N generally increase rates of mass loss for litter that has a relatively low content of lignin, tannin and other secondary plant compounds. Mass loss rates for lignified or humified material generally do not increase with added N and often decline. Recent studies link these observations to changes in the distribution of microbial extracellular enzyme activity (EEA): cellulase and other glycosidase activities associated with litter and soil organic matter tend to increase in response to N additions, while the activities of oxidative enzymes needed to degrade aromatic secondary compounds and humus tend to decline (Carreiro et al. 2000; Saiya-Cork et al. 2002; Michel and Matzner 2003; Gallo et al. 2004; DeForest et al. 2004).

Although there is some generality in the microbial EEA responses, the underlying mechanisms are unclear, and predictions about the magnitude and even direction of enzyme responses for specific litter, organic matter fractions or ecosystems are problematic. At present, the best predictor of the effect of N deposition on decomposition rates is the phenol oxidase response: when activity increases so does turnover rate, when activity decreases turnover slows (Carreiro et al. 2000; Waldrop et al. 2004a).

For some white rot basidiomycetes, it is well established that high N availability blocks expression of lignin-degrading peroxidases (Hammel 1997). This phenomena has often been discussed as a possible mechanism for N inhibition of decomposition (Fog 1988; Berg and Matzer 1997). However, not all white rot fungi exhibit this N-dependent expression, and at least some white rot fungi use laccase, rather than peroxidase, as their primary delignification agent (Bermek et al. 1998; Li et al. 1999). In addition, losses or gains of both phenol oxidase and peroxidase activities occur in systems that vary widely in microbial community composition and fungal abundance (DeForest et al. 2004; Gallo et al. 2004).

In 2001, we began studying the effects of N deposition in three northern hardwood forest ecosystems. In the first growing season, oxidative enzyme activities in mineral soil declined in all three ecosystems in response to experimental N addition (Gallo et al. 2004). Over the next two seasons, changes in soil organic matter content, dissolved organic matter production, and microbial metabolism showed that sugar maple-dominated and oak-dominated ecosystems were responding divergently to the N treatments (Sinsabaugh et al. 2004; Waldrop et al. 2004a, b). In this report, we present data on extracellular

enzyme activities in litter and mineral soil from the third growing season of treatment. Our goal was to link the organic matter dynamics of these ecosystems to nitrogen-driven changes in microbial community activity.

Methods

Experimental sites

The experimental sites are located in the Manistee National Forest (Manistee and Wexford Counties) in northern Michigan. The nine sites represent three types of forest ecosystems common in the Upper Great Lakes region: sugar maple–basswood (SMBW, *Acer saccharum–Tilia americana*), sugar maple–red oak (SMRO, *A. saccharum–Quercus rubra*) and black oak–white oak (BOWO, *Q. velutina–Q. alba*) ecosystems (Zak et al. 1986, 1989). Although similar in overstory age (approximately 85 years), the three forest types differ in microbial community composition, N cycling rates, overstory composition, and litter chemistry. Comparative data and GPS coordinates for these sites were presented by Gallo et al. (2004).

The sites are located on sandy till-derived soils: typic haplorthods of the Kalkaska series underlie the SMBW and SMRO sites and entic haplorthods of the Rubicon series underlie the BOWO ecosystem (Host et al. 1988). These sites are a subset of those examined by Zak et al. (1989), and have been used in previous studies (Zak and Pregitzer 1990; Holmes and Zak 1999; Myers et al. 2001). Mean annual precipitation for the study area is 81 cm; mean annual temperature is 7.2 °C;. the growing season varies from 100 to 150 d depending on distance from Lake Michigan (Albert et al. 1986). Mean ambient N deposition is 11.7 kg ha^{-1} y^{-1} (MacDonald et al. 1993).

Three experimental plots (10 × 30 m) were established at each of the nine forest sites (three sites per ecosystem type) in 2001. One plot per site was assigned to each of the three N treatments (ambient, +30 and +80 kg N ha^{-1} y^{-1}). Because nitrate is the dominant form of N entering these ecosystems (Burton et al. 1991), we applied N in the form of $NaNO_3$ pellets, which were broadcast over the soil surface in six monthly increments during the growing season.

Sample collection and preparation

Soil and litter samples from each plot were collected on 20 May, 16 June, 15 July, 10 September and 20 October 2003. The plots were visually divided into quadrants; two soil cores (2 cm × 20 cm) and two litter (O horizon) grab samples (5–10 g) were randomly taken from each quadrant. The eight soil cores (exclusive of O horizon material) for each plot were combined into one composite sample and sieved through 2 mm mesh. The eight litter samples

were also pooled to form a composite sample for each plot. Litter samples were cut into smaller pieces using scissors, placed into a dry food blender, and chopped into particles 1–2 mm in size. Subsamples of soil and litter were dried at 60 °C to determine moisture content and combusted at 500 °C to determine organic matter content. The remaining material was sealed in plastic bags, kept cool and shipped on ice overnight to University of New Mexico for analysis.

Enzyme assays

Soil and litter samples for each plot were assayed for acid phosphatase, β-1,4-glucosidase, cellobiohydrolase, leucine aminopeptidase, phenol oxidase, and peroxidase using 4-methyumbelliferyl (MUB) phosphate, MUB-β-glucoside, MUB-cellobioside, L-leucine 7-amido-4 methyl coumarin, and L-3,4-dihydroxyphenylalanine (DOPA) as substrates, respectively, following published protocols (Saiya-Cork et al. 2002; Sinsabaugh et al. 2003). Sample suspensions were prepared by placing 0.5 g litter or 1.0 g soil in a 125 ml Nalgene bottle. Acetate buffer (50 mM, pH 5) 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 phenol oxidase and peroxidase assays were conducted in clear 96-well microtiter plates. 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 10 mM DOPA substrate. The negative sample control wells contained 200 μl aliquots of sample suspension and 50 μl of acetate buffer. The negative substrate control wells received 200 μl aliquots of acetate buffer and 50 μl substrate. For peroxidase assays, each well also received 10 μl of H_2O_2 (0.3%). 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.

The hydrolase assays were conducted on black 96-well microtiter plates. The assay design was similar to that described above except that reference standards (eight wells) and quench controls (eight wells per sample) were added to each plate. The reference standard for the acid phosphatase, β-1,4-glucosidase, and cellobiohydrolase assays was 10 μM 4-methylumbelliferone; for the leucine aminopeptidase assay 10 μM 7-amino-4-methylcoumarin was used. Quench control wells contained 200 μl of sample suspension and 50 μl of reference standard. The assays were incubated at 20 °C. The reactions were terminated by adding 10 μl of 1.0 M NaOH to each well. Fluorescence was measured using a Molecular Devices fmax spectrofluorometer with set to excitation 365 nm and emission set to 460 nm. Because there were statistically significant differences in soil organic matter content between ecosystems and N treatments (Waldrop et al. 2004a, b), all enzyme activities were calculated as μmol h^{-1} gOM^{-1}.

Data analysis

The assay data were analyzed using multivariate analysis of variance (MANOVA). There were 135 soil samples and 135 litter samples (3 forest types × 3 sites/forest type × 3 N treatments/site × 5 sampling dates). Activities, expressed in units of μmol h^{-1} gOM^{-1}, were LN-transformed to normalize variance prior to MANOVA. Ecosystem type, N treatment, and sampling date were designated fixed effects; the six enzyme activities measured were designated dependent variables. Tukey's tests were used for post hoc comparisons. Litter and soil EEA data were analyzed separately.

Results

Mean values and standard deviations for soil and litter EEA are presented in Table 1 by ecosystem type and N treatment. For both litter and soil, MANOVA showed significant differences in activity (Wilk's Lambda, $\pi < 0.01$) in relation to ecosystem type, N treatment and sampling date.

For soil, all six enzyme activities varied significantly by ecosystem type and sampling date ($p < 0.001$). Phenol oxidase and peroxidase activities declined significantly in response to N treatment; cellobiohydrolase activity showed a marginally significant increase (Tables 1 and 2). Post hoc comparisons for these enzymes indicated significant differences between ambient and high N treatments. In the case of phenol oxidase there was also a significant difference between the ambient and low N treatments. For oxidative enzymes, there was a strong seasonal pattern in activity with peaks during spring and autumn. Trends were less clear for hydrolytic activities.

Oxidative activities in soil generally declined in response to N amendment. Phenol oxidase activity in the high N treatment declined by 47% relative to the ambient treatment in the BOWO ecosystem, declined by 82% in the SMRO ecosystem, and was undetectable in the SMBW ecosystem (Table 1). For peroxidase, which accounted for >95% of the soil oxidative enzyme potential, the corresponding declines were 38% for the BOWO ecosystem and 29% for the SMRO ecosystem; in the SMBW ecosystem, activity in the high N treatment was unchanged relative to the ambient treatment (Figure 1).

Cellobiohydrolase activity in soil generally increased in response to N amendment. For the BOWO ecosystem, activity was 18% higher in the high N treatment, relative to the ambient treatment; corresponding values for the SMRO and SMBW ecosystems were 29 and 2%, respectively (Figure 2).

For litter, all six enzyme activities varied significantly by date ($\alpha < 0.001$). β-Glucosidase, cellobiohydrolase and leucine aminopeptidase activities showed significant differences by ecosystem type ($p < 0.001$). Phenol oxidase activity showed a significant decline with N addition (Table 2). Cellobiohydrolase activity showed a marginally significant increase in response to N treatment and peroxidase had a significant treatment × date interaction. In general,

Table 1. Mean enzyme activities across all dates, with standard deviations (SD) by ecosystem type and treatment.

Ecosystem	Treatment	*n*	AP	βG	CBH	LAP	Phox	Perox
Soil								
SMBW	Amb	15	4.11	4.92	1.05	0.40	0.09	24.9
	SD		1.15	0.67	0.34	0.03	0.04	8.23
	LoN	15	4.22	4.45	0.99	0.38	ND	26.9
	SD		1.21	0.97	0.32	0.04		6.07
	HiN	15	4.87	4.65	1.07	0.36	ND	25.8
	SD		0.30	0.56	0.12	0.08		9.98
SMRO	Amb	15	4.07	3.23	0.64	0.19	0.99	86.5
	SD		0.47	0.46	0.11	0.01	0.47	21.8
	LoN	15	4.42	3.57	0.74	0.24	0.48	65.9
	SD		0.70	0.54	0.17	0.05	0.54	18.0
	HiN	15	3.87	3.56	0.83	0.21	0.18	61.7
	SD		0.92	0.30	0.08	0.02	0.31	25.0
BOWO	Amb	15	2.44	2.22	0.45	0.13	2.85	80.6
	SD		0.84	0.55	0.15	0.06	0.44	20.0
	LoN	15	2.70	3.10	0.47	0.13	0.70	65.6
	SD		0.82	1.18	0.10	0.04	0.36	4.85
	HiN	15	2.57	2.36	0.53	0.15	1.52	49.6
	SD		1.04	0.39	0.15	0.03	0.61	10.7
Litter								
SMBW	Amb	15	14.5	25.0	9.03	0.78	4.85	0.52
	SD		4.95	8.03	2.72	0.32	1.76	0.86
	LoN	15	14.8	24.2	8.47	0.68	3.13	3.46
	SD		2.10	5.21	1.37	0.19	3.30	4.03
	HiN	15	14.4	22.4	8.33	0.70	2.64	4.76
	SD		2.10	2.83	1.46	0.30	1.01	2.98
SMRO	Amb	15	12.2	20.0	7.52	0.54	6.25	3.40
	SD		2.33	2.03	0.28	0.27	2.99	1.49
	LoN	15	15.4	21.1	8.69	0.57	4.38	6.50
	SD		2.93	0.54	0.29	0.25	1.65	0.99
	HiN	15	15.4	20.3	8.00	0.75	4.06	4.17
	SD		2.67	1.42	0.62	0.12	1.76	5.58
BOWO	Amb	15	12.1	14.5	5.62	0.36	4.49	4.26
	SD		1.78	2.71	0.64	0.02	2.02	5.36
	LoN	15	11.6	15.2	6.54	0.42	5.08	4.09
	SD		1.14	0.74	0.63	0.11	3.86	5.82
	HiN	15	12.0	19.3	7.80	0.38	2.15	4.60
	SD		2.25	1.96	0.86	0.07	1.07	4.69

Ecosystems: SMBW = sugar maple/basswood, SMRO = sugar maple/red oak, BOWO = black oak/white oak. Treatments: Amb = ambient N deposition, LoN = +30 kg N ha^{-1} y^{-1}, HiN = +80 kg N ha^{-1} y^{-1}. Enzymes: AP = acid phosphatase, βG = β-glucosidase, CBH = cellobiohydrolase, Phox = phenol oxidase, Perox = peroxidase. Activities are presented as μmol h^{-1} gOM^{-1}.

enzyme activities per unit mass decline in late summer and autumn as a new litter cohort is deposited.

As in soil, litter phenol oxidase generally declined in response to N amendment (Figure 1). Relative to the ambient treatment, activity in the high

Table 2. MANOVA results for litter and soil enzyme activities.

Enzyme	Effect	*F*-value	α
Soil			
Phenol oxidase	N treatment	8.49	<0.001
	Amb vs. LoN		<0.001
	Amb vs. HiN		0.009
Peroxidase	N treatment	3.83	0.025
	Amb vs. HiN		0.020
Cellobiohydrolase	N treatment	2.741	0.070
	Amb vs. HiN		0.060
Litter			
Phenol oxidase	N treatment	5.67	0.005
	Amb vs. HiN		0.003
Cellobiohydrolase	N treatment	2.43	0.095
	Amb vs. HiN		0.093
Peroxidase	Treatment × date	2.34	0.040

Ecosystem type, N treatment, and sampling date were fixed effects. Acid phosphatase, β-glucosidase, cellobiohydrolase, leucine aminopeptidase, phenol oxidase and peroxidase activities were dependent variables. Ecosystem type, N treatment and sampling date were statistically significant effects for both litter and soil ($\alpha < 0.001$). Tukey's tests were used for post hoc comparisons. Only statistically significant comparisons ($\alpha < 0.1$) related to N treatment are shown. Amb and HiN refer to treatments with ambient and $+80$ kg N ha^{-1} y^{-1} deposition.

N plots declined by 46% in the SMBW ecosystem, 35% in the SMRO ecosystem, and 48% in the BOWO ecosystem. However, peroxidase activity tended to increase with N amendment (Table 1), so the total oxidative potential (phenol oxidase + peroxidase) associated with SMBW litter increased by 38% in the high N treatment, relative to the ambient treatment. In the BOWO and SMRO ecosystems, increments in peroxidase activity did not offset losses in phenol oxidase so total oxidative potential in the high N treatment declined by 23 and 15%, respectively. As with soil, cellobiohydrolase activity in litter tended to increase in the BOWO and SMRO ecosystems (Figure 2): values in the high N treatments were, respectively, 39 and 6% greater than those in the ambient treatments.

Discussion

Over the first 3 years of experimental treatment (2001–2003) there have been ecosystem-specific changes in soil carbon storage. For the SMBW ecosystem, which produces relatively labile litter, organic matter in the upper 20 cm of mineral soil has declined by 25% in the high N treatment, relative to ambient (Waldrop et al. 2004b). In contrast, SOM content in the BOWO ecosystem, which produces relatively lignified litter, has significantly increased by 10% (Waldrop et al. 2004b). SOM in the intermediate SMRO ecosystem has tended to increase, but the response is smaller than that of BOWO ecosystem. The

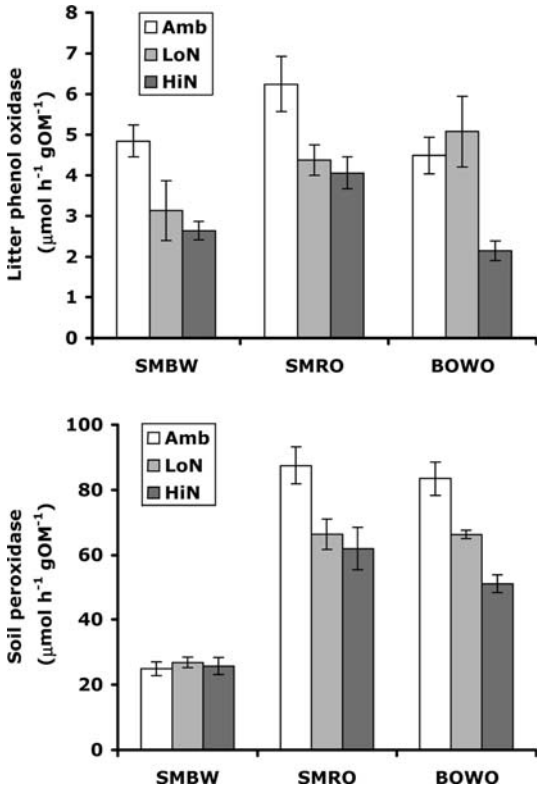

Figure 1. Oxidative enzyme activity in relation to N deposition rate for three temperate forest ecosystems. Phenol oxidase activity in the litter horizon was significantly lower in the high N treatment ($+80$ kg N ha^{-1} y^{-1}), relative to the ambient treatment (Table 2). In the upper 20 cm of mineral soil, peroxidase activities were significantly lower in the high N treatment for the black oak/white oak (BOWO) and sugar maple/red oak (SMRO) ecosystems (Table 2).

purpose of this study was to link these divergent ecosystem responses to changes in microbial activity.

Sinsabaugh et al. (2002) examined the effect of N deposition on the decomposition rates of leaf litter of varying composition. Their results suggested that N alters microbial decomposition by uncoupling the degradation of polysaccharides and polyphenols. In this model, high N availability stimulates cellulolysis, which tends to accelerate the decomposition of labile litter, and inhibits the expression of oxidative enzymes required for the breakdown of lignin and other secondary compounds. The magnitude of these effects varies with litter type and microbial community composition. In general, communities associated with labile or newly senescent litter show increased cellulolytic activity in response to high N availability with little change, or even gains, in oxidative activity (Carreiro et al. 2000).

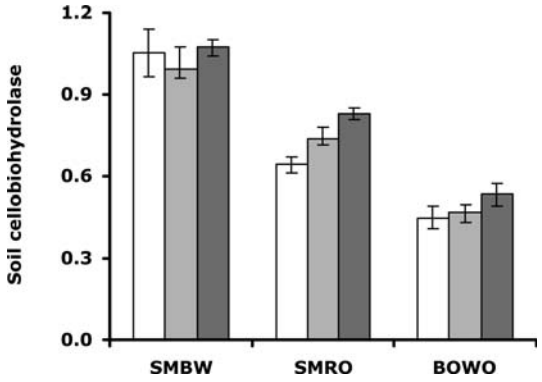

Figure 2. Cellobiohydrolase activity in relation to N deposition for three temperate forest ecosystems. In both litter and soil, there was a marginally significant increase in activity between ambient and high N treatments in the black oak/white oak (BOWO) and sugar maple/red oak (SMRO) ecosystems (Table 2). Activity in the sugar maple/basswood (SMBW) ecosystem did not respond to N addition.

Communities associated with lignified or humified organic matter may show less increase in cellulolytic activity but typically exhibit large declines in the phenol oxidase and peroxidase activities needed to breakdown aromatic molecules. If extended to the ecosystem scale, the predicted result is that labile organic matter stocks will turnover more rapidly, thus shrink in abundance, while humified fractions accumulate. Such changes in organic matter composition have been reported for grassland ecosystems in response to experimental N addition (Neff et al. 2002), but they were not linked to specific microbial responses.

To evaluate the connection between EEA and SOM responses, we reduced our six soil enzyme variables to a single factor using principal components analysis (for this analysis enzyme activities were expressed per g soil dry mass). The EEA factor accounted for 63% of the total variance and was positively correlated with hydrolytic activities (acid phosphatase 0.90, β-1,4-glucosidase 0.92, cellobiohydrolase 0.93, leucine aminopeptidase 0.86) and negatively correlated with oxidative activities (phenol oxidase -0.49, peroxidase -0.46). For all ecosystem types, differences in SOM content in relation to N treatment were directly proportional to the EEA response ($r^2 = 0.92$). For the BOWO and SMRO ecosystems, positive shifts in the EEA factor were associated with increases in SOM; for the SMBW ecosystem, negative shifts in the EEA factor were associated with SOM losses (Figure 3). These results are consistent with extrapolations from decomposition studies, however, many aspects of this relationship remain unclear.

One question is whether responses observed at high N deposition rates are qualitatively similar to those occurring at deposition rates more representative of anthropogenically affected areas. A related question is the role of microbial

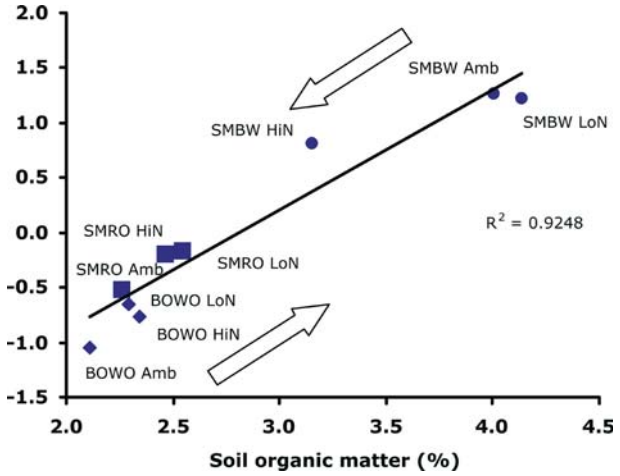

Figure 3. Extracellular enzyme activity (EEA) in relation to soil organic matter content expressed as a percentage of mass (%SOM). EEA is represented by principal components factor that is positively correlated with hydrolytic enzyme activities and negatively correlated with oxidative enzyme activities. Each point represents a N treatment within an ecosystem type: BOWO = black oak/white oak, SMRO = sugar maple/red oak (SMRO), SMBW = sugar maple/basswood; Amb = ambient, Lo N = +30 kg N ha^{-1} y^{-1}, Hi N = +80 kg N ha^{-1} y^{-1}. For all ecosystem types, changes in SOM content in response to N addition are directly proportion to the change in EEA.

community composition in directing or limiting the N response at a particular site.

In regard to the first question, many of the enzyme responses we have observed have been reported in other systems and at lower deposition rates. In particular, Saiya-Cork et al. (2002) and DeForest et al. (2004) studied enzyme responses at sugar maple-dominated forest sites in northern Michigan that have received N treatments (+30 kg N ha^{-1} y^{-1}) since 1994. Compared to ambient treatment plots, Saiya-Cork et al. (2002) found higher cellulase and phosphatase activities in both litter and soil. Phenol oxidase activity in soils from N-treated plots was lower than that of ambient plots, whereas litter from N-treated plots had elevated activity. DeForest et al. (2004), who studied four sites, including the one used by Saiya-Cork et al. (2002) found that N treatment increased cellulase activities in litter, decreased cellulase activities in soil, and decreased oxidative enzyme activities in both litter and soil. In general, these responses are consistent with our working model, but for the most part they were not statistically significant. Yet, in these systems rates of dissolved organic matter production and soil carbon storage have increased significantly (Pregitzer et al. 2004).

The forest sites used by Saiya-Cork et al. (2002) and DeForest et al. (2004) are similar in age and composition to our SMRO sites and the N treatment applied was identical to our low N treatment. In our project, the SMRO ecosystem is considered 'intermediate' between the SMBW and BOWO ecosystems in the sense that it receives substantial inputs of both labile litter

(sugar maple) and recalcitrant litter (red oak). Because of these varied inputs, the SMRO ecosystem has been the most difficult system in which to detect statistically significant responses to N treatment, particularly at the lower deposition rate. However, a meta-analysis of the enzyme data from the three studies indicates that phosphatase, α-glucosidase and cellulase (cellobiohydrolase, β-glucosidase) activities in the litter layer have increased significantly ($\alpha < 0.05$) in response to N addition (Table 3). In the mineral soil, α-glucosidase, cellobiohydrolase and leucine aminopeptidase activities increased significantly while phenol oxidase and peroxidase activities showed significant declines (Table 3). Overall, activities directed toward acquisition of labile carbon and phosphorus increased while activities directed toward the oxidative breakdown of lignin and humus declined. These results are similar to those observed at high levels of N addition; they are just more difficult to resolve statistically at low addition rates because enzyme activities are highly dynamic.

Perhaps the biggest unknown in predicting ecosystem response to N loading is the role of microbial community composition. A longstanding hypothesis for reduced decomposition rates and lower oxidative enzyme activity under N enrichment is suppression of white rot basidiomycetes (Fog 1988; Berg and Matzer 1997). For some taxa, it is well established that high N availability blocks expression of lignin-degrading peroxidases (Hammel 1997). However, this mechanism may not be sufficient to explain field results. Not all white rot fungi exhibit N-dependent expression, and losses (or gains) of both phenol oxidase and peroxidase activities occur in systems that vary widely in microbial community composition and fungal abundance (DeForest et al. 2004; Gallo et al. 2004; Waldrop et al. 2004b). In addition, there is evidence that hydrolytic enzyme responses, e.g. cellulase, phosphatase, also play a role in the decomposition response.

Resident microbial communities can potentially respond to N addition at multiple levels of organization. On a molecular scale, there may be direct effects on gene expression, as has been demonstrated for peroxidases in white rot fungi. It is possible that other, less investigated, microbial groups also show N-dependent expression patterns. At the organismal scale, N availability may affect growth, and thereby constitutive enzyme expression, by removing N limitation, or altering carbon availability (Schimel and Weintraub 2003). Changes in carbon availability may also include changes in the abundance of inhibitory phenols or increased humification of litter through geochemical reactions. At the community scale, it seems probable that high N availability simply selects different taxa whose collective EEA is distinct from that of a community adapted to low N availability (Dix and Webster 1995). It is likely that all these mechanisms contribute to community response; however, their relative importance may vary among ecosystems or change through time.

For cellulase activities, N addition alleviates a limitation associated with decomposition of material that has a high C:N ratio relative to microbial biomass. All else being equal, one might predict that cellulase response to N addition should be directly related to the C:N ratio of litter, but empirical data

Table 3. A meta-analysis of enzyme responses to N addition in the sugar maple/red oak ecosystem of northern Michigan.

Enzyme	Saiya-Cork et al.			DeForest et al.			This study			Mean grand		
	n	*d*	var(*d*)	*n*	*d*	var(*d*)	*n*	*d*	var(*d*)	*d*	var	+CI
Soil												
αG*	21	1.529	0.154	36	0.072	0.056				0.801	0.041	0.404
βG	21	0.444	0.100	36	−0.306	0.057	15	0.748	0.153	0.295	0.029	0.343
CBH*	21	0.272	0.097	36	−0.111	0.056	15	0.848	0.159	0.336	0.029	0.340
βX	21	0.683	0.107	36	−0.039	0.056				0.322	0.037	0.382
NAG	21	0.368	0.099	36	−0.186	0.056				0.091	0.036	0.378
LAP*	21	−0.704	0.108				15	4.000	0.705	1.648	0.093	0.611
AP	21	0.256	0.097	36	0.124	0.056	15	0.076	0.134	0.152	0.028	0.335
PhOx*	21	−0.423	0.100	36	−0.042	0.056	15	−1.086	0.175	−0.517	0.030	0.344
Perox*	21	0.063	0.095	36	−0.189	0.056	15	−0.970	0.167	−0.365	0.029	0.341
Litter												
αG*	9	0.583	0.243	36	0.351	0.057				0.467	0.046	0.431
βG*	9	1.053	0.292	36	0.122	0.056	12	0.564	0.181	0.580	0.037	0.386
CBH*	9	1.072	0.294	36	0.095	0.056	12	4.106	0.933	1.785	0.045	0.422
βX	9	0.171	0.224	36	0.171	0.056				0.171	0.045	0.423
NAG	9	−0.091	0.223	36	0.254	0.056				0.082	0.045	0.425
LAP	9	0.773	0.260				12	0.117	0.167	0.445	0.102	0.638
AP*	9	0.816	0.264	36	0.167	0.056	12	1.384	0.254	0.789	0.039	0.395
PhOx	9	0.486	0.237	36	−0.456	0.059	12	−0.625	0.184	−0.198	0.037	0.387
Perox	9	−0.022	0.222	36	−0.320	0.057	12	0.827	0.198	0.162	0.037	0.384

The study by Saiya-Cork et al. (2002) focused on a single site over a three growing seasons. DeForest et al. (2004) sampled four widely separated sites over a single growing season. Effect size (*d*) is the difference in activity between ambient and N-amended treatments expressed in units of standard deviation. Effect sizes, variances, and 95% confidence intervals (CI) were calculated as described by Saiya-Cork et al. (2002). Enzymes: αG = α-glucosidase, βG = β-glucosidase, CBH = cellobiohydrolase, βX = β-xylosidase, NAG = β-*N*-acetylglucosaminidase, LAP = leucine aminopeptidase, AP = acid phosphatase, PhOx = phenol oxidase, Perox = peroxidase. * indicates a statistically significant response (α = 0.05).

do not show this pattern. Activities increase in both litter and soil across a wide range of C:N ratio and different enzyme components of 'cellulase' may show differential, and sometimes negative, responses (DeForest et al. 2004, Michel and Matzner 2003).

High N availability can also alter the activities of enzymes involved in the breakdown of organic N and P compounds. Increased phosphatase activity in response to N addition probably reflects increased P demand, a likely consequence of reduced N limitation on microbial activity (Johnson et al. 1998; Ajwa et al. 1999; Turner et al. 2002; Sinsabaugh et al. 2002). The complementary hypothesis – that activities directed toward acquisition of organic N should decline when inorganic N availability is high – appears not to hold. In field studies, chitinase and peptidase activities often increase with N deposition (Saiya-Cork 2002; Michel and Matzner 2003). An alternate hypothesis for chitinase is that activity is predominantly associated with fungi and changes in activity reflect shifts in the relative abundance or activity of fungi. For peptidases, high N availability and relief from N limitation may stimulate protein production and biomass turnover increasing the environmental abundance of labile protein.

Initial responses to N addition are probably biochemical or organismal, but over time, it is likely that microbial community composition will shift in response to changing nutrient availability and organic matter characteristics. For mycorrhizal fungi, declines in diversity or infection rates have been reported along N deposition gradients (Egerton-Warburton and Allen 2000; Lilleskov et al. 2002; Treseder 2004). Decomposer communities have not received much study. PLFA analyses have been used to compare microbial community composition between control and N-amended treatments (DeForest et al. 2004; Gallo et al. 2004; Waldrop et al. 2004b). Declines in biomass have been noted, but no marked differences in community composition have been found using this technique, even for soils treated for several years. Higher resolution techniques will be needed to establish whether process changes are the result of altered community composition. Even if compositional changes can be documented and tied to process changes, PLFA results suggest that the range of compositional plasticity within each ecosystem is limited and that the draw down of labile soil carbon eventually reduces microbial biomass and respiration.

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