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Chronic N deposition does not apparently alter the biochemical composition of forest floor and soil organic matter

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ABSTRACT

Future rates of atmospheric N deposition have the potential to slow litter decay and increase the accumulation of soil organic matter by repressing the activity of lignolytic soil microorganisms. We investigated the relationship between soil biochemical characteristics and enzymatic responses in a series of sugar maple (*Acer saccharum*)-dominated forests that have been subjected to 16 yrs of chronic N deposition (ambient + 3 g NO_3^-–N m^{-2} yr^{-1}), in which litter decay has slowed and soil organic matter has accumulated in sandy spodosols. Cupric-oxide-extractable lignin-derived phenols were quantified to determine the presence, source, and relative oxidation state of lignin-like compounds under ambient and experimental N deposition. Pools of respired C and mineralized N, along with rate constants for these processes, were used to quantify biochemically labile substrate pools during a 16-week laboratory incubation. Extracellular enzymes mediating cellulose and lignin metabolism also were measured under ambient and experimental N deposition, and these values were compared with proxies for the relative oxidation of lignin in forest floor and surface mineral soil. Chronic N deposition had no influence on the pools or rate constants for respired C and mineralized N. Moreover, neither the total amount of extractable lignin (forest floor, $P = 0.260$; mineral soil, $P = 0.479$), nor the relative degree of lignin oxidation in the forest floor or mineral soil (forest floor $P = 0.680$; mineral soil $P = 0.934$) was influenced by experimental N deposition. Given their biochemical attributes, lignin-derived molecules in forest floor and mineral soil appear to originate from fine roots, rather than leaf litter. Under none of the studied circumstances was the presence or relative oxidation of lignin correlated with the activity of cellulolytic and lignolytic extracellular enzymes. Although chronic atmospheric N deposition has slowed litter decay and increased organic matter in our experiment, it had little effect on biochemical composition of lignin-derived molecules in forest floor and surface mineral soil suggesting organic matter has accumulated by other means. Moreover, the specific dynamics of lignin phenol decay is decoupled from short-term organic matter accumulation under chronic N deposition in this ecosystem.

1. Introduction

Since the start of the Industrial Revolution, fossil fuel combustion and agricultural fertilizer production have more than doubled the rate at which inert pools of nitrogen (N) are converted into biologically reactive forms such as NO_x and NH_3 (Galloway et al., 2004). Atmospheric N emissions deposited into the biosphere can cascade through a number of different reservoirs, often impacting the cycling and storage of carbon (C) in terrestrial ecosystems. In quantifying the influence of N deposition on ecosystem C storage, few studies adequately consider the mechanisms by which anthropogenic N might alter soil C storage. Soil organic matter is a major component of the global C cycle, representing the largest C pool on land (Schimel, 1995). Nonetheless, the mechanisms by which atmospheric N deposition could influence soil C cycling in different ecosystems are not well understood. For example, studies which experimentally increase the supply of N to simulate anthropogenic N deposition in terrestrial ecosystems report variable responses, indicating that N deposition might increase, decrease, or have no significant effect on rates of soil C cycling and storage (Knorr et al., 2005).

In forest ecosystems, experimental N deposition has been observed to increase the decomposition of cellulose-rich plant

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litter, as well as slow the decay of lignin-rich detritus and secondary humic compounds whose metabolism often dominate later stages of decay (Janssens et al., 2010; Knorr et al., 2005). Although lignin can be rapidly metabolized during the initial phase of decay in the presence of soluble co-metabolites, the depletion of these metabolites over time slows lignin metabolism as leaf decay progresses (Klotzbücher et al., 2011); however, such a response is not universal (Rinkes et al., 2011). Despite different rates of lignin metabolism during decay, recent analyses reveal that a decline in the extent (i.e., degree of modification) of lignin metabolism can increase organic matter accumulation under experimental N deposition (Whittinghill et al., 2012).

If higher levels of atmospheric N deposition diminish the physiological capacity of saprotrophic fungi to completely metabolize lignin as well as other compounds contained in above- and belowground litter, then such a response could increase the proportion of lignin-like compounds in soil organic matter (Grandy et al., 2008); physical interactions between lignin decay products and soil minerals may also increase their abundance (Amelung et al., 2008). In deciduous forests, the proportion of lignin, suberin, and cutin varies among plant organ as well as among tree species. For example, lignin increases from leaf petiole > root > leaf body; cutin or suberin follow a similar trend (leaf body > root > leaf petiole (Crow et al., 2009). Plant roots, in general, contain higher concentrations of lignin and suberin than leaf litter, a characteristic that contributes to the longer residence time of root-derived C in the soil (Rasse et al., 2005). Due to differences in the biochemical composition of leaf and root litter, declines in lignolytic metabolism, elicited by atmospheric N deposition, could differently influence the proportion of lignin-like molecules composing soil organic matter that originate from fine roots. For example, enzymatic responses to short-term N deposition parallel changes in organic matter biochemistry, wherein an increase in lignin derivatives contained in soil organic matter paralleled a decline in lignolytic extracellular enzyme activity (Grandy et al., 2008). Similarly, short-term enhancement of inorganic N availability increased the proportion of plant-derived, alkyl organic compounds in the mineral soil, despite an increase or no change in lignin oxidation (Feng et al., 2010). In combination, these observations suggests that greater rates of atmospheric N deposition, like those projected to occur in the near future, could increase organic matter accumulation, at least initially in soil fractions that respond rapidly to environmental change.

We hypothesized that the suppression of lignin oxidation under experimental N deposition should increase the incorporation of unmodified lignin-derived phenols into soil organic matter. We further reasoned that most of organic matter accumulating in forest floor (Oe/Oa horizon) in response to experimental N deposition would be predominantly fine root-derived, due to the low lignin content of sugar maple leaf litter. And lastly, that the accumulation of unmodified lignin-derived phenols in the forest floor should be inversely related to the activity of extracellular enzymes with lignolytic activity, especially under experimental N deposition. To test our hypotheses, we quantified biochemical characteristics of forest floor and soil organic matter, together with the activity of cellulolytic and lignolytic extracellular enzymes, in wide-spread forest ecosystem that has received experimental N deposition since 1994.

2. Methods

2.1. Site description and sample collection

Previously, we have demonstrated that experimental N deposition has increased leaf litter N concentration, with no effect on above- or belowground litter production in a series of sugar maple dominated northern hardwood forests (Fig. 1; Pregitzer et al., 2008). Moreover, lignolytic enzyme activity has decreased in the forest floor and mineral soil, concurrent with the reduced transcription of lignocellulolytic genes by ascomycete and basidiomycete fungi (Edwards et al., 2011). The above-mentioned responses occurred in concert with a decline in soil respiration (Burton et al., 2004), the increased production of phenolic DOC (Pregitzer et al., 2004; Smemo et al., 2007) and a rapid accumulation of organic matter in both forest floor and surface mineral soil (Zak et al., 2008).

The four *Acer saccharum*-dominated forest stands span a latitudinal gradient across the lower and upper peninsulas of Michigan (Fig. 1). Overstory associates include: *Quercus rubra* L., *Fraxinus americana* L., *Betula allaghaniensis* Marsh., and *Prunus serotina* Ehr. The Oi horizon is composed of sugar maple leaf litter, and the Oe horizon is penetrated by numerous sugar maple fine roots. Soils are 85–90% sand and are weakly developed Spodosols (typic Haplorthods). The forests encompass a gradient in ambient N deposition (0.68–1.17 g N m^{-2} yr^{-1}), mostly consisting of NO_3^-. Each of the four sites contains six 30-m × 30-m plots surrounded by a 10-m buffer. Three of the six plots receive ambient plus 3 g NO_3^-–N m^{-2} yr^{-1}, and the remaining three plots receive only ambient N deposition. The added NO_3^- is delivered in 6 separate application of $NaNO_3$ over the course of the growing season; the treatment also is applied to the buffer surrounding each plot.

Samples of above- and belowground litter, forest floor (Oe/Oa), and mineral soil were collected in May 2009 and again in May 2010. At each site, six randomly sampled 10-cm cores were collected in the buffer zone of each of the six plots. The six cores collected in each plot were homogenized into a single composite sample. Within each composited sample, forest floor (Oe/Oa) was then separated from mineral soil (A and E horizons) by ocular

Fig. 1. Location of four sugar maple dominated northern hardwood forest sites receiving experimental atmospheric N deposition since 1994.

examination to distinguish these horizons by organic vs. mineral content; fine roots were removed, rinsed, and air-dried. At each site, a composite sample of Oi horizon was collected from plots receiving ambient and experimental N deposition. A subsample was weighed and oven-dried to obtain soil moisture content. Oven-dried samples were ground with a ball-mill and total C and N content quantified with a Europa Scientific CN Analyzer. A second subset of samples was air-dried and ground for biochemical analyses. One gram of the field-fresh, composite forest floor and mineral soil samples was placed in a plastic 100 mL Nalgene® bottle and frozen for enzyme analysis.

2.2. Respired C and mineralized N: pools and rates

To determine readily metabolized and stable organic matter pools, we conducted a 16-week laboratory incubation of forest floor and surface mineral soil. Samples of these soil horizons were collected in 2009 and incubated in 5-cm Buchner funnels containing 2 glass fiber filters. Each funnel was filled with ~15 g of forest floor or mineral soil packed to attain field bulk density. Funnels were placed into 1-L Mason jars with lids containing septa for headspace gas sampling. Jars were incubated at 20 °C for 16 weeks in the dark.

To estimate microbial respiration during the 16-week incubation, the CO_2 concentration of headspace gas was determined at Day 3, Day 7, 1-week intervals for 1 month, and at 2-week intervals thereafter. Samples of headspace gas were analyzed using a Trace 2000 gas chromatograph equipped with a Porapak Q column with a thermal conductivity detector. The column was maintained at 55 °C during the analysis and He was used as a carrier gas.

Forest floor and mineral soil were extracted with 100 mL of 0.01 M $CaCl_2$ to determine the amount of NH_4^+–N and NO_3^-–N produced over time. Soils were initially extracted (0 days) and after 3 days and after 1, 2, 3, 4, 6, 8, 10, 12, 14 and 16 weeks. Following the extraction, we added 25 mL of minus-N nutrient solution (Stanford and Smith, 1972) to each sample, and brought the samples to field capacity (−0.03 MPa) using a vacuum pump. NH_4^+–N and NO_3^-–N were determined colorimetrically using an OI Analytical FS 3000 rapid flow analyzer. Following the extractions, incubations were left open for 1 h to equalize CO_2 concentrations with the ambient atmosphere. Respired C was expressed per unit of soil organic C, and mineralized N was likewise express per unit of soil organic N; soil organic C and N were determined prior to incubation using a Carlo Erba CN Analyzer.

2.3. Biochemical analyses

Alkaline cupric-oxide oxidation (CuO) was used to quantify and characterize lignin-derived phenols in forest floor and surface mineral soil samples collected in 2010 (*sensu* Hedges and Mann, 1979; Filley et al., 2008a). Litter and soil samples were weighed to an equivalent of approximately 4 mg of organic C. Ethyl vanillin and DL-12 hydroxystearic acid were added as internal standards following the initial alkaline reaction and prior to solvent phase extraction. Organic matter was extracted through a process of acidification, centrifugation, and separation with ethyl acetate. Lignin oxidation products were concentrated and lignin-derived phenols analyzed as trimethylsilane derivatives of vanillyl (V)-based (i.e., vanillin, acetovanillone, and vanillic acid), syringyl (S)-based (i.e., syringaldehyde, acetosyringone, and syringic acid), and cinnamyl (Ci)-based (i.e., p-hydroxycinnamic and ferulic acids) monomers using ion-extracted internal calibration curves. Structural and quantitative analysis was performed on a Shimadzu QP2010-plus gas chromatograph quadrupole mass spectrometer system.

2.4. Extracellular enzyme analyses

Extracellular enzyme assays were performed on each field-fresh sample of forest floor and surface mineral soil samples collected in May 2010 (*sensu* Saiya-Cork et al., 2002). Specifically, we measured the activities of β-1,4-glucosidase, cellobiohydrolase, xylosidase, phenol oxidase, and peroxidase; β-1,4-glucosidase hydrolyzes cellobiose into glucose, and cellobiohydrolase hydrolyzes cellulose into cellobiose. The degradation of polyphenols, such as lignin and lignin degradation products, is controlled by the activity of oxidative enzymes.

Extracellular enzyme assays were conducted in 96-well plates, allowing for 16 replicates per sample. To measure activity of cellobiohydrolase, xylosidase, and β-1,4-glucosidase, we used 200 μm methylumbellyferyl MUB-linked substrates. A 5-mm L-dihydroxyphenlyalanine (L-DOPA) substrate was used to assay phenol oxidase and peroxidase. A 3% H_2O_2 solution (25 μL) was included to assay peroxidase activity. Enzyme assays included 1 g of soil + 100 μL of 50 mM sodium acetate buffer, in addition to 50 μL of substrate. Controls included soil plus buffer, buffer plus substrate, and, for MUB substrates, soil + MUB.

Cellobiohydrolase, xylosidase, and β-1,4-glucosidase assays were incubated in the dark at 20 °C for 2 h. Afterwards, 25 μL of 0.2M NaOH was added to each well to stop the reaction and increase fluorescence. Enzyme activity was measured in a Molecular Devices *f* MAX fluorometer set at 365 nm excitation wavelength and 460 nm emission wavelength. Phenol oxidase and peroxidase assays were incubated for 24 h and rates were estimated spectrophotometrically (Saiya-Cork et al., 2002).

2.5. Statistical analyses

Accumulation curves for both microbial respiration and net N mineralization were fit to a first-order rate equation [$y = A(1 - e^{-kt})$] using a non-linear least squares regression. Model parameters A (substrate pool) and k (the first-order rate constant) were compared between sites and N deposition treatments using two-way ANOVA with a site by treatment interaction. Two-way ANOVA also was used to compare the total amount of lignin-derived phenols ((Cinnamyl (Ci) + Vanillyl (V) + Syringyl (S)), as well as the relative degree of lignin oxidation (Acid/Aldehyde; Ac/Al_v and Ac/Al_s). Differences in extracellular enzyme activity were also determined using the same ANOVA model. Regression analysis was used to examine the relationship between the total amount of lignin-derived phenols and oxidative enzyme activity. All data analysis was completed using PASW SPSS, and statistical significance was accepted at $\alpha = 0.05$.

3. Results

3.1. Pools and rates of respired C and mineralized N

The first-order rate equation accounted for most of the variation in the accumulation of CO_2 and inorganic N over time, wherein R^2 values ranged from 0.88 to 0.97. We found no effect of experimental N deposition on the pool of respired C produced during our laboratory incubation, as estimated by A ($F = 0.773$; df = 23; $P = 0.773$; Table 1). Respired C pools in forest floor (Oe/Oa horizons) also did not vary significantly among sites ($F = 1.47$; df = 23; $P = 0.260$). In mineral soil (A and E horizons), neither site nor N deposition treatment had an effect on the pool of respired C (Table 1). Moreover, first-order rate constant (k) for microbial respiration in the forest floor did not differ among sites, nor did atmospheric N deposition significantly alter k ($F = 1.31$; df = 23; $P = 0.305$; Table 2). In mineral soil, first-order rate constants varied

Table 1
Mean estimates of A (potential substrate pool) (±1 SD) derived from fitting microbial and respiration and N mineralization in forest floor and mineral soil samples to a first-order rate equation ($y = A\ (1 - e^{-kt})$. Mean estimates of A are arranged by N deposition treatment.

	Ambient N	Experimental N	P
Microbial respiration	mg CO_2–C/g SOC		
Forest floor	75.2 (±31.2)	71.4 (±34.5)	0.773
Mineral soil	83.8 (±58.9)	64.5 (±32.5)	0.304
N mineralization	mg N/g SOC		
Forest floor	14.1 (±9.53)	10.5 (±4.48)	0.243
Mineral soil	13.7 (±13.57)	11.4 (±11.36)	0.579

significantly among sites ($F = 4.47$; df = 23; $P = 0.018$), although not between ambient N and experimental N deposition (Table 2).

Mineralized N in forest floor and mineral soil failed to differ among sites and did respond to experimental N deposition. (Table 1). Furthermore, the first-order rate constant for net mineralization did not differ between N deposition treatments in either forest floor or mineral soil (Table 2). These observations indicate that experimental N deposition did not alter the pool of organic matter metabolized during our incubation, nor did it alter the rate at which the microbial community metabolized this pool. By extension, these observations suggest that experimental N deposition also has not altered the pool of relatively stable organic matter in forest floor and in these sandy mineral soils.

3.2. Soil organic matter biochemical composition

Averaged across study sites, CuO-extractable lignin (S + V + Ci) ranged from 0.85 to 6.07 mg/100 mg OC (organic C) in the forest floor and from 0.17 to 5.52 mg/100 mg OC in the mineral soil (Table 3). Significantly more lignin was recovered from forest floor samples than from mineral soil samples (forest floor = 3.26 ± 1.6 mg/100 mg OC; mineral soil = 2.21 ± 1.08 mg/100 mg OC; $F = 5.36$; df = 47; $P = 0.025$). However, in forest floor, the amount of lignin-derived phenols did not differ among sites, nor did it differ under ambient and experimental N deposition. In mineral soil, the amount of lignin-derived phenols was not influenced by experimental N deposition (Table 3), but significant variation in lignin was observed between sites ($F = 3.90$; df = 23; $P = 0.03$). The amount of Ci-based lignin-derived monomers, which tend to disappear early during lignin degradation (Filley et al., 2008b), also differed marginally between sites in the mineral soil ($F = 3.13$; df = 23; $P = 0.057$).

The ratio of acid and aldehyde lignin in syringyl and vanillyl-based monomers ($Ac/Al_{(v,s)}$), provides insight into the relative degree of lignin oxidation; elevated ratios indicate a more advanced state of oxidation (Ertel and Hedges, 1984; Kögel, 1986). Ratios were generally higher in the mineral soil; however, this trend was not significant (Table 3). Ac/Al_v and Ac/Al_s in the mineral soil displayed

Table 2
Mean estimates of k (first-order rate constant) (±1 SD) derived from fitting microbial respiration and N mineralization accumulation curves from forest floor and mineral soil samples to a first-order rate equation ($y = A\ (1 - e^{-kt})$. Mean estimates of k are arranged by N deposition treatment.

	Ambient N (week^{-1})	Experimental N (week^{-1})	P
Respired C			
Forest floor	0.043 (±0.022)	0.035 (±0.014)	0.333
Mineral soil	0.055 (±0.049)	0.046 (±0.044)	0.579
Net N mineralized			
Forest floor	0.079 (±0.017)	0.087 (±0.026)	0.284
Mineral soil	0.058 (±0.014)	0.077 (±0.055)	0.260

Table 3
Mean amount of total CuO-extractable lignin-derived phenols and cinnamyl-based lignin-derived phenols (mg/100 mg OC), and mean ratio of acid to aldehyde vanillyl and syringyl-based lignin-derived phenols (±1 SD) in forest floor and mineral soil under ambient and simulated N deposition.

	Ambient N	Simulated N	P
	mg/100 mg SOC		
Total lignin (S + V + Ci)			
Forest floor	3.53 (±1.301)	2.99 (±0.991)	0.260
Mineral soil	2.10 (±1.332)	2.34 (±0.7581)	0.479
Cinnamyl-based lignin			
Forest floor	0.440 (±0.1810)	0.393(±0.1527)	0.472
Mineral soil	0.292 (±0.1621)	0.355(±0.1056)	0.227
	Acid/Aldehyde		
Ac/Al_v			
Forest floor	0.4701 (±0.089)	0.4965 (±0.098)	0.520
Mineral soil	0.5441 (±0.083)	0.5389 (±0.085)	0.810
Ac/Al_s			
Forest floor	0.4346 (±0.088)	0.4472 (±0.113)	0.786
Mineral soil	0.5423 (±0.081)	0.5534 (±0.086)	0.876

significant variation among sites, but not in forest floor (forest floor: $F = 0.748$; df = 23; $P = 0.539$; mineral soil: $F = 6.734$; df = 23; $P = 0.005$; Table 3). However, ambient N and experimental N treatments produced no difference in Ac/Al_v or Ac/Al_s in the forest floor or mineral soil (Table 3). Ratios were generally higher under experimental N than under ambient N deposition, although again, this response was not significant. The acid to aldehyde ratio of lignin monomers in the leaf and fine root litter collected from each site varied from 0.50 to 0.60. The composition of lignin-derived phenols in both the forest floor and mineral soil was overall far more similar to that of fine roots than to that of leaf litter (Fig. 2), suggesting that the lignin contributing to soil organic matter formation was primarily root-derived.

3.3. Extracellular enzyme analyses

β-1,4 glucosidase, cellobiohydrolase, and β-xylosidase generally displayed trends of greater activity under experimental N deposition. However, under none of the studied circumstances were these trends statistically significant (Table 4). The activity of phenol oxidase in the forest floor was marginally higher under ambient N deposition than experimental N deposition ($F = 3.48$; df = 23; $P = 0.081$), although activity did not differ in mineral soil between N deposition treatments (Table 4). Peroxidase displayed significantly greater activity under ambient N deposition in the mineral soil ($F = 7.51$; df = 23; $P = 0.015$). Under no circumstances was the activity of extracellular enzymes correlated with the biochemical characteristics of the forest floor or mineral soil ($P = 0.518$).

4. Discussion

After over a decade of experimental N deposition, organic matter has accumulated in forest floor and mineral soil in our experiment (Pregitzer et al., 2008; Zak et al., 2008). This response is coincident with a reduced lignolytic extracellular enzyme activity and a corresponding decrease litter decay in forest floor and surface soil (DeForest et al., 2005; Zak et al., 2008). Some conceptions of the decay process reason that high inorganic N concentrations inhibit the ability of soil decomposers to metabolize litter containing high amounts of lignin (Carreiro et al., 2000). Our results indicate that experimental N deposition can slow decay while having little effect on the degree of lignin oxidation and the concentration lignin-

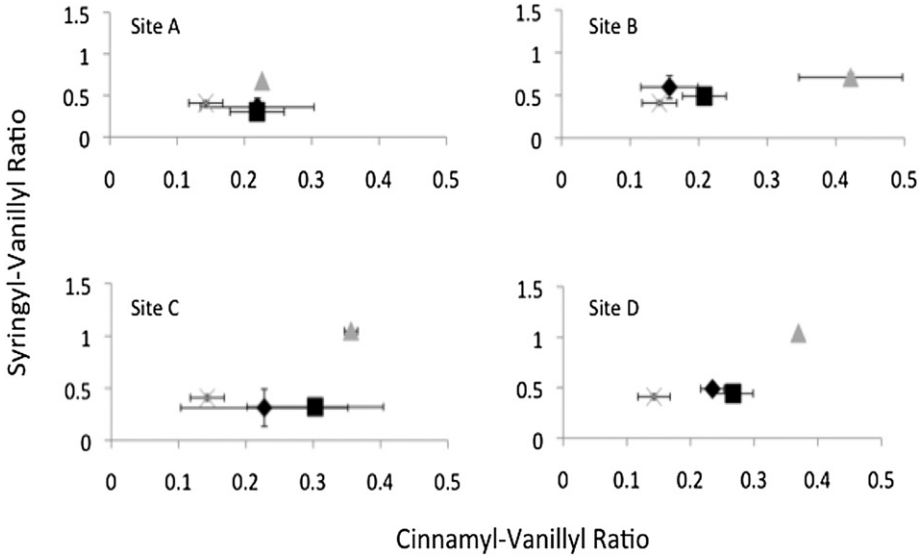

Fig. 2. The average biochemical composition, as estimated by the ratio of S/V and Ci/V lignin-derived phenols, compared across forest floor, mineral soil, leaf litter, and fine root litter in four study sites. Forest floor is represented by a black diamond, black squares represent mineral soil, grey triangles represent leaf litter, and a grey crosses represent fine roots. In each study site, the biochemical composition of leaf litter differs from fine roots, forest floor and soil organic matter. Error bars are standard deviations.

derived phenols in soil organic matter, which does not support our overall hypothesis.

For example, 17 years of experimental N deposition had little effect on the total concentration of lignin-derived phenols (S + V + Ci) recovered in forest floor and surface mineral soil. A significantly smaller amount of lignin-like compounds were present in mineral soil under ambient N and experimental N deposition, suggesting that forest floor lignin is a proportionately equivalent fraction of soil organic matter regardless of inorganic N availability and differential organic matter accumulation rates. The similar amounts of lignin-derived phenols recovered under ambient and experimental N deposition in forest floor fails to support the hypothesis that experimental N deposition is causing organic matter to accumulate through the selective preservation of lignin. In both forest floor and surface mineral soil, our laboratory incubation experiment indicates that labile pools of organic matter also have not been reduced by chronic N deposition, because neither rate constants for microbial respiration nor pools of respired C differed between N deposition treatments; the same was true for rates and pools of mineralized N. Moreover, we found no relationship between the activity of extracellular enzymes with lignocellulolytic activity and the biochemical composition of soil organic matter, at least as characterized by our methods. Recent studies suggest that lignin is not as "stable" as once thought (Thevenot et al., 2010), wherein rapid mass loss can occur during the initial phase of decay (Klotzbücher et al., 2011). In our study, regardless of when lignin decay is most rapid (Klotzbücher et al., 2011; Rinkes et al., 2011), the microbial metabolism of lignin-derived compounds has not been dramatically altered by experimental N deposition, given the insights provided by the biochemical analyses we report here.

Table 4
Mean activity (mmol h^{-1} g C^{-1}) (±1 SD) of five extracellular enzymes mediating plant litter decay in forest floor and mineral soil exposed to ambient N and experimental N deposition.

	Ambient N (mmol h^{-1} g C^{-1})	Experimental N (mmol h^{-1} g C^{-1})	*P*
β-1,4 Glucosidase			
Forest floor	6479 (±4248.2)	11,789 (±12286.4)	0.159
Mineral soil	9467 (±4685.4)	8134 (±4685.4)	0.444
Cellobiohydrolase			
Forest floor	1651 (±1331.9)	2255 (±1839.2)	0.380
Mineral soil	1485 (±1147.0)	1559 (±1417.3)	0.893
β-Xylosidase			
Forest floor	1640 (±1304.5)	2342 (±2004.6)	0.268
Mineral soil	3022 (±1952.5)	3390 (±1472.2)	0.609
Phenol oxidase			
Forest floor	13 (±12.9)	7 (±5.7)	0.081
Mineral soil	26 (±16.5)	22 (±16.1)	0.560
Peroxidase			
Forest floor	25 (±19.2)	37 (±41.1)	0.395
Mineral soil	110 (±45.30)	72 (±38.6)	0.015

The presence of an increased Ac/Al_v or Ac/Al_s in lignin-derived phenols, caused by the microbial oxidation of aldehyde to acid functional groups over the course of decomposition (Ertel and Hedges, 1984; Kögel, 1986), also did not vary between ambient and experimental N deposition. Lignin-derived phenols recovered from the forest floor moreover appeared equally modified as lignin recovered from the mineral soil. However, variation in lignin oxidation did exist between the four sugar maple forest sites. The similarity in forest floor and mineral soil might be explained by the sandy soil texture and that most lignin is not associated with mineral surfaces. In general, Ac/Al ratios increased from north to south among our study sites, suggesting that lignin is more decomposed in southern sites than in northern sites. Such a trend could be due differences in climate and microbial community composition among study sites (Eisenlord and Zak, 2010; Edwards et al., 2011). It is important to note that our approximation of degradation only detects side-chain oxidation of lignin monomers and not ring decomposition, which can be facilitated by different basidiomycete fungi (Filley, 2003). Experimental N deposition might also have altered microbial activity in a way that causes the decomposition of lignin to be undetected by CuO oxidation. This method can only quantify the oxidation of lignin, and cannot quantify the relative modification of lignin through decomposers such as brown rot fungi (Hedges et al., 1988; Filley et al., 2000).

Comparison of the lignin phenol monomer composition among sources (i.e., leaf and root) with that in forest floor and soil

suggest that lignin appears to originate more from fine-root detritus than from aboveground leaf litter. This observation supports our hypothesis that lignin remaining in the soil under ambient and experimental N deposition is primarily root-derived, although more data, such as can be obtained by tracking substituted fatty acids from cutin and suberin (Crow et al., 2009), would needed to fully substantiate this claim. Recent research suggests that fine-root litter has an overall longer resistance time in the soil, due to its biochemical composition and to greater physio-chemical protection than leaf litter (Rasse et al., 2005). Previous results from our experiment indicate that experimental N deposition did not alter the lignin concentration or the production of fine-root litter (Eikenberry and Pregitzer, *unpublished data*). Therefore, the exact mechanisms by which fine root litter is stabilized in this long-term experiment remain to be determined.

Although we quantified the recovery of lignin-derived phenols under experimental N deposition, this approach provides no insight into the accumulation of other types of organic compounds as well as the production of long-lived microbial byproducts, which may accumulate as soil organic matter (Kleber, 2010; Schmidt et al., 2011; Amelung et al., 2008). It is possible that experimental N deposition has simply shifted the saprotrophic microbial community in a way that slows decomposition without causing significant changes into the composition of the accumulating organic matter (Edwards et al., 2011). It is also possible that experimental N deposition is causing an initial acceleration of low-lignin sugar maple litter, thereby increasing the formation of stable, microbially-derived products which are contributing to organic matter accumulation that are not reflected in our biochemical analyses (Amelung et al., 2008). Further, is it plausible that the equivalent recovery of lignin-derived phenols under ambient and experimental N deposition results from the increased export of phenolic-rich DOC under experimental N deposition (Pregitzer et al., 2004; Smemo et al., 2007). Additional research on the mechanisms by which organic matter has accumulated under experimental N deposition is essential for determining the means by which this agent of global change has increased soil C storage in our experiment.

5. Conclusion

Despite the fact that several independent measures indicate a decline in lignolytic activity (i.e., lower laccase gene expression and extracellular enzyme activity; Edwards et al., 2011), we have no evidence to support the hypothesis that lignin-like molecules are accumulating in forest floor or mineral soil receiving experimental N deposition. We also found no relationship between extracellular enzyme activity and the biochemical characteristics of organic matter in these soil horizons. Because the biochemical composition of lignin-derived compounds in forest floor and surface mineral soil was more similar to fine roots than leaf litter, changes in the microbial transformation of root detritus may underlie the accumulation of organic matter under experimental N deposition. We suggest that further work is needed to elucidate how shifts in microbial composition and function, as a result of experimental N deposition, feedback to modify the preservation of a wide range of organic compounds in the forest soils causing an unusually rapid accumulation of organic matter in forest floor and surface soil.

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