

Soil organic matter and litter chemistry response to experimental N deposition in northern temperate deciduous forest ecosystems

M. E. GALLO*, C. L. LAUBER*, S. E. CABANISS†, M. P. WALDROP‡, R. L. SINSABAUGH* and D. R. ZAK‡

*Biology Department, University of New Mexico, 167A Castetter Hall, Albuquerque, NM, 87131, USA, †Chemistry Department, University of New Mexico, Albuquerque, NM, 87131, USA, ‡School of Natural Resources, University of Michigan, Ann Arbor, MI, 48109, USA

Abstract

The effects of atmospheric nitrogen (N) deposition on organic matter decomposition vary with the biochemical characteristics of plant litter. At the ecosystem-scale, net effects are difficult to predict because various soil organic matter (SOM) fractions may respond differentially. We investigated the relationship between SOM chemistry and microbial activity in three northern deciduous forest ecosystems that have been subjected to experimental N addition for 2 years. Extractable dissolved organic carbon (DOC), DOC aromaticity, C:N ratio, and functional group distribution, measured by Fourier transform infrared spectra (FTIR), were analyzed for litter and SOM. The largest biochemical changes were found in the sugar maple–basswood (SMBW) and black oak–white oak (BOWO) ecosystems. SMBW litter from the N addition treatment had less aromaticity, higher C:N ratios, and lower saturated carbon, lower carbonyl carbon, and higher carboxylates than controls; BOWO litter showed opposite trends, except for carbonyl and carboxylate contents. Litter from the sugar maple–red oak (SMRO) ecosystem had a lower C:N ratio, but no change in DOC aromaticity. For SOM, the C:N ratio increased with N addition in SMBW and SMRO ecosystems, but decreased in BOWO; N addition did not affect the aromaticity of DOC extracted from mineral soil. All ecosystems showed increases in extractable DOC from both litter and soil in response to N treatment. The biochemical changes are consistent with the divergent microbial responses observed in these systems. Extracellular oxidative enzyme activity has declined in the BOWO and SMRO ecosystems while activity in the SMBW ecosystem, particularly in the litter horizon, has increased. In all systems, enzyme activities associated with the hydrolysis and oxidation of polysaccharides have increased. At the ecosystem scale, the biochemical characteristics of the dominant litter appear to modulate the effects of N deposition on organic matter dynamics.

Keywords: dissolved organic matter, extracellular enzyme activity, litter chemistry, nitrogen deposition, soil organic matter

Received 2 July 2004; revised version received 30 March 2005; accepted 1 April 2005

Introduction

The amount of bioavailable nitrogen (N) produced annually through human activities is more than double of that produced naturally in terrestrial ecosystems (Aber *et al.*, 2003; Galloway *et al.*, 2003). Much of this

anthropogenic N is deposited near industrialized regions of the eastern US and Europe, where it can accumulate in soil to levels that saturate plant demand and alter decomposition processes (Aber *et al.*, 1998; Matson *et al.*, 2002).

Exogenous N inputs affect decomposition by influencing the dynamic interaction between microbial activity and organic matter composition. The general observation is that mass loss rates for cellulosic litter tend to

Correspondence: Marcy E. Gallo, tel. + 505-277-0304, e-mail: megallo@unm.edu

increase with N availability, particularly in the early stages of decomposition, while rates for lignified litter, and humified organic matter tend to decline (Fog, 1988). For a defined litter cohort, the transition from N-enhanced decomposition to N-inhibited decomposition occurs when the acid insoluble fraction of the organic material reaches about 70% (Berg & Meentemeyer, 2002; Sinsabaugh *et al.*, 2002). Analyses of the extracellular enzyme activity (EEA) of microbial decomposers indicate that high N availability tends to stimulate the production of cellulolytic enzymes. The effects on polyphenol-oxidizing enzymes are more variable; these activities tend to increase with N availability for labile litter, then decline as litter composition approaches 70% acid insoluble material (Carreiro *et al.*, 2000). Measurements of microbial EEA at the ecosystem scale are generally consistent with the results from litter cohort studies, but more variable because soil and litter contain a heterogeneous admix of organic materials, including living roots, that are associated with microbial assemblages of diverse composition (Saiya-Cork *et al.*, 2002; DeForest *et al.*, 2004; Gallo *et al.*, 2004; Sinsabaugh *et al.*, 2005).

Several mechanisms, none mutually exclusive, have been proposed to explain the N-inhibition phenomenon. The most direct mechanism focuses on the regulation of extracellular oxygenase and peroxidase expression. In some white-rot basidiomycetes, ligninase expression is down regulated by N. If these populations are prominent in late succession decomposer communities, or if other microbial groups exhibit similar regulation, mass loss rates and microbial respiration should decline in proportion to the acid insoluble content of the substrate. Another potential direct effect is the incorporation of ammonium or nitrate directly into humic complexes through geochemical reactions, thereby increasing the recalcitrance of the residual organic matter (Stevenson, 1994).

It is also possible that high N availability during the early stages of decomposition enhances microbial growth efficiency, which may accelerate the incorporation of microbial biomass residues into humus. Another potential indirect effect of N is to increase the concentration of soluble phenolic compounds, as a consequence of lower oxidative enzyme activity. These compounds may inhibit microbial activity, or select for tolerant populations, leading to slower rates of decomposition.

At the ecosystem scale all of these mechanisms may be relevant to organic matter dynamics, particularly if we consider that the litter, rhizosphere and soil humus components of the system differ in substrate characteristics and microbial community composition. One way to resolve the mechanics of N effects on decomposition

is to analyze how organic matter composition within these functional soil compartments responds to elevated N inputs.

Experimental plots at Niwot Ridge, an alpine tundra ecosystem, have been experimentally fertilized since 1990 ($10 \text{ gN m}^{-2} \text{ yr}^{-1}$); although net primary production has increased, there has been no observable change in bulk carbon concentration in the soil. However, Neff *et al.* (2002) using ^{13}C tracer studies found that the turnover rate of the reactive soil organic matter (SOM) fraction has increased, concurrent with an increase in the quantity of plant carbon stabilized within the mineral soil. Dignac *et al.* (2002), using ^{13}C nuclear magnetic resonance and chemical fractionation techniques, compared SOM from the Oh and A horizons of Norway spruce ecosystems, using C:N ratio as an indicator of N accumulation. In the A horizon, lignin content increased with N accumulation; in the Oh horizon it declined. Increasing N content was associated with higher amino acid content, suggesting that the products of microbial growth were becoming a larger component of SOM. Michel & Matzner (2002) sampled Oa horizon material from the same network of sites used by Dignac *et al.* (2002). Measurements of microbial respiration, N mineralization and dissolved organic carbon (DOC) release, done in microcosms, indicated that SOM recalcitrance increased with N content.

In previous reports, we described functional and compositional changes in soil microdecomposer communities in response to N addition in three types of northern deciduous forest ecosystems. Experimental N deposition significantly reduced phenol oxidase and peroxidase activity in soils, but not litter, with only modest changes in microbial community composition, as measured by analysis of phospholipid fatty acids (Gallo *et al.*, 2004). In the second year of treatment (2002), we found that DOC concentration in N-treated soils had increased in all ecosystem types (Sinsabaugh *et al.*, 2004). Despite these commonalities, there have been divergent responses in SOM content across ecosystems: SOM is declining in the sugar maple/basswood ecosystem, and increasing in the black oak/white oak and sugar maple/red oak ecosystems (Waldrop *et al.*, 2004).

To further resolve the mechanisms underlying these changes in organic matter dynamics, we conducted comparative analyses of litter, SOM, and dissolved organic matter (DOM) composition on samples collected from ambient and N-amended sites of each ecosystem type. One hypothesis was that the aromatic content of DOM would change in proportion to the responses in oxidative enzyme activity (i.e. loss of oxidative activity should increase the concentration of potentially inhibitory phenolic compounds). A second

hypothesis was that the C:N ratio of SOM would decline in proportion to the increment in microbial cellulolytic activity because faster microbial growth on labile substrate would increase the incorporation of microbial detritus into humus. Finally, we predicted that the composition of O horizon litter would be more affected where cellulolytic and oxidative enzyme responses were in opposition, than where both responded in the same direction.

Methods

Site description

We studied three northern forest ecosystems common in the Upper Great Lakes region: the 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). Three replicate stands in each ecosystem type are distributed across a two county area in the Manistee National Forest in northern Lower Michigan. Although similar in overstorey age (approximately 88 years) and soil parent material, the three forest types differ in overstorey composition, N cycling rates and microbial community composition. These stands are a subset of those examined by Zak *et al.* (1989), and have been used in previous studies, which contain detailed site descriptions (Zak & Pregitzer, 1990; Holmes & Zak, 1999; Myers *et al.*, 2001; Gallo *et al.*, 2004).

In April 2001, we established three plots (10 m × 30 m) in each stand (three stands per ecosystem) and randomly assigned them to three levels of N treatment (ambient, +30 and +80 kg N ha⁻¹ yr⁻¹). Mean ambient N deposition at these sites is 12 kg ha⁻¹ yr⁻¹ (MacDonald *et al.*, 1993). Because NO₃⁻ is the dominant form of atmospheric N entering these ecosystems (Burton *et al.*, 1991), we applied NaNO₃ pellets over the soil surface in six monthly increments during the growing season. Treatment began in April 2001 and continued through 2004.

Sampling

Litter and soil samples were collected on 20 May 2003; 2 years after experimental treatment began. Each plot was divided into quadrants and two samples were randomly removed from each. Soil samples were removed with a 2 cm diameter × 20 cm deep soil corer; the litter (the whole O horizon) was collected as grab samples adjacent to each core. 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. In October 2003, newly senescent litter was removed from the top of the O horizon as grab samples in order to assess whether N treatment was affecting initial litter composition through changes in tree physiology.

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 60 °C to determine moisture content and combusted at 500 °C to determine organic matter content. Additional subsamples were shipped overnight, on ice, to the Sinsabaugh lab at University of New Mexico where they were stored at –20 °C prior to biochemical analyses. Analyses for this study were performed on samples from the ambient and high N-treated plots within 3 weeks of collection.

Fourier transform infrared analysis of litter samples

Litter samples from the ambient and +80 kg N ha⁻¹ yr⁻¹ treatment plots, collected 20 May 2003, and freshly senescent litter collected in October 2003 were milled (Wiley Mill fitted with a #20 mesh collection vessel; Thomas Scientific, Swedesboro, NJ, USA) then dried for 48 h at 60 °C immediately prior to analysis. Spectra were collected using a Bruker (Bruker Biopsin, Rheinstetten, Germany) Tensor 27 FTIR equipped with DLaTGS detector and a Pike Technologies diamond MIRacle single-bounce attenuated total reflectance sampling device. Subsamples of the litter were pressed onto the diamond surface of the MIRacle accessory using a pressure clamp. Spectra were collected at 4 cm⁻¹ resolution, averaging 30 scans per spectrum. Three subsample spectra were acquired and averaged to generate a single mean spectrum per sample. Difference spectra (data from high N treatments minus data from ambient treatments) were used to identify peaks showing responses (Bellamy, 1975). Newly senescent litter was analyzed to assess whether alterations in litter chemical composition were because of differences in the decomposition process or changes in foliar chemistry caused by plant physiological responses.

UV-Vis absorbance spectra of extractable organic matter

DOC was extracted from soil samples by placing 1.0 g soil in a 50 mL centrifuge tube with 10 mL of 50 mM sodium bicarbonate buffer (pH 8.2). The tubes were vortexed for 1 min at maximum speed. The resulting slurry was centrifuged for 10 min at 5000 × g. The supernatant was filtered through glass fiber and 0.22 µm membrane filters to remove bacteria and other colloids. Litter samples were extracted in the same manner except that 20 mL of sodium bicarbonate buffer

was used. Three replicate subsamples were extracted for each litter and soil sample.

For each DOC extract, absorbance was measured from 200 to 500 nm (quartz cuvette, 1 cm path) using a Shimadzu 1601 UV-Vis spectrophotometer; 50 mM sodium bicarbonate buffer was used as a baseline reference. Litter extracts were diluted 10^{-1} in bicarbonate buffer prior to scanning. Mean absorbances, at 10 nm increments, were calculated from the replicate subsamples. Linear regression (LN (ABS) vs. wavelength) was used to calculate a first order rate constant (S) for each sample. The value of this rate constant declines as the relative abundance of aromatic molecules increases (Vodacek *et al.*, 1997).

Extractable DOC

To quantify DOC from soil and litter samples; 0.5 g soil or litter was extracted with 20 mL of deionized water. The tubes were vortexed for 1 min at maximum speed. The resulting slurry was centrifuged for 10 min at $5000 \times g$. The supernatant was filtered through 0.22 μ m membrane. Three replicate subsamples were extracted for each litter and soil sample. DOC concentrations of the filtrates, as well as negative controls and internal standards, were determined using a Shimadzu TOC-5000A analyzer.

Elemental analysis

Elemental analyses (carbon and nitrogen) of litter and soil organic matter were performed using a CE

Instruments Thermoquest NC 2100 CN analyzer. There were three replicates for each sample.

Data analysis

The molar C:N ratio, DOC content and S values for litter and soil were compared in relation to ecosystem type and treatment using two factor, fixed effects, analysis of variance (ANOVA). Treatment effects were also compared for each ecosystem type, using two-factor (treatment \times site), fixed effect, ANOVA. This was done because our previous work has shown that microbial responses to N addition in the SMBW ecosystem diverge from those in the BOWO and SMRO ecosystems (Gallo *et al.*, 2004; Sinsabaugh *et al.*, 2004; Waldrop *et al.*, 2004). For FTIR data, multivariate analysis of variance (MANOVA) was used to assess treatment effects on newly senescent and decomposed litter by comparing total peak areas for selected functional groups.

Results

The C:N ratio of litter was similar across ecosystems (range 29–34) and showed little response to treatment. The only statistically significant response was in the SMRO ecosystem, where C:N ratio increased from 29 to 31 with N addition (Table 1). The trend toward higher C:N ratio in the SMRO ecosystem, as well as the BOWO ecosystem, was the result of significant increases in the carbon content of litter, rather than a change in nitrogen content (Table 1). In the SMBW

Table 1 Chemical characteristics of litter and soil organic matter in relation to ecosystem type and N treatment

	Trt	%C	%N	C:N	%DOC	S
Litter						
SMBW	AMB	42.5 (3.8)	1.71 (0.39)	29.5 (4.1)	2.72 (0.47)	-0.0113 (0.0002)
	HiN	39.9 (6.1)	1.67 (0.44)	28.4 (2.9)	4.13 (0.96)*	-0.0114 (0.0002)*
SMRO	AMB	45.2 (3.5)	1.85 (0.20)	28.6 (0.9)	2.55 (0.59)	-0.0128 (0.001)
	HiN	49.0 (0.4)*	1.83 (0.12)	31.4 (2.3)*	2.90 (0.63)*	-0.0128 (0.001)
BOWO	AMB	46.3 (2.6)	1.61 (0.09)	33.9 (3.9)	2.56 (0.87)	-0.0147 (0.001)
	HiN	49.3 (0.4)*	1.62 (0.14)	35.8 (3.6)	3.14 (0.80)*	-0.0128 (0.001)*
Soil						
SMBW	AMB	1.64 (0.20)	0.11 (0.02)	17.5 (1.1)	10.2 (1.1)	-0.0124 (0.001)
	HiN	1.47 (0.04)	0.09 (0.01)*	18.6 (1.7)*	13.7 (2.0)*	-0.0126 (0.001)
SMRO	AMB	1.07 (0.44)	0.06 (0.02)	20.4 (1.3)	15.6 (3.9)	-0.0119 (0.0005)
	HiN	1.01 (0.18)	0.06 (0.00)	20.4 (4.2)	19.9 (4.1)*	-0.0117 (0.0003)
BOWO	AMB	1.16 (0.06)	0.05 (0.00)	29.2 (1.0)	15.7 (4.3)	-0.0127 (0.001)
	HiN	1.23 (0.28)	0.05 (0.01)	29.7 (3.1)	14.4 (2.7)	-0.0126 (0.0003)

%C and %N are carbon and nitrogen content as a fraction of dry mass. %DOC is the fraction of organic carbon that was water extracted. S is a first order rate constant calculated from regressions of LN (absorbance) vs. λ from 200–500 nm. All values are means from three sites (\pm SD).

Statistically significant treatment effects ($\alpha < 0.05$) are indicated by *.

BOWO, black oak/white oak; SMRO, sugar maple/red oak; SMBW, sugar maple/basswood.

ecosystem, neither the C nor N content of litter varied in relation to N treatment.

In all three ecosystems, the extractable DOC content of litter was significantly greater in the N addition plots, relative to control plots (Table 1). For BOWO litter, the DOC extracted from the N addition plots had a higher content of aromatic carbon ($P = 0.000$), as indicated by the first order rate constant (S) for absorbance as a function of wavelength. SMBW litter showed a small, but significant, decline in the aromaticity of DOC in response to N addition ($P = 0.048$). The S values for SMRO litter did not vary in relation to treatment.

The C:N ratio of SOM varied more widely across ecosystems than litter C:N (range 17–30). Only the SMBW ecosystem showed a significant treatment response: C:N ratio was greater in the N addition plots as a result of a decline in the N content of SOM ($P = 0.002$) (Table 1). N addition increased the quantity of DOC extracted from SOM in the SMBW ($P = 0.002$) and SMRO ($P = 0.002$) ecosystems, but there was no change in DOC aromaticity.

From FTIR difference spectra of O horizon litter samples, we identified six peak areas for statistical analysis: OH stretch, CH₂, CH₃ stretch, carbonyl stretch, carboxylates & amides, C–O stretch and Si–OH (Table 2). MANOVA indicated a significant ecosystem effect ($F = 3.95$, $P = 0.04$), treatment effect ($F = 4.44$, $P = 0.04$), and a forest \times treatment interaction effect ($F = 4.32$, $P = 0.03$). For the BOWO ecosystem (Fig. 1), peaks associated with CH₂ and CH₃ stretch (saturated compounds such as lipids and waxes) increased in area

by 157% on average, with N treatment. For SMRO litter, the increase was 39%. However, in the SMBW ecosystem these peak areas decreased by 33% with N treatment. Peak areas corresponding to the carbonyl region (esters, ketones, and aldehydes) decreased by 22% with N treatment in the BOWO ecosystem, by 13% in the SMRO ecosystem, and by 51% in SMBW ecosystem. Peak areas associated with carboxylates and amides increased by 140%, 103% and 20% in the BOWO, SMRO and SMBW ecosystems, respectively, in response to N addition. MANOVA results for newly senescent litter showed a significant ecosystem effect ($F = 6.22$, $P = 0.01$), but no differences in regard to N treatment, indicating that changes in O horizon litter composition with respect to N were the result of decomposition processes, rather than differences in initial foliar chemistry (Table 2).

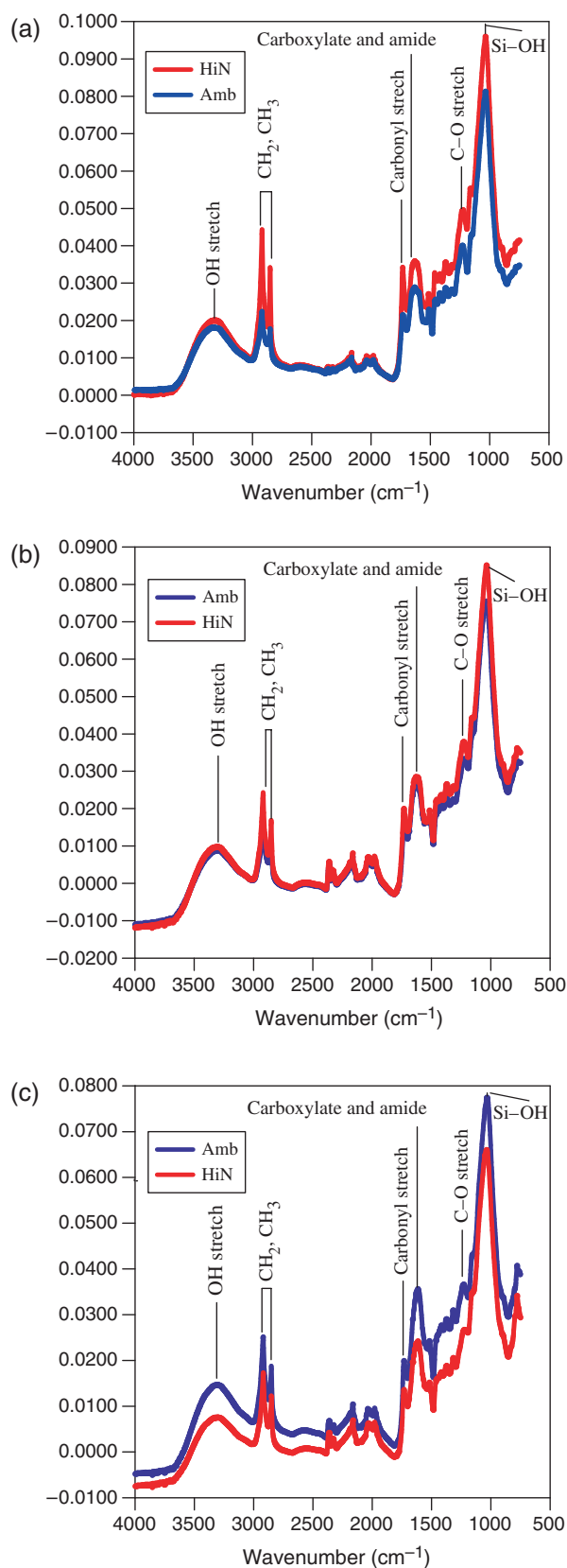
Discussion

Our results suggest that at the ecosystem scale soil carbon storage, in response to increased N deposition, is a function of initial litter composition, which, in turn, is established by the overstory plant community. At the process level, all three of the northern hardwood ecosystems show some commonality in microbial response to N deposition (Table 3): cellulase and other glycosidase activities associated with SOM tend to increase, while the activities of oxidative enzymes needed to breakdown, aromatic secondary compounds and humus tend to decline (Saiya-Cork *et al.*, 2002; DeForest *et al.*, 2004; Sinsabaugh *et al.*, 2005). The

Table 2 FTIR peak areas for functional groups within three northern forest ecosystems

	Trt	OH stretch	CH ₂ , CH ₃ stretch	Carbonyl stretch	Carboxylates, Amides	C–O stretch	Si–OH
<i>Newly senescent litter</i>							
SMBW	AMB	7.256	0.319	0.264	0.205	0.449	4.878
	HiN	5.400	0.336	0.362	0.295	0.361	5.195
SMRO	AMB	6.033	0.366	0.151	0.372	0.364	4.904
	HiN	6.387	0.403	0.066	0.310	0.435	4.896
BOWO	AMB	7.784	0.368	0.149	0.500	0.473	4.984
	HiN	4.674	0.597	0.000	0.156	0.413	4.883
<i>Decomposed litter</i>							
SMBW	AMB	4.171	0.598	1.195	0.158	0.263	6.011
	HiN	3.055	0.403	0.586	0.189	0.103	7.121
SMRO	AMB	3.802	0.488	1.061	0.144	0.294	6.562
	HiN	4.332	0.681	0.926	0.292	0.353	7.485
BOWO	AMB	3.495	0.488	1.061	0.146	0.533	6.363
	HiN	4.820	0.735	0.687	0.351	0.461	5.881

BOWO, black oak/white oak; SMRO, sugar maple/red oak; SMBW, sugar maple/basswood. The values are the mean of three replicates. Functional group areas correspond to the following peak maxima wavenumbers (cm⁻¹): –OH = 3330–3350, –CH = 2850, 2918, carbonyl stretch (aldehydes, ketones, esters) = 1730–1750, carboxylates and amides = 1550–1610, C–O stretch = 1020–1250, Si–OH = 1027–1033.



similarity in enzyme response across ecosystems does not extend to the litter layer (O horizon). Oxidative enzyme activity (phenol oxidase + peroxidase) on litter from the SMBW ecosystem has increased in response to N addition, while activities in BOWO and SMRO ecosystems have declined (Table 3, Sinsabaugh *et al.*, 2005). Paralleling this trend, SOM content in the SMBW system has declined by 20%, while contents have remained steady or increased in the BOWO and SMRO ecosystems (Waldrop *et al.*, 2004).

Taken collectively, our results indicate that the SOM changes we are observing are affected through changes in the reactivity of C rather than N in the system. It is surprising that the C:N ratio of litter and SOM is relatively unresponsive to N, despite the high level of addition (80 kg ha⁻¹ yr⁻¹). In cases where statistically significant shifts in C:N ratio were observed, the direction of response was toward higher C:N values. This was a result of increased C content. The lack of change in %N suggests that reactions that directly incorporate N into SOM are not a major mechanism in ecosystem response. The principal reactions underlying SOM responses appear to be ones that alter the oxidative state of carbon.

In the BOWO and SMRO ecosystems, where organic matter is accreting, litter from N-amended sites showed large increases in saturated carbon, carboxylic acids and amides, and a decline in carbonyl carbon. In the SMBW ecosystem, saturated carbon and carbonyl carbon declined, while peaks associated with carboxylic acids and amides increased slightly. Because the FTIR spectra of newly senescent litter showed no treatment effect for initial plant composition, these changes in functional group distribution are the result of altered decomposer activities. These divergent responses in litter carbon chemistry between the oak-dominated and sugar maple-dominated ecosystems also extended to the composition of extractable DOC. The aromatic content of DOC extracts increased with N treatment for BOWO litter and declined for SMBW litter. The same trends occurred in DOC extracts of SOM, but the responses were not statistically significant.

For the BOWO and SMRO ecosystems, the increased abundance of saturated carbon in litter and the increased aromaticity of DOC with N addition corresponds to observed declines in extracellular oxidative

Fig. 1 FTIR spectra of decomposing litter exposed to N addition from three northern hardwood ecosystems (a) Black oak/White oak ecosystem (b) Sugar maple/red oak ecosystem (c) Sugar maple/Basswood ecosystem. HiN, high N treatment (80 kg NO₃ ha⁻¹ yr⁻¹); Amb, ambient treatment. The spectra are mean of three replicates.

Table 3 Response ratios (HiN treatment: Amb treatment) for extracellular enzyme activities in the sugar maple/basswood and black oak/white oak (BOWO) ecosystems in relation to N addition

Ecosystem	Year	Horizon	N	AP	β G	CBH	LAP	Phox	Perox
SMBW	2003	Litter	15	0.99	0.90	0.92	0.90	0.54	9.15
		Soil	15	1.18	0.95	1.02	0.90	0.00	1.04
	2001	Litter	15					1.23	0.70
		Soil	15					0.29	0.58
BOWO	2003	Litter	15	0.99	1.33	1.39	1.06	0.48	1.08
		Soil	15	1.05	1.06	1.18	1.15	0.53	0.62
	2001	Litter	15					0.96	1.08
		Soil	15					0.52	0.83

The ratios are mean values from 2003 and 2001 ($n = 15$, three sites per ecosystem \times 5 sampling dates). Response ratios for the sugar maple/red oak ecosystem were similar to those observed in BOWO. Ratios were calculated from a subset of data included in a meta-analysis presented by Sinsabaugh *et al.* (2005). Ratios calculated for 2001 were adapted from Gallo *et al.* (2004) using activities presented as $\mu\text{mol h}^{-1} \text{gOM}^{-1}$.

AP, acid phosphatase; β G, β -glucosidase; CBH, cellobiohydrolase; Phox, phenol oxidase; Perox, peroxidase.

enzyme activity (-29% and -12% , respectively). Likewise, the lower saturated carbon content of SMBW litter and the lower aromaticity of DOC in these systems as a result of N addition is consistent with higher oxidative activity in the O horizon ($+38\%$) (Sinsabaugh *et al.*, 2005). The decreases in carbonyl carbon coupled with increases in carboxyl carbon, observed in all ecosystems, correspond to the increased activity of enzymes associated with the hydrolysis and oxidation of polysaccharides (Gallo *et al.*, 2004; Sinsabaugh *et al.*, 2005).

Higher cellulase activity may also be responsible for the increased DOC content of litter and SOM, which we observed in all ecosystems (Sinsabaugh *et al.*, 2004). Over the course of our studies it is the only enzymatic response to N addition that has been consistently higher across ecosystems (Saiya-Cork *et al.*, 2002; DeForest *et al.*, 2004; Sinsabaugh *et al.*, 2005). Increased export of DOC, recorded by lysimeters, has also been reported by Pregitzer *et al.* (2004) for N-amended forest ecosystems of the Upper Great Lakes region. Similarly, Hagedorn *et al.* (2004) reported an increase in water extractable organic carbon in soils from beech and spruce forests receiving exogenous N. Enzyme activity data from the sites monitored by Pregitzer *et al.* (2004) also show increased glycosidase activity (DeForest *et al.*, 2004; Sinsabaugh *et al.*, 2005). This association between glycosidase activity and DOC suggests that N affects the retention of soil carbon through hydrolytic enzyme expression, as well as oxidative. This is particularly significant because the capacity to degrade cellulose and other structural polysaccharides is much more widely distributed across microbial diversity than the capacity to oxidize lignin and humus.

Despite prior knowledge of microbial and SOM responses to N addition, our initial hypotheses about the mechanisms relating these responses were largely unsupported. As predicted, we did find a trend linking increases in the aromaticity of DOC to losses of oxidative enzyme activity. But, we found no evidence for our second hypothesis: that higher cellulolytic activity would decrease the C:N ratio of SOM by increasing the incorporation of microbial detritus into SOM via faster growth. The principal effect of higher cellulase and other glycosidase activities appeared to be increased DOC, while the major influence on C:N ratio appeared to be carbon composition. Our third hypothesis was that cellulase and oxidative enzyme responses would have synergistic effects on litter composition, but FTIR analyses suggest that each response has a discrete effect.

From these findings, we propose that changes in oxidative enzyme activity are the most likely mechanism affecting long-term C storage in these hardwood forest ecosystems, while changes in glycosidase activities controls short-term C dynamics (i.e. the decomposition of labile organic matter), and DOC export.

Acknowledgements

This research was supported by the Office of Science (BER), US Department of Energy, Grant No. DE-FG02-03ER63591.

References

- Aber JD, Goodale CL, Ollinger SV *et al.* (2003) Is nitrogen deposition altering the nitrogen status of northeastern forests? *Bioscience*, **53**, 375–389.

- Aber JD, McDowell W, Nadelhoffer K *et al.* (1998) Nitrogen saturation in temperate forest ecosystems: hypotheses revisited. *Bioscience*, **48**, 1–14.
- Bellamy LJ (1975) *The Infra-red Spectra of Complex Molecules*. Chapman & Hall, London.
- Berg B, Meentemeyer V (2002) Litter quality in a north European transect vs. carbon storage potential. *Plant and Soil*, **242**, 83–92.
- Burton AJ, Pregitzer KS, Reed DD (1991) Leaf-area and foliar biomass relationships in northern hardwood forests located along an 800 km acid deposition gradient. *Forest Science*, **37**, 1041–1059.
- Carreiro MM, Sinsabaugh RL, Repert DA *et al.* (2000) Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology*, **81**, 2359–2365.
- DeForest JL, Zak DR, Pregitzer KS *et al.* (2004) Anthropogenic NO₃⁻ deposition alters microbial community function in northern hardwood forests. *Soil Science Society of America Journal*, **68**, 132–138.
- Dignac M-F, Kögel-Knabner I, Michel K, Matzner E *et al.* (2002) Chemistry of soil organic matter as related to C:N in Norway spruce forest (*Picea abies* (L.) Karst.) floors and mineral soils. *Journal of Plant Nutrition and Soil Science*, **165**, 281–289.
- Fog K (1988) The effect of added nitrogen on the rate of decomposition of organic matter. *Biological Reviews*, **63**, 433–462.
- Gallo ME, Amonette RN, Lauber CL *et al.* (2004) Microbial community structure and oxidative enzyme activity in nitrogen-amended north temperate forest soils. *Microbial Ecology*, **48**, 218–229.
- Galloway JN, Aber JD, Erisman JW *et al.* (2003) The nitrogen cascade. *Bioscience*, **53**, 341–356.
- Hagedorn F, Saurer M, Blaser P (2004) A C-13 tracer study to identify the origin of dissolved organic carbon in forested mineral soils. *European Journal of Soil Science*, **55**, 91–100.
- Holmes WE, Zak DR (1999) Soil microbial control of nitrogen loss following clear-cut harvest in northern hardwood ecosystems. *Ecological Applications*, **9**, 202–215.
- MacDonald NW, Witter JA, Burton AJ *et al.* (1993) Relationships among atmospheric deposition, throughfall, and soil properties in oak forest ecosystems. *Canadian Journal of Forest Research*, **23**, 2348–2357.
- Matson P, Lohse KA, Hall SJ (2002) The globalization of nitrogen deposition: consequences for terrestrial ecosystems. *Ambio*, **31**, 113–119.
- Michel K, Matzner E (2002) Nitrogen content of forest floor Oa layers affects carbon pathways and nitrogen mineralization. *Soil Biology & Biochemistry*, **34**, 1807–1813.
- Myers RT, Zak DR, White DC *et al.* (2001) Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. *Soil Science Society of America Journal*, **65**, 359–367.
- Neff JC, Townsend AR, Gleixner G *et al.* (2002) Variable effects of nitrogen additions on the stability and turnover of soil carbon. *Nature*, **419**, 915–917.
- Pregitzer KS, Zak DR, Burton AJ *et al.* (2004) Chronic nitrate additions dramatically increase the export of carbon and nitrogen from northern hardwood ecosystems. *Biogeochemistry*, **68**, 179–197.
- Saiya-Cork KR, Sinsabaugh RL, Zak DR (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biology & Biochemistry*, **34**, 1309–1315.
- Sinsabaugh RL, Carreiro MM, Repert DA (2002) Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. *Biogeochemistry*, **60**, 1–24.
- Sinsabaugh RL, Zak DR, Gallo ME *et al.* (2004) Nitrogen deposition and dissolved organic carbon production in northern temperate forests. *Soil Biology & Biochemistry*, **36**, 1509–1515.
- Sinsabaugh RL, Gallo ME, Lauber CL *et al.* (2005) Extracellular enzyme activities and soil carbon dynamics for northern hardwood forests receiving simulated nitrogen deposition. *Biogeochemistry* (in press).
- Stevenson FJ (1994) *Humus Chemistry: Genesis, Composition, Reactions*. John Wiley & Sons, Inc, New York.
- Vodacek A, Blough NV, Degrandpre MD *et al.* (1997) Seasonal variation in CDOM and DOC in the middle Atlantic bight: terrestrial inputs and photooxidation. *Limnology and Oceanography*, **42**, 674–686.
- Waldrop MP, Zak DR, Sinsabaugh RL *et al.* (2004) Communication: nitrogen deposition modifies soil carbon storage through changes in microbial enzyme activity. *Ecological Applications*, **14**, 1172–1177.
- Zak DR, Host GE, Pregitzer KS (1989) Regional variability in nitrogen, nitrification, and overstory biomass in northern lower michigan. *Canadian Journal of Forest Research*, **19**, 1521–1526.
- Zak DR, Pregitzer KS (1990) Spatial and temporal variability of nitrogen cycling in northern lower Michigan. *Forest Science*, **36**, 367–380.
- Zak DR, Pregitzer KS, Host GE (1986) Landscape variation in nitrogen mineralization and nitrification. *Canadian Journal of Forest Research*, **16**, 1258–1263.