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Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions

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Abstract Recent research has dramatically advanced our understanding of soil organic matter chemistry and the role of N in some organic matter transformations, but the effects of N deposition on soil C dynamics remain difficult to anticipate. We examined soil organic matter chemistry and enzyme kinetics in three size fractions (>250 μm, 63–250 μm, and <63 μm) following 6 years of simulated atmospheric N deposition in two ecosystems with contrasting litter biochemistry (sugar maple, *Acer saccharum*—basswood, *Tilia americana* and black oak, *Quercus velutina*—white oak, *Q. alba*). Ambient and simulated (80-kg NO_3^-–N ha^{-1} $year^{-1}$) atmospheric N deposition were studied in three replicate stands in each ecosystem. We found striking, ecosystem-specific effects of N deposition on soil organic matter chemistry using pyrolysis gas chromatography/mass spectrometry. First, furfural, the dominant pyrolysis product of polysaccharides, was significantly decreased by simulated N deposition in the sugar maple–basswood ecosystem (15.9 vs. 5.0%) but was increased by N deposition in the black oak–white oak ecosystem (8.8 vs. 24.0%). Second, simulated atmospheric N deposition increased the ratio of total lignin derivatives to total polysaccharides in the >250 μm fraction of the sugar maple–basswood ecosystem from 0.9 to 3.3 but there were no changes in other size classes or in the black oak–white oak ecosystem. Third, simulated N deposition increased the ratio of lignin derivatives to N-bearing compounds in the 63–250 and >250 μm fractions in both ecosystems but not in the <63 μm fraction. Relationships between enzyme kinetics and organic matter chemistry were strongest in the particulate fractions (>63 μm) where there were multiple correlations between oxidative enzyme activities and concentrations of lignin derivatives and between glycanolytic enzyme activities and concentrations of carbohydrates. Within silt-clay fractions (<63 μm), these enzyme-substrate correlations were attenuated by interactions with particle surfaces. Our results demonstrate that variation in enzyme activity resulting from atmospheric N deposition is directly linked to

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changes in soil organic matter chemistry, particularly those that occur within coarse soil size fractions.

Keywords Nitrogen deposition · Enzymes · Carbon structure · Pyrolysis gas chromatography/mass spectrometry · Soil organic matter

Introduction

Changes in soil ecosystem function associated with elevated atmospheric N deposition include lower respiration rates, shifts in microbial community composition, and lower activities of ligninolytic extracellular enzymes (Burton et al. 2004; Frey et al. 2004; Waldrop et al. 2004a; Olsson et al. 2005). These and other potential changes (e.g., Phillips and Fahey 2007) in response to atmospheric N deposition can alter litter decomposition and soil C storage (Hobbie et al. 2002; Waldrop et al. 2004b; Zeglin et al. 2007). Considerable progress has been made in understanding the microbial and molecular level responses leading to these changes in soil C cycling (e.g., Smemo et al. 2007; Blackwood et al. 2007), in particular the role extracellular enzymes play in modifying decomposition dynamics (Saiya-Cork et al. 2002; Gallo et al. 2004; DeForest et al. 2004).

Summarizing this work, Sinsabaugh et al. (2005) propose that soil N differentially affects the microbial degradation of polysaccharides and polyphenols, resulting in an uncoupling of these processes. Greater N availability can stimulate cellulolysis, which accelerates the decomposition of labile litter components, but inhibit the degradation of lignin and its derivatives due to lowered oxidative enzyme activity. One consequence of these changes is that atmospheric N deposition might influence the chemistry of soil organic matter by altering the activities of extracellular enzymes that depolymerize plant cell wall components (i.e., cellulose and lignin). For example, lower oxidase enzyme activity might result in an accumulation of lignin derivatives in soil and accelerated cellulolytic activity could lead to lower concentrations of some polysaccharides in soils.

Soil size fractions exhibit distinct biological and chemical properties (Six et al. 2001; Sollins et al. 2006), including differences in enzyme kinetics (Kandeler et al. 1999, 2000; Sessitsch et al. 2001; Marx et al. 2005) as well as organic matter concentration and chemistry (Guggenberger et al. 1995; Grandy et al. 2007). Coarse soil fractions (>53 μm) typically contain higher proportions of lignin derivatives than smaller fractions, whereas fine fractions (<53 μm) contain a greater abundance of N-bearing compounds, polysaccharides and lipids (Baldock et al. 1992, 1997; Grandy et al. 2007). These differences suggest that atmospheric N deposition might alter soil organic matter chemistry differentially by size fraction.

The hypothesis that N deposition changes soil enzyme activities and subsequently soil C chemistry has been supported by analyses of whole soils, and the strength of enzyme-soil organic matter (SOM) chemistry correlations, which often vary across ecosystems (Grandy et al. 2007; Leinweber et al. 2008). In this study, we investigate whether enzyme and SOM responses to simulated atmospheric N deposition vary across soil size fractions and ecosystems. Our specific objectives are to determine: (1) how enzyme kinetics in different soil size fractions respond to N deposition; (2) whether changes in enzyme kinetics due to N deposition are related to changes in SOM chemistry; and (3) the degree to which soil C and enzyme responses to N within soil size fractions vary with vegetation and litter chemistry. We use our results and those of others to develop a conceptual model highlighting N deposition effects on enzymes and SOM chemistry in different soil size fractions and the potential implications for SOM turnover dynamics.

Methods

Experimental site

Our experimental sites were located in the Manistee national forest, Michigan, described in detail elsewhere (Zak et al. 1986, 1989; Gallo et al. 2004; Waldrop et al. 2004b). We studied two ecosystems, each replicated at three different landscape locations: a sugar maple, *Acer saccharum*—basswood, *Tilia americana* ecosystem (SMBW) and a black oak, *Quercus velutina*—white oak, *Q. alba* ecosystem (BOWO). The ecosystems are similar in their overstory age (~90 years), but differ in microbial community composition and rates of soil C and N cycling (Zak et al. 1986, 1989; Gallo et al. 2004; Blackwood et al. 2007).

The soils at our experimental sites formed in sandy glacial drift. The SMBW soils are classified as typic

haplorthods of the Kalkaska series and are loamy sands; the BOWO soils are entic haplorthods of the Rubicon series and are also loamy sands (Host et al. 1988). Mean annual precipitation in the area is 81 cm and mean temperature is 7.2°C. Annual N deposition is ~12 kg N ha^{-1} $year^{-1}$, largely in the form of nitrate (Burton et al. 2000). In 2001, three plots (10 m × 30 m) were established in each replicate stand to study the effect of elevated atmospheric N deposition. One plot received ambient N deposition, one received ambient plus 30 kg NO_3^-–N ha^{-1} $year^{-1}$, and the remaining plot in each stand received ambient plus 80-kg NO_3^-–N ha^{-1} $year^{-1}$. The additional N was delivered as $NaNO_3$ pellets, which were broadcast over the forest floor in six equal applications over the growing season (May–October). In this study, we examined soil organic matter chemistry and extracellular enzyme activity (EEA) in the ambient and ambient plus 80 kg NO_3^-–N ha^{-1} $year^{-1}$ treatments in all three replicates of each ecosystem.

Soil fractionation

We collected mineral soil samples (2-cm-diameter × 20-cm-deep cores) from each plot receiving ambient and simulated NO_3^- deposition in June 2006. Eight cores collected in each plot were pooled to create a composite sample. Because the soils have high sand contents, a gentle and rapid fractionation method was used to separate them into size classes. One hundred to 130 g of soil were combined with an equivalent (w/v) amount of deionized water at a temperature of ~1°C. The slurries were shaken on a rotary action shaker for 20 min. To minimize the impact of the separation process on enzymes, cold water temperature was maintained with ice packs. After shaking, soil slurries were poured through a 250 μm sieve, and the liquid and soil <250 μm was collected in a pan. This slurry was then poured through a 63 μm sieve. The water and soil that passed through this sieve was centrifuged at 3,300 g for 15 min to collect a pellet of soil in the <63 μm class. This created three size classes: >250 μm, 63–250 μm, and <63 μm. To minimize the redistribution of soluble C, enzymes, and microbes among soil classes during sieving, the >250 μm and 63–250 μm size classes were rinsed with 400 ml of deionized water (1°C) and the <63 μm size class with 200 ml of deionized water by centrifugation and decanting. Soil size classes were frozen (−20°C) immediately after separation. Soil carbon and N concentrations were measured with an EA 1110 CNS combustion analyzer (Thermo Electron Corporation, Waltham, MA).

SOM characterization

We used pyrolysis gas chromatography/mass spectrometry to determine the molecular structure of organic matter (Hempfling and Schulten 1990; White et al. 2004; Kaal et al. 2007; Adani et al. 2007) in whole soils and soil fractions using methods described in detail elsewhere (Neff et al. 2005; Grandy et al. 2007). Samples were pyrolyzed at 590°C in pyrofoils (Pyrofoil F590, Japan Analytical Company, Tokyo, Japan) using a Curie-Point pyrolyzer (Pyromat, Brechbühler Scientific Analytical Solutions, Houston, TX). The products of pyrolysis were transferred online to a gas chromatograph (ThermoQuest Trace GC, Thermo Finnegan, San Jose, CA). The interface temperature was 250°C with a split injection (split ratio 50:1, He flow rate 1.0 ml/min). Pyrolysis products were separated on a BPX 5 column (60 m × 0.25 mm, film thickness 0.25 μm) using a temperature program of 40°C for 5 min, 5°C min^{-1} to 270°C followed by a jump (30°C min^{-1}) to a final temperature of 300°C. The column outlet was connected with a Thermo Polaris-Q ion-trap mass spectrometer (Polaris Q, Thermo Finnigan, San Jose CA) operated at 70 eV in the EI mode. We heated the transfer line to 270°C and the source temperature was held at 200°C.

We compared peaks corresponding to pyrolysis products with reference spectra after deconvolution and extraction using AMDIS v. 2.64, the National Institute of Standards and Technology mass spectral libraries, and published literature. Our results are expressed as relative abundance by normalizing results to the largest peak measured in the chromatogram. These data do not provide insight into the absolute abundance of compounds across samples, an approach that would require multiple internal standards and which has, to date, been used only for specific types of molecules. The relative abundance approach, although not quantitative, provides a broad molecular profile of the organic composition of soils similar to the approach used in the studies mentioned below. As well as reporting data for the dominant individual moieties, we grouped compounds into five chemical

classes based on their origin (Saiz-Jimenez 1994; Stankiewicz et al. 1997; Nierop et al. 2001; Gleixner et al. 2002; de Alcantara et al. 2004; Buurman et al. 2005; Wickland and Neff 2007): polysaccharides (e.g., furfural; furfural, 5-methyl), lignin derivatives (e.g., benzene, 1,2-dimethoxy; phenol, 2-methoxy; phenol, 2-methoxy-4-vinyl-), N bearing compounds (e.g., pyrrole, pyridine); lipids (e.g., n-dodecane and n-eicosane), and unknown compounds.

Enzyme analyses

A frozen subsample of each soil fraction was thawed and assayed for the activities of four enzymes: β-1,4-glucosidase (BG, EC 3.2.1.21), β-1,4-*N*-acetylglucosaminidase (NAG, EC 3.2.1.14), phenol oxidase (POX, EC 1.10.3.2), and peroxidase (PER, EC 1.11.1.7), following microplate protocols described in other work (Saiya-Cork et al. 2002; Sinsabaugh et al. 2005; Grandy et al. 2007). Maximum reaction rates (V_{max}) and half-saturation constants (K_m) (at 20°C, pH 5) were estimated for each enzyme by measuring potential activity at multiple substrate concentrations. For BG and NAG, activity was measured using fluorigenic methylumbelliferyl substrates at concentrations of 2, 5, 10, 15, 20, 25, 30 and 40 μM. For POX and PER, activity was measured colorimetrically using *L*-3,4-dihydroxyphenylalanine at concentrations of 0.25, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0 mM. K_m was calculated by linear regression using Eadie–Hofstee transformation of the Michaelis–Menten equation: $V = -K_m\ V/S + V_{max}$, where V is reaction rate and S is the substrate concentration.

Data analysis

Field sites were arranged as a factorial combination of N deposition treatment (0 vs. 80 kg N ha^{-1} $year^{-1}$) and ecosystem (BOWO and SMBW) with three replicates. Soil size classes were not independent of one another, and to account for correlations between fractions, we used a repeated measures (size fraction: >250 μm, 63–250 μm and <63 μm) factorial to analyze the effects of size fraction, N deposition, ecosystem, and their interaction. We tested for differences among whole soil samples separately from fractions using a two-way ANOVA to test the effects of simulated N deposition, ecosystem, and their interaction. Where there were significant effects, a Tukey's HSD test was used as a post hoc comparison to determine differences among individual treatment means. Because of the complexity of the SOM chemical data, we also used principal component analysis (PCA) to evaluate patterns of SOM response among treatments and size classes.

Results

SOM concentration and distribution

We found no effects ($P < 0.05$) or trends ($P < 0.1$) indicating that N deposition treatment, ecosystem, or their interaction altered soil C concentrations, which averaged 16.2 ± 1.2 g kg^{-1} when averaged across sites. Ecosystem type did affect total soil N concentrations (SMBW: 1.13 ± 0.47, BOWO: 0.58 ± 0.08 g kg^{-1}). While we found no significant differences in whole soil organic C between ecosystems or N deposition treatments, there were significant differences among size fractions. The <63 μm fraction was 10% or less of the whole-soil mass in all soil samples. However, the total C concentration of this fraction was 6- to 14-fold greater than that in the >250 μm fraction and at least double that of the 63–250 μm fraction, resulting in similar contributions from the >250 and <63 μm fractions to C content on a whole soil basis (Table 1).

SOM composition

We analyzed the organic chemistry of 48 samples and identified a total of 186 compounds as well as an additional 59 unidentified compounds. Most compounds were present in very low abundance (<1%) and in only a limited number of samples. Consistent with other studies using pyrolysis gas chromatography/mass spectrometry (e.g., Wickland and Neff 2007), the dominant compounds in soil organic matter were derived from polysaccharides, lignin, or unknown sources, but there was one N-containing compound (pyrrole; Table 1, supplemental).

PCA of the distribution of compounds by chemical classes suggests soils in the BOWO ecosystem contained more polysaccharides and less lignin and N-bearing compounds than soils in the SMBW ecosystem (Fig. 1). PCA of the distribution of

Table 1 C and N concentration and distribution among ecosystems, simulated N deposition rates, and soil fractions

Treatment[a]	C concentration (%)	N concentration (%)	Mass proportion	C content (mg g soil^{-1})	N content (mg g soil^{-1})
Black oak-white oak					
Ambient Nitrogen					
>250	0.80(0.17)	0.03(0.00)	0.84(0.02)	6.56(1.23)	0.18(0.03)
63–250	1.08(0.15)	0.05(0.01)	0.13(0.02)	1.37(0.36)	0.05(0.01)
<63	11.0(0.57)	0.54(0.09)	0.04(0.00)	4.41(0.60)	0.22(0.04)
Added Nitrogen					
>250	1.10(0.31)	0.03(0.01)	0.83(0.02)	8.93(2.27)	0.24(0.07)
63–250	1.41(0.21)	0.05(0.01)	0.12(0.02)	1.66(0.20)	0.05(0.01)
<63	10.8(4.35)	0.44(0.18)	0.06(0.01)	6.36(2.75)	0.26(0.12)
Sugar maple-basswood					
Ambient Nitrogen					
>250	0.93(0.30)	0.05(0.01)	0.72(0.07)	7.05(2.97)	0.36(0.12)
63–250	4.02(3.21)	0.29(0.24)	0.19(0.06)	3.94(2.02)	0.27(0.15)
<63	8.77(1.06)	0.65(0.10)	0.10(0.01)	8.19(1.03)	0.59(0.06)
Added Nitrogen					
>250	1.53(0.78)	0.09(0.06)	0.73(0.06)	10.5(4.64)	0.60(0.36)
63–250	2.05(0.97)	0.13(0.07)	0.18(0.05)	2.67(0.57)	0.17(0.04)
<63	9.52(2.60)	0.58(0.31)	0.10(0.01)	9.31(3.67)	0.59(0.38)
ANOVA *F*-tests[b]	*S*	*S*	*S*, *E* × *S*	*E*, *S*	*E*

Numbers in parentheses are standard errors

[a] Treatments are organized by ecosystem, N treatment (Ambient Nitrogen = no added N and Added Nitrogen = 80 kg N ha^{-1} year^{-1}) and soil size classes (>250 μm, 63–250 μm, and <63 μm)

[b] Significant analysis of variance results at ($P < 0.05$) for ecosystem (*E*), nitrogen (N), size fraction (*S*) or their interaction

compounds by soil size fraction shows differences in chemistry among fractions. The <63 μm fraction generally contained more polysaccharides, N-bearing compounds, and other compounds while the >250 μm fraction contained more lignin derivatives. The chemical structure of the 63–250 μm fraction was generally intermediate, containing a lower abundance of lignin than the >250 μm fraction and lower polysaccharide abundance than the <63 μm fraction.

The effects of N deposition in whole soils and soil fractions are seen using both PCA (Fig. 1) and analysis of changes in specific chemical compounds. PCA shows that in four of the six comparisons looking at the effects of N deposition within fractions, there were declines in polysaccharide abundance and increases in the abundance of lignin derivatives in the simulated N deposition treatment (Fig. 1). Analysis of specific compounds indicates that there was a significant ecosystem by N interaction ($P < 0.01$) for the relative abundance of furfural in whole soils (Fig. 2). In the BOWO ecosystem, there was more than 2.5-fold greater whole-soil furfural abundance in the experimental N deposition treatment, relative to the ambient treatment. In contrast, whole-soil furfural abundance in the SMBW ecosystem was more than three-fold greater in the ambient treatment relative to the simulated N deposition treatment.

We also found a significant three-way interaction among ecosystem, N deposition treatment and soil fraction for lignin/polysaccharide ratios (Fig. 3). In the >250 μm fraction of SMBW soil, the lignin/polysaccharide ratios were significantly greater in the experimental N deposition treatment (3.29 vs. 0.86). These effects were not observed in other size classes or in the BOWO ecosystem. There was also a significant particle size by N treatment interaction for the ratio of lignin derivatives to N-bearing compounds (Fig. 4). Simulated N deposition increased this ratio from 3.12 to 14.7 in the 63–250 μm fraction

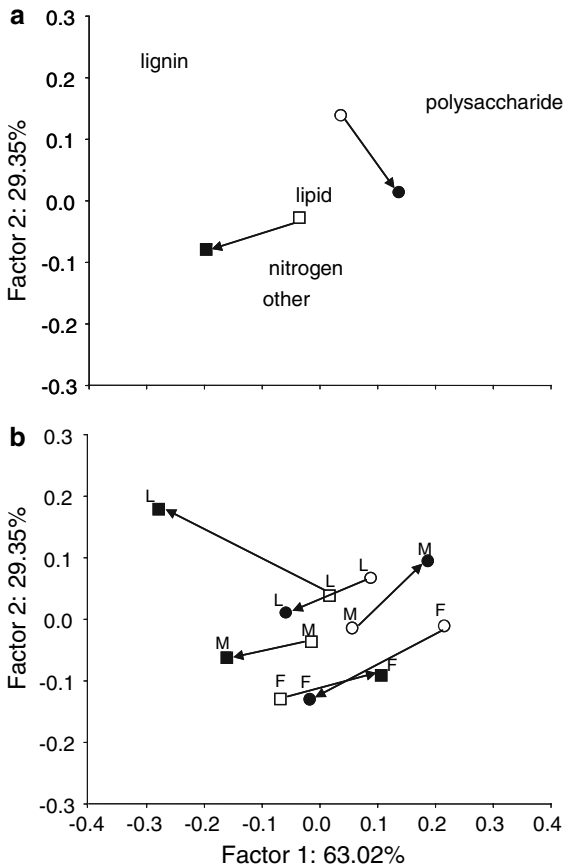

Fig. 1 Principal components analysis (*PCA*) of the distribution of chemical groups in whole soils (**a**) or soil size fractions (**b**). *Squares* indicate SMBW ecosystems and *circles* BOWO ecosystems; *shading* indicates simulated atmospheric N deposition. *Letters* indicate size fractions: *L* large (>250 μm); *M* medium (63–250 μm); and *S* small (<63 μm). *Arrows* are drawn between N treatments within an ecosystem and size class. Contributing variables are designated by their factor scores

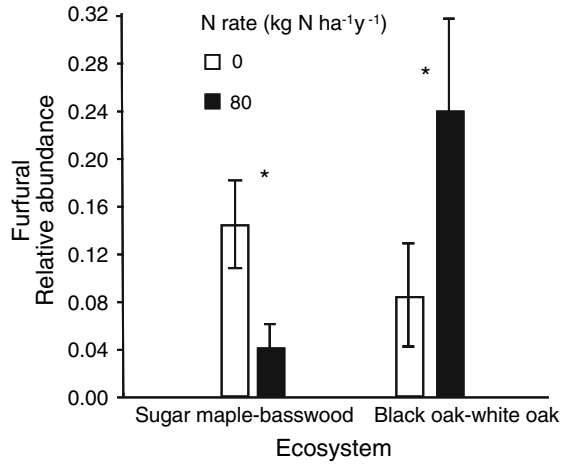

Fig. 2 Simulated atmospheric N deposition effects on the relative abundance of furfural in whole soils from SMBW and BOWO ecosystems. '*' Indicates significant differences between N treatments using Tukey's HSD post hoc comparison. *Bars* are means ± SE

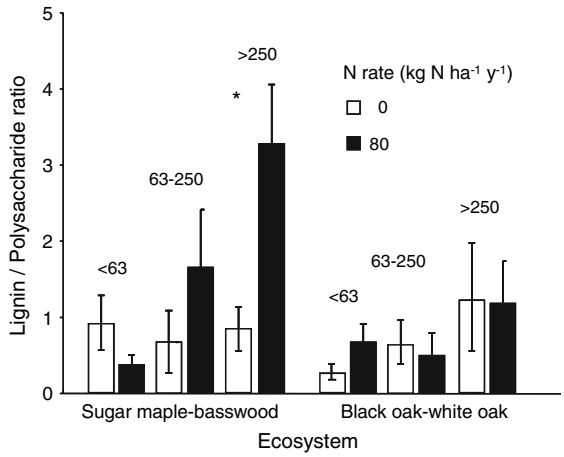

Fig. 3 Simulated atmospheric N deposition effects on the ratio of total lignin derivatives to polysaccharides across ecosystems and soil size fractions. '*' Indicates significant N deposition effects using Tukey's HSD post hoc comparison. *Bars* are means ± SE

and from 5.23 to 14.4 in the >250 μm fraction. There were no effects of simulated N deposition on the ratio of lignin derivatives to N-bearing compounds in the <63 μm fraction.

Enzyme kinetics and relationships to SOM

Extracellular enzyme activities ranged widely across soil fractions with V_{max} values for BG from 8–640 nmol h^{-1} g $soil^{-1}$, NAG from 17 to 260 nmol h^{-1} g $soil^{-1}$, POX from 89 to 2,600 nmol h^{-1} g $soil^{-1}$, and PER from 230 to 18,000 nmol h^{-1} g $soil^{-1}$ (Table 2). In general, EEA was proportional to organic carbon content (r^2 values for linear regressions of V_{max} vs. %C: BG 0.44, NAG 0.82, POX 0.91, PER 0.98). Half-saturation constants (K_m) varied more narrowly, ranging from 20 to 120 nM for BG and from 73 to 265 nM for NAG, and were not significantly correlated with organic C content. POX and PER activities generally increased in relation to substrate concentration but the data did not fit the Michaelis–Menten model, so we were unable to calculate half-saturation values.

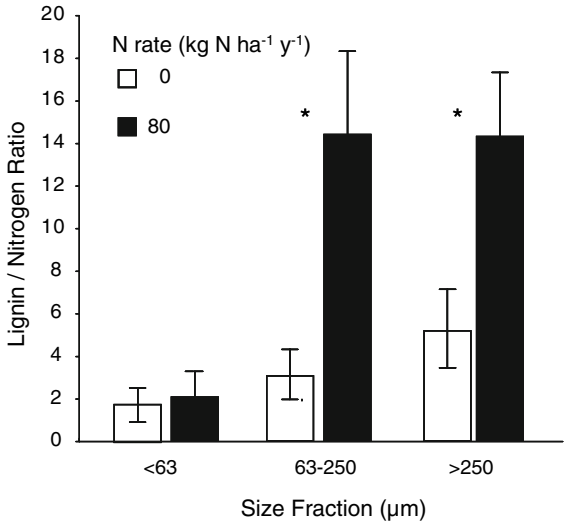

Fig. 4 Simulated atmospheric N deposition effects on the ratio of total lignin derivatives to N-containing compounds across soil size fractions. '*' Indicates significant N deposition effects using Tukey's HSD post hoc comparison. *Bars* are means ± SE

Simulated N deposition did not have significant effects on enzyme activity but did result in a trend ($P < 0.1$) toward lower POX activities and higher K_m for BG (Table 2) in N-enriched sites (Table 2). There were significant ecosystem by soil fraction interactions for BG and NAG activity and significant size fraction effects for POX and PER. K_m values exhibited significant ecosystem by soil fraction interactions for BG and significant fraction effects for NAG.

There were 9 significant correlations between enzyme activities and SOM components in the >250 μm fraction, 14 in the 63–250 μm fraction, 5 in the <63 μm fraction, and 4 in the whole soil (Table 3). There were consistent and strong correlations between oxidative enzymes and phenolic compounds, especially in the particulate soil fractions >63 μm. Peroxidase was significantly negatively correlated with the total abundance of lignin derivatives and POX activity was negatively correlated with phenol, 2-methoxy-4-(1-propenyl) in the >250 μm fraction. Both PER and POX activities were positively correlated with toluene in the >250 μm fraction. In the 63–250 μm fraction, there were positive relationships between the pyrroles, toluene, and the oxidative enzymes and negative relationships between the oxidative enzymes and vanillin.

Pyrrole, an aromatic N-containing compound, was closely tied to EEA. In the >250 μm fraction pyrrole was correlated with BG (0.81) and NAG (0.64). In the 63–250 μm fraction pyrrole was correlated with POX (0.72), PER (0.82), and BG (0.82). Averaged across all soil fractions, there was a negative relationship between the total abundance of lignin derivatives and the potential activities of POX and PER.

Discussion

Sinsabaugh et al. (2004) argued that our understanding of SOM chemistry limits our ability to predict how soil C turnover and other ecosystem processes respond to N enrichment. Our study addresses this limitation and is the first to find that N deposition effects on SOM chemistry vary across ecosystems and soil size fractions. Specifically, we found N deposition altered: (1) whole soil furfural abundance but that the effects varied in SMBW and BOWO ecosystems; (2) the ratio of lignin derivatives to polysaccharides but that the effects varied by ecosystems and size fractions; and (3) the ratio of lignin derivatives to N-bearing compounds but that the effects varied by soil size fraction. These changes in SOM chemistry in whole soils and in soil size fractions appear to be linked to long-term changes in enzyme activities following N deposition. We further show that N deposition has particularly strong effects on the chemistry of organic matter in particulate fractions, where enzyme-SOM interactions are not attenuated by silt and clay surfaces.

We found that nitrogen deposition significantly decreased furfural relative abundance in the SMBW ecosystem and increased furfural in the BOWO ecosystem. These results indicate that in response to N deposition furfural, the dominant pyrolysis product of polysaccharides, is declining relative to other compounds in the SMBW ecosystem but increasing in the BOWO ecosystem. Consistent with our results, Neff et al. (2002) found that experimental N deposition (100 kg N ha^{-1} $year^{-1}$) in an alpine dry meadow decreased two pyrolysis products of polysaccharides (5-methyl-2-furanone and 2-hydroxy-3-methyl-2-cyclopentenone) by 91%. Although we did not detect changes in glycanolytic enzyme activities, SOM chemistry reflects historical as well as

Table 2 Ecosystem, simulated N deposition rate, and soil fraction effects on enzyme activities and half saturation constants (K_m)

Treatment[a]	Phenol oxidase activity (nmol h^{-1} g^{-1})	Peroxidase activity (nmol h^{-1} g^{-1})	β-1,4-glucosidase		β-1,4-*N*-acetyl-glucosaminidase	
			Activity (nmol h^{-1} g^{-1})	K_m (nmol)	Activity (nmol h^{-1} g^{-1})	K_m (nmol)
Black oak–white oak						
Ambient Nitrogen						
>250	123(63.9)	367(18.6)	8.17(1.60)	19.7(3.79)	25.0(7.17)	156(54.6)
63–250	420(126)	1,580(234)	20.9(2.28)	36.7(3.83)	88.1(23.9)	266(74.6)
<63	2,330(146)	17,800(1,980)	140(19.0)	47.1(10.7)	264(77.0)	184(45.8)
Added Nitrogen						
>250	128(61.6)	343(144)	11.9(4.26)	34.7(0.84)	39.1(25.5)	73.3(25.4)
63–250	234(17.6)	1,200(56.9)	23.8(2.62)	48.7(13.2)	76.2(10.3)	230(13.1)
<63	1,930(261)	16,400(2,240)	130(14.2)	39.2(4.12)	168(48.6)	169(25.8)
Sugar maple-basswood						
Ambient Nitrogen						
>250	158(45.4)	423(115)	48.7(13.9)	38.0(1.05)	141(91.4)	155(28.9)
63–250	596(220)	4,340(2,350)	257(177)	54.0(14.8)	39.2(7.80)	175(15.8)
<63	2,570(140)	13,700(4,230)	562(134)	90.2(7.87)	237(60.1)	156(20.1)
Added Nitrogen						
>250	88.7(52.8)	230(23.1)	34.9(6.59)	43.0(5.46)	95.5(55.3)	122(41.4)
63–250	425(177)	2,660(988)	206(109)	68.1(21.2)	17.2(5.50)	203(11.9)
<63	2,210(118)	12,200(4,630)	637(114)	120(17.8)	171(51.8)	174(27.0)
ANOVA *F*-Tests[b]	*N*(0.1), *S*	*S*	*E*, *S*, *E* × *S*	*E*, *N*(0.1), *E* × *S*	*S*, *E* × *S*	*S*

Numbers in parentheses are standard errors

[a] Treatments are organized by ecosystem, N treatment (Ambient Nitrogen = no added N and Added Nitrogen = 80 kg N ha^{-1} $year^{-1}$) and soil size classes (>250 μm, 63–250 μm, and <63 μm)

[b] Significant analysis of variance results at ($P < 0.05$) for ecosystem (*E*), nitrogen (N), size fraction (*S*) or their interaction. Results suggesting a trend ($P < 0.1$) are labeled as such

contemporary activities, and previous studies at these sites have shown that glycanolytic activities were increased by simulated N deposition in the SMBW ecosystem. Increases in cellulase activity and decreases in oxidative activity are, in fact, predictable responses to chronic N deposition in temperate hardwood forests, but the magnitude of response is temporally variable and ecosystem-specific (e.g., DeForest et al. 2004; Gallo et al. 2004; Waldrop et al. 2004b; Blackwood et al. 2007).

Previous research has suggested that N deposition will accelerate decomposition in ecosystems in which the plant litter has a relatively high concentration of polysaccharides, but inhibit decomposition in ecosystems where litter contains high concentrations of lignin derivatives and secondary compounds (Waldrop et al. 2004b; Knorr et al. 2005). The different furfural responses to N enrichment in the SMBW and BOWO ecosystems support this notion. In the SMBW ecosystem, litter has a relatively low concentration of lignin derivatives and decomposes rapidly (Waldrop and Firestone 2004), which likely explains the decline in furfural abundance due to N deposition. In the BOWO ecosystem, the relatively high lignin content of litter controls decomposition (Moorhead and Sinsabaugh 2006).

Simulated N deposition also altered the abundance of total lignin derivatives relative to polysaccharides but the effects varied by ecosystem and soil size fraction. There was a significant increase in this ratio for the >250 μm fraction of the SMBW ecosystem, mostly due to the high abundance of lignin derivatives in this fraction (Fig. 1). These changes in total abundance of lignin derivatives are consistent with previously reported changes in POX and PER activities in response to experimental N deposition

Table 3 Significant correlations ($P < 0.05$) between enzymes and soil C

	Phenox	Perox	Bgluc	NAG
Soil fraction: >250 μm				
Lignin	–	−0.42	–	–
Lipid	–	–	–	–
N-bearing	–	–	0.86	–
Levoglucosenone	–	–	−0.74	–
Phenol, 2-methoxy-4-(1-propenyl)-	−0.59	–	–	–
Phenol, 2-methoxy-4-methyl-(Methylguaiacol)	–	–	–	0.73
Phenol, 2-methoxy-4-vinyl-(Vinylguaiacol)	–	–	–	–
Pyrrole	–	–	0.81	0.64
Toluene	0.66	0.71	–	–
Soil fraction: 63–250 μm				
N-Bearing	–	0.72	0.76	–
Furan, 3-methyl-	–	0.78	0.70	–
Levoglucosenone	–	–	−0.58	–
Pyrrole	0.72	0.82	0.82	–
Toluene	0.64	0.71	0.66	–
Vanillin	−0.69	−0.53	−0.57	–
Soil fraction: <63 μm				
N-Bearing	–	–	0.74	–
Other	–	–	–	–
Polysaccharide	–	0.60	–	–
Phenol, 2,6-dimethoxy-(Syringol)	–	−0.66	–	–
Phenol, 2-methoxy-4-(1-propenyl)-	–	−0.63	–	–
Vanillin	−0.63	–	–	–
Soil fraction: whole soil				
N-bearing	–	–	0.69	–
Polysaccharide	–	–	−0.62	–
Phenol, 2,6-dimethoxy-(Syringol)	–	–	0.86	–
Phenol, 2-methoxy-4-(1-propenyl)-	–	–	0.58	–
All soil fractions				
Lignin	−0.42	−0.42	−0.29	–
N-bearing	0.45	–	0.66	–
Phenol, 2-methoxy-	−0.30	–	–	–
Phenol, 2-methoxy-4-(1-propenyl)-	–	−0.35	–	−0.30
Phenol, 2-methoxy-4-methyl-(Methylguaiacol)	−0.38	−0.32	–	–
Phenol, 2-methoxy-4-vinyl-(Vinylguaiacol)	−0.35	−0.38	−0.35	–
Pyrrole	–	–	0.50	–
Vanillin	−0.38	−0.31	−0.31	−0.30

(Gallo et al. 2004; DeForest et al. 2004; Waldrop and Firestone 2004) and corroborate research at other sites linking declines in oxidative activity with changes in the decomposition of lignin-derived C (Saiya-Cork et al. 2002; Sinsabaugh et al. 2002; Smemo et al. 2007).

Simulated N deposition changed the ratio of lignin derivatives to N-containing compounds, in addition to changing furfural abundance and the ratio of lignin to polysaccharides. Averaged across the SMBW and BOWO ecosystems, there were significant increases in the ratio of lignin derivatives to N-containing

compounds in the particulate SOM classes (63–250 and >250 μm classes). The decline in N relative to lignin derivatives is, in some respects, surprising considering the high rates of N applied and the potential for increased plant N uptake; however, lignin derivatives increased in the >250 μm fraction of both ecosystems and in the 63–250 μm fraction of the SMBW ecosystem (Fig. 1). Although lignin derivatives did not increase in 63–250 μm fraction of the BOWO ecosystem, N abundance tended to decrease (Fig. 1).

Grandy and Neff (2008) proposed that changes in SOM chemistry as a result of altered microbial processes should be more apparent in coarse than in fine soil fractions. Our results provide support for this hypothesis as increases in the ratio of lignin derivatives to polysaccharides were detectable only in the >250 μm fraction and the ratio of lignin derivatives to N-containing compounds increased only in the 63–250 and >250 μm classes. This hypothesis is also supported by our finding that enzyme activities and SOM chemistry were more consistently correlated in the coarse fractions >63 μm than in the fine fractions (Table 3). In the San Juan Mountains of Colorado, Grandy et al. (2007) similarly found that the chemistry of the particulate fraction correlated more consistently with enzyme activity than did the composition of the fine fraction. These results indicate that although C concentrations and enzyme activities are high in mineral-associated fractions, C cycling and microbial processes may be disconnected. This would occur if sorption or aggregation is stabilizing enzymes and SOM but limiting their availability to freely interact (Kandeler et al. 1999; Sessitsch et al. 2001). While several authors have found that enzyme potentials are highest in the fine fractions in the lab, this may not reflect field conditions where the physical properties of silt and clay may effectively suppress the activity of enzymes (Stemmer et al. 1999; Allison and Jastrow 2006).

N deposition and SOM chemistry: a conceptual model

Our study shows that changes in enzymes translate into changes in SOM chemistry in different size classes following simulated N deposition. Here we use these results to build on previous experimental results (e.g., Sinsabaugh et al. 2005; Grandy and Neff 2008) and hypotheses (e.g., Fog 1988) to develop a new conceptual model that provides a more synthetic and mechanistic explanation of SOM responses to N deposition (Fig. 5). Two observations form the basis for this model: (1) enzyme-SOM interactions and the effects of N deposition are strongest in particulate fractions; and (2) changes in microbial activity and SOM chemistry in coarse particulate fractions may have 'downstream' effects that result in changes to the SOM chemistry of fine mineral fractions that tend to be the inverse of the effects observed in the coarse fractions. We propose that following chronic N deposition, the least degraded organic matter, which is contained in the litter and sand-sized pools, experiences increases in the relative abundance of lignin derivatives due to declines in oxidative enzyme activities. This results in a bottleneck that slows the rates at which lignin derivatives are transferred to mineral-associated pools over time and reduces the abundance of lignin derivatives in fine fractions.

In contrast, polysaccharide abundance declines in the coarse fraction and increases in the fine fraction due to accelerated glycanolytic enzyme activity. One of the striking elements of this model is that changes in enzyme production and activity have the strongest direct effect on SOM in particulate fractions but lead to indirect effects on SOM chemistry in silt- and clay-associated fractions. In silt and clay associated fractions, enzyme immobilization and protection by mineral particles limits their responses to N deposition and their subsequent effects on SOM chemistry. This finding may partly explain some of the variation in reported whole soil enzyme responses to N deposition.

Our hypotheses have interesting implications for the effects of N deposition on SOM dynamics in soils with different texture and litter quality, in which N deposition effects have proven highly variable (Wang and Fernandez 1999; Parker et al. 2001; Waldrop et al. 2004b). In coarse-textured soils receiving plant litter with low lignocellulose concentrations, accelerated decomposition of sand-sized organic matter in response to N deposition could rapidly deplete soil C concentrations because there is little capacity for mineral-stabilization of microbial polysaccharides and other by-products of decomposition. In coarse-textured soils receiving plant litter with high lignocellulose concentrations, we would expect an increase in organic matter concentrations. In these soils, mineral

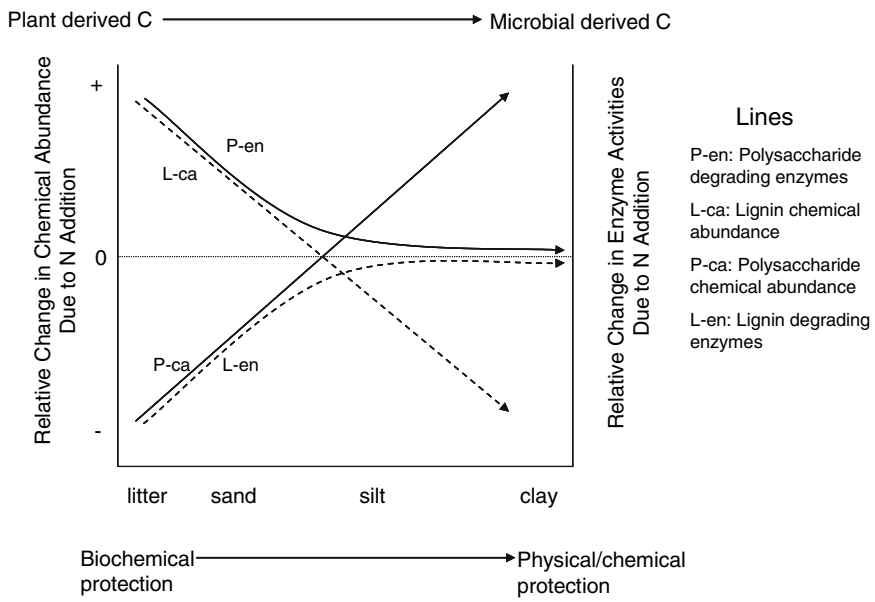

Fig. 5 Hypothesized relative changes in soil organic matter chemistry and the contribution of enzymes to those changes in different soil size classes due to N deposition. Changes are relative so the line at *zero* indicates either no effect of N enrichment on SOM chemistry (*left* *y*-axis) or no direct contribution by changes in enzyme activities to SOM chemical changes (*right* *y*-axis). Changes in enzyme activities have the strongest direct effect on SOM chemistry in particulate fractions but these effects may translate into 'downstream' changes in the chemistry of silt- and clay-associated SOM pools

stabilization of organic C would be less important relative to the biochemical protection provided by lignin, which would be enhanced due to suppressed oxidative enzyme activity. In finer textured soils receiving plant inputs with low lignocellulose concentrations, the acceleration of coarse-fraction polysaccharide decomposition may be offset to an extent by C stabilization on minerals resulting in little change in total C. Atmospheric N deposition in fine textured soils receiving plant litter with high lignocellulose concentrations will decrease C stocks, but in this case the effects may take decades to measure. This would be due to reduced lignin turnover that results in an accumulation of unprotected, particulate organic matter and reduced production of mineral-stabilized microbial byproducts.

Summary and conclusions

Previous studies have demonstrated that elevated atmospheric N deposition results in changes in extracellular enzyme activities and total soil C. This study is the first study to use chemical approaches (c.f. Gallo et al. 2004) combined with soil fractionation (c.f. Neff et al. 2002) to understand the mechanisms linking these changes. Our results provide support for the hypothesis that carbohydrate and lignin breakdown is decoupled by increased N availability (Sinsabaugh et al. 2004) but that specific effects vary depending on litter biochemistry. We further demonstrate that SOM chemical responses to N deposition vary by soil size class.

Evidence includes the following responses to N deposition: (1) decreases in whole soil furfural abundance in the sugar maple–basswood ecosystem but increases in the black oak–white oak ecosystem; (2) increases in the ratio of lignin derivatives to polysaccharides in the >250 μm fraction of the sugar maple-basswood ecosystem but not in other size fractions or in the black oak–white oak ecosystem; and (3) increases in lignin to N ratios in the 63–250 and >250 μm fractions in both ecosystems. These changes in organic matter chemistry are related to changes in enzyme activity and are modified by the composition of litter inputs and the properties of specific soil size fractions. Efforts to predict the effects of enhanced N availability on soil organic

matter need to consider the effects of organic matter chemical changes.

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