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NITROGEN DEPOSITION MODIFIES SOIL CARBON STORAGE THROUGH CHANGES IN MICROBIAL ENZYMATIC ACTIVITY

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Abstract. Atmospheric nitrogen (N) deposition derived from fossil-fuel combustion, land clearing, and biomass burning is occurring over large geographical regions on nearly every continent. Greater ecosystem N availability can result in greater aboveground carbon (C) sequestration, but little is understood as to how soil C storage could be altered by N deposition. High concentrations of inorganic N accelerate the degradation of easily decomposable litter and slow the decomposition of recalcitrant litter containing large amounts of lignin. This pattern has been attributed to stimulation or repression of different sets of microbial extracellular enzymes. We hypothesized that soil C cycling in forest ecosystems with markedly different litter chemistry and decomposition rates would respond to anthropogenic N deposition in a manner consistent with the biochemical composition of the dominant vegetation. Specifically, oak-dominated ecosystems with low litter quality should gain soil C, and sugar maple ecosystems with high litter quality should lose soil C in response to high levels of N deposition (80 kg $N \cdot ha^{-1} \cdot yr^{-1}$). Consistent with this hypothesis, we observed over a three-year period a significant loss of soil C (20%) from a sugar maple-dominated ecosystem and a significant gain (10%) in soil C in an oak-dominated ecosystem, a result that appears to be mediated by the regulation of the microbial extracellular enzyme phenol oxidase. Elevated N deposition resulted in changes in soil carbon that were ecosystem specific and resulted from the divergent regulatory control of microbial extracellular enzymes by soil N availability.

Key words: carbon sequestration; forests, northern temperate; Michigan (USA); N deposition; soil enzyme activities.

INTRODUCTION

Atmospheric nitrogen (N) deposition, resulting primarily from fossil-fuel combustion, has increased N availability in forest ecosystems in the northeastern United States and Europe (Bouwman et al. 2002). Nitrogen deposition is likely to increase over the current century as the rate of fossil-fuel combustion continues to increase within industrialized countries and as land clearing and biomass burning continue in developing regions. Elevated levels of N deposition can make a small contribution to aboveground C sequestration (Nadelhoffer et al. 1999), but questions still remain concerning the effect of greater N deposition on soil C sequestration. Soil C storage is controlled in part by decomposition, which can increase or decrease following N additions to soil and litter (Melillo et al. 1982, Magill and Aber 1998, Carreiro et al. 2000, Hobbie 2000, Neff et al. 2002). These divergent trends may be explained by the opposing responses of different sets of extracellular microbial enzymes that degrade plant litter and soil organic matter (Sinsabaugh et al. 2002).

Lignin and cellulose are the two most abundant biochemical constituents of plant litter and they are decomposed by different classes of microbial extracellular enzymes. Lignin is degraded oxidatively by phenol oxidases and peroxidases. In some white-rot basidiomycetes, expression of these enzymes is down regulated by high N availability, leading to a reduction in the rates of lignin and humus degradation (Fog 1988, Hammel 1997). Cellulose is degraded hydrolytically by exoglucanases, endoglucancases, and β-glucosidases (Sinsabaugh and Liptak 1997); in natural systems, cellulose degradation is often limited by N availability (Fog 1988, Berg and Matzner 1997). Because high N availability may stimulate cellulose degradation and inhibit lignin degradation, the net result for decomposition varies with litter biochemistry (Sinsabaugh et al. 2002). Extrapolating to the ecosystem scale, we reasoned that N deposition would slow decomposition in ecosystems with highly lignified litter due to the reduction in oxidative enzyme activity and accelerate decomposition in ecosystems containing litter with low lignin content. As a result, we hypothesized that ecosystems with highly lignified litter would accrue soil C and ecosystems with litter low in lignin would lose soil C. To test our hypothesis, we initiated a field experiment to study the effect of experimental N deposition on microbial enzyme activities and soil C in three northern temperate forests that broadly differ in leaf-litter biochemistry.

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TABLE 1. Results of repeated-measures ANOVA using natural-log-transformed soil carbon concentrations with ecosystem (BOWO, SMRO, and SMBW) and treatment (control vs. N amended) as independent variables.

Source of variation	df	MS	*F*	*P*
Between subjects				
Intercept	1	198.0	843.7	0.000
Ecosystem	2	1.897	8.094	0.007
Treatment	1	0.004	0.015	0.905
Ecosystem × Treatment	2	0.206	0.880	0.442
Error	11	0.234		
Within subjects				
Time	10	0.871	11.173	0.000
Time × Ecosystem	20	0.037	2.197	0.005
Time × Treatment	10	0.008	0.494	0.891
Time × Ecosystem × Treatment	20	0.029	1.705	0.043
Error	110	0.016		

Note: Ecosystem refers to one of three forest types in northwestern Lower Michigan, USA: BOWO = black oak–white oak, SMRO = sugar maple–red oak, and SMBW = sugar maple–basswood.

METHODS

Our study took place in three upland forests ecosystems in northwestern Lower Michigan, USA, that are representative of forests throughout the Upper Lake States region. The ecosystems we studied are the black oak–white oak (BOWO), the sugar maple–red oak (SMRO), and the sugar maple–basswood (SMBW) ecosystems (Zak and Pregitzer 1990). The BOWO forest is located on glacial outwash classified as an entic haplorthod soil and the SMRO and SMBW forests are located on sandy moraine-derived soils classified as typic haplorthods. Although these forests are similar in age (range = 83–91 yr), the SMBW forest has the greatest aboveground biomass (209 ± 36 Mg/ha [mean ± 1 SE]), 15–28% greater than the aboveground biomass in SMRO and BOWO ecosystems. Leaf-litter production is greatest in the SMRO ecosystem (2.90 ± 0.55 $Mg \cdot ha^{-1} \cdot yr^{-1}$), intermediate in the SMBW ecosystem (2.36 ± 0.65 $Mg \cdot ha^{-1} \cdot yr^{-1}$), and lowest in the BOWO ecosystem (1.63 ± 0.75 $Mg \cdot ha^{-1} \cdot yr^{-1}$). Litter quality also is lowest in the BOWO ecosystem (C:N = 133), intermediate in the SMRO ecosystem (C:N = 104), and highest in the SMBW forest (C:N = 80). Soil C:N ratios were highest in the BOWO ecosystem (C:N = 23), intermediate in the SMRO ecosystem (C:N = 21), and lowest in the SMBW ecosystem (C:N = 19). The bulk density for all three ecosystem types is 0.7 Mg/m^3 (Zak and Pregitzer 1990).

There were three replicate stands (i.e., blocks) for each ecosystem that were separated by ~8 km. Within each stand we randomly selected two 15 × 30 m plots. One plot was randomly assigned to the control treatment, and to the other we added 80 kg $N \cdot ha^{-1} \cdot yr^{-1}$ as $NaNO_3$. We began N additions in April of 2001 and we delivered the experimental treatment in six increments over the growing season. Our first soil sampling occurred within a month of the first fertilizer application. Eight soil samples per plot were collected using a 2-cm diameter by 20-cm deep soil corer, then composited and frozen, on 11 occasions from May 2001 to July 2003. The litter and O-horizon material was not included in the subsequent analysis. Soil organic-matter content was measured as loss on ignition, and it was converted to soil C by using a conversion factor of 0.47. Each soil sample was assayed for β-glucosidase, N-acetyl-glucosaminidase (NAGase), phenol oxidase, and peroxidase activity using standard fluorometric and colorimetric techniques. Briefly, enzyme assays were conducted using 0.5 g soil in sodium acetate buffer (50 mmol/L, pH 5.0) using either methylumbelliferyl enzyme substrates (for β-glucosidase and N-acetyl glucosamidsase) or L-3, 4-dihydroxyphenylalanine (L-DOPA, 10 mmol/L, [Sigma-Aldrich, St. Louis, Missouri, USA]) as the substrate (for phenol oxidase and peroxidase) (Saiya-Cork et al. 2002). We used 16 analytical replicates per sample, and assays were conducted at 20°C, for up to 3–8 h, using both sample and substrate controls.

We used repeated-measures ANOVA to determine the effect of time, ecosystem type, and treatment (control and N amendment) on soil carbon and enzyme activities (Norusis 2001). We used JMP statistical software for linear regression analysis (SAS Institute 2000). We chose statistical significance to be $P < 0.10$ due to the known difficulty of observing changes in enzyme activities and soil carbon stocks over short periods of time.

RESULTS

Experimental N deposition produced changes in soil C (Table 1, Fig. 1) that became statistically significant over time. Soil carbon content was not different among the control and N-amended plots for the first two sampling dates. Thereafter, the interaction among time, ecosystem, and treatment ($F = 1.705$, $P = 0.043$), revealed that N additions increased soil C in the BOWO and SMRO ecosystems and decreased soil C in the SMBW ecosystem (Fig. 1), but this change was only

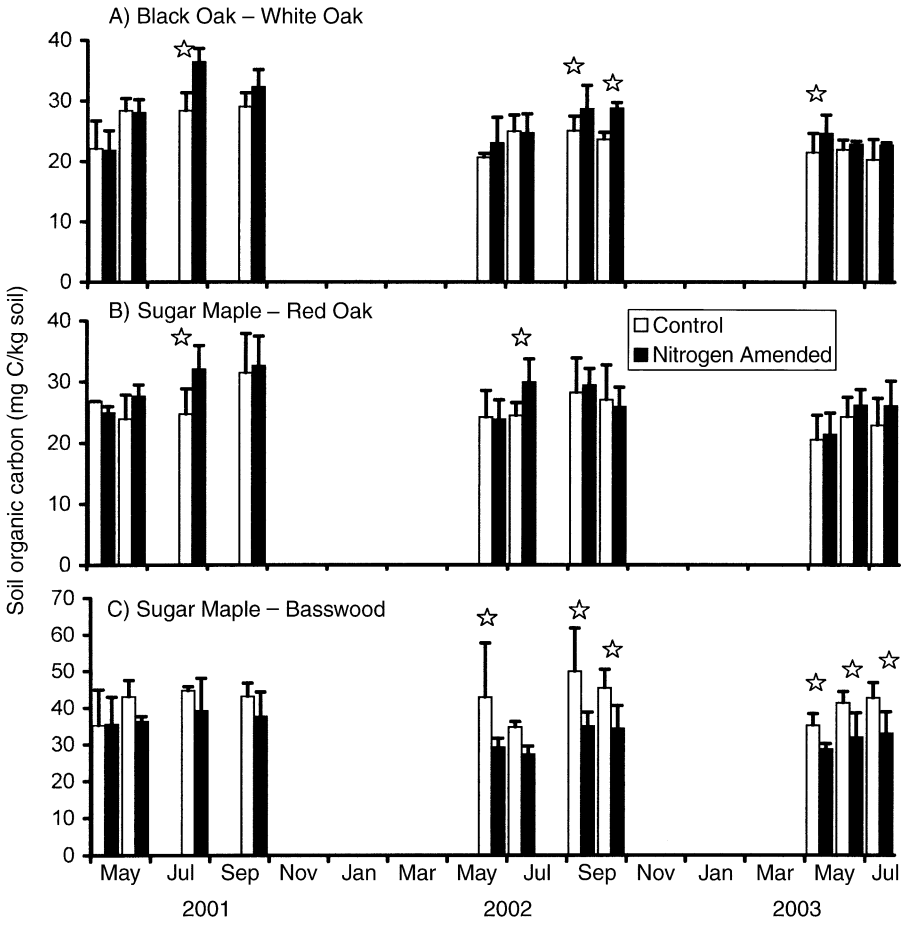

FIG. 1. Soil C in control and N-amended plots of (A) black oak–white oak (BOWO), (B) sugar maple–red oak (SMRO), and (C) sugar maple–basswood (SMBW) forest ecosystems. Soil C was measured down to 20 cm at eight intervals over a three-year period. N deposition consistently increased soil C in the BOWO ecosystem and consistently decreased soil C in the SMBW ecosystem. Changes in soil carbon in the SMRO ecosystem in response to N deposition were equivocal. Each bar represents three observations (mean and 1 SE). A star indicates significant differences ($P < 0.10$) between control and N-amended plots, calculated from a Fisher's protected least-significant difference.

significant at specific time points. Within the oak-dominated BOWO ecosystem, soil C was significantly greater in the N-deposition treatment relative to the control treatment at four time points, but all later time points tended toward greater soil C in the N-amended plots. In the SMRO ecosystem, increases in soil C tended to appear intermittently, and sometimes mean soil C was lower in the N-deposition treatment compared to the control (May and October 2002). Within the SMBW ecosystem, losses of soil C in the N-amended treatment increased over time, becoming significant in years 2 and 3 (Fig. 1). We observed a high amount of variability in soil C content over time that was likely due to variability associated with field sampling and not representative of intra-annual variability in soil C. Overall, after three years of elevated N deposition, the SMBW ecosystem lost 5.46 kg soil C/ha (a 20% decrease), whereas the BOWO ecosystem gained 1.68 kg soil C/ha (a 10% increase), and the SMRO ecosystem gained 1.26 kg soil C/ha (a 6% increase) (Fig. 1). Since we sampled the soil to 20 cm, soil C in the surface soil may be experiencing larger changes than soil C further down the soil profile. In one instance, we sampled the soils of all the plots to only 10 cm, and the observed change in soil C to 10 cm were 50–300% greater than that quantified here (Waldrop et al., *in press*).

The activity of β-glucosidase, an enzyme that degrades cellulose, was unaffected by our N-amended treatment (Fig. 2A). β-glucosidase activity was highest in the SMBW ecosystem, intermediate in the SMRO ecosystem, and lowest in the BOWO ecosystem, and this pattern was reflective of the greater quantity of soil C within the SMBW soil (Fig. 1). On the other hand, the activity of NAGase, an enzyme that degrades chitin, was reduced in the BOWO ecosystem N-amended treatments and elevated in the SMBW ecosystem N-amended treatments compared to controls (Fig. 2B; $P < 0.05$). Activities of phenol oxidase and peroxidase, two enzymes that degrade lignin, were also reduced in the BOWO ecosystem N-amended treatment and elevated

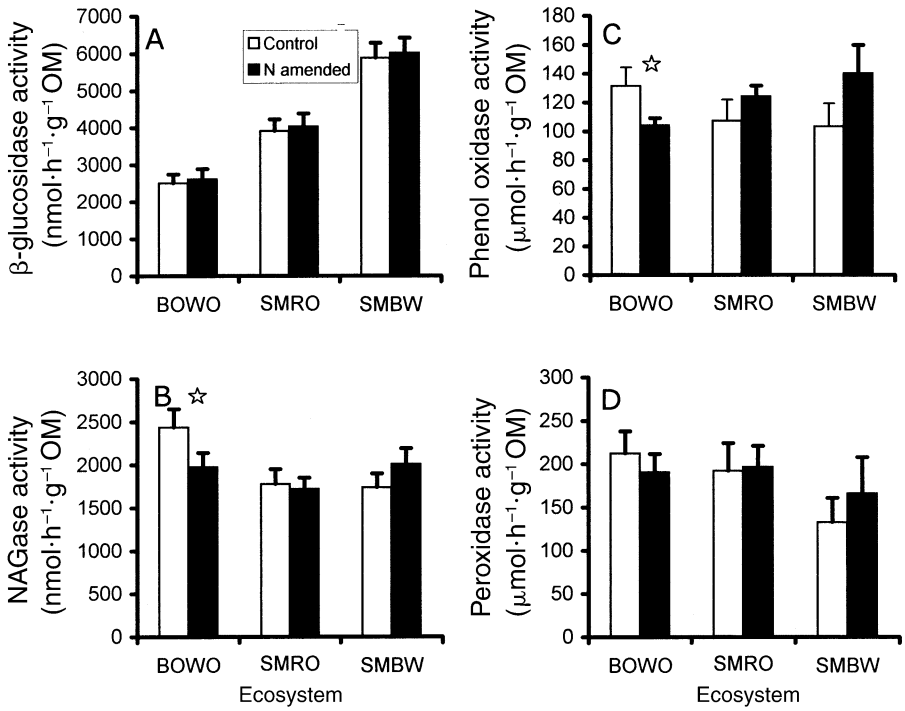

FIG. 2. Effect of N deposition on the activity of soil enzymes: (A) β-glucosidase, (B) N-acetyl-glucosaminidase (NAG), (C) phenol oxidase, and (D) peroxidase in black oak–white oak (BOWO), sugar maple–red oak (SMRO), and sugar maple–basswood (SMBW) forest ecosystems. A star indicates statistical significance at $P < 0.10$. Each bar represents 24 (A and B) or 12 (B and C) observations; the data are means and 1 SE. OM stands for organic matter.

in the SMBW ecosystem N-amended treatment relative to control (Fig. 2C and D). Only the decrease in NAGase and phenol oxidase activity in the BOWO ecosystem was statistically significant, however (Fig. 2B and C; $P < 0.10$; ecosystem × treatment interaction). We used regression analysis to determine if changes in enzyme activities could explain changes in soil C between control and N-amended plots. Changes in soil C were not explained by changes in β-glucosidase, NAGase, or peroxidase activities ($P > 0.10$; data not shown). However, we observed a significant linear relationship between a change in phenol oxidase and changes in soil C among ecosystems (Fig. 3).

DISCUSSION

Understanding the mechanisms controlling soil C storage in response to N deposition is important for accurately predicting ecosystem C sequestration. The changes in soil C storage we observed occurred over a relatively short period of time, indicating that, at least within northern temperate forests, soil C storage is very sensitive to atmospheric N deposition. Several groups of researchers have examined the effect of atmospheric N deposition (15–150 kg N·ha^{-1}·yr^{-1}) on soil C storage without finding a significant effect (Ohtonen 1994, Smolander et al. 1994, Tietema 1998, Magill et al. 2000, Neff et al. 2002), with the exception of Nohrstedt

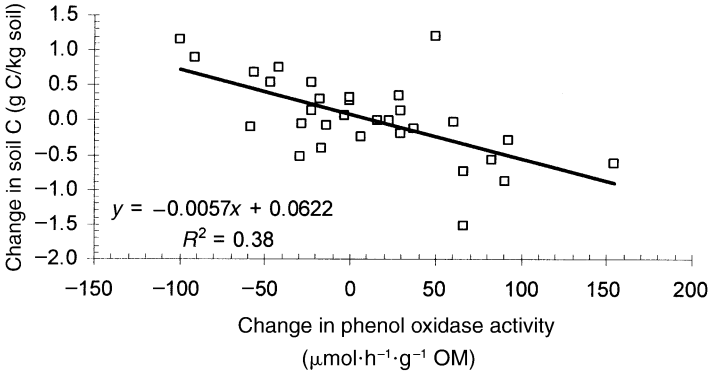

FIG. 3. Regression analysis of changes in phenol oxidase activity and changes in soil C following high levels of simulated N deposition (80 kg N·ha^{-1}·yr^{-1}). OM stands for organic matter.

et al. (1989) who found a significant increase in soil C in a pine forest 11 years after applying up to 600 kg $N \cdot ha^{-1} \cdot yr^{-1}$. We believe the ecosystems we studied are more sensitive to N deposition because of the sandy loam texture of the soils and the lack of soil structure (Zak and Pregitzer 1990). Sandy soils have lower capacity to adsorb organic matter and to protect soil C within aggregates compared to soils of finer texture (Hassink et al. 1993). Without structural protection, a large portion of soil organic matter is subject to changes in microbial activity. The data support our hypothesis that soil in ecosystems with low-quality litter and soil (BOWO) would accrue C in response to N amendment, whereas ecosystems with high-quality litter and soil (SMBW) would lose soil C. However, our hypothesized mechanism, that N would stimulate cellulosic enzyme activity and repress oxidative enzyme activity in *all* ecosystems was incorrect. Rather, our observations demonstrate that N additions reduced chitinase and oxidative enzyme activities in the BOWO ecosystem and tended to increase the activity of these enzymes in the SMBW ecosystem, but had no apparent affect on β-glucosidase activity. Our regression analysis (Fig. 3) suggests that the stimulation of oxidative enzyme activity by N in the SMBW soil may have increased the degradation rate for aromatic compounds within soil organic matter whereas reduced oxidase activity within the BOWO ecosystem may have led to lower decomposition rates and an increase in soil C storage. Carreiro et al. (2000) have also found that phenol oxidase responses, both positive and negative, were the best indicator of changes in litter decomposition rate stemming from N deposition. The significant relationship between changes in phenol oxidase activity and changes in soil carbon content across all three ecosystems provides additional support for the idea that phenol oxidase may act as an ''enzymatic latch'' controlling C storage across many ecosystem types (Freeman et al. 2001).

The N amendments may have also affected soil C storage by increasing aboveground production or improving litter quality by reducing the litter C:N ratio. However, several N-deposition studies have shown little or no increase in aboveground and belowground production or nutrient ratios of litter material in northern forests, at least for several years (Nadelhoffer et al. 1999, Zak et al. 2004.). Furthermore, changes in soil organic-matter content began to occur within the first six months following N amendments, thus there was little time for a litter-production feedback to develop. A second possible reason for changes in soil carbon content follow N amendments is an increase in N condensation reactions with phenolic compounds, thereby making organic matter less available for microbial metabolism. However, we do not believe this is an important mechanism because an increase in N condensation reactions would result in a decrease in microbial activity, whereas we observed an increase in the metabolic quotient (microbial respiration per unit microbial biomass) in all three ecosystem types following N amendments (Waldrop et al., *in press*). Thus we feel that the response of oxidative enzyme activities to N amendments is a plausible mechanism for observed alterations in soil carbon storage.

Ligninolytic oxidative enzymes are typically considered to be produced under N-limiting conditions (Fog 1988), but research also indicates that some groups of microorganisms (including some white-rot basidiomycetes) can produce phenol oxidase in response to high levels of inorganic N in soil solution (Fog 1988, Collins and Dobson 1997, Hammel 1997). Previous data from our three ecosystems indicate that the composition of the microbial community differs among the three ecosystems (Myers et al. 2001), but because the methods employed were not specific to fungal taxa we cannot make any association between fungal organisms and enzyme regulation. The importance of fungal community dynamics to soil C storage is further emphasized through the pattern of NAGase activity observed. The ecosystem-specific response in NAGase activity indicates that there is either a change in fungal litter inputs to soil, a change in the capacity of the microbial community to degrade fungal litter, or both. Therefore, it becomes paramount to understand the distribution of soil fungi, how they are associated with different ecosystem types, and how they respond to high levels of inorganic N. Our data point to one important fact: the composition of the soil microbial community and its response to N deposition may control soil C storage in temperate forests.

In summary, our study demonstrates that high levels of N deposition lead to significant and rapid changes in soil C in northern forest ecosystems, a response that was ecosystem specific. The oak-dominated BOWO forest gained soil C and the sugar-maple-dominated SMBW forest lost unprecedented quantities of soil C. Our enzymatic analysis suggests that the mechanism for the observed pattern is ecosystem-specific regulation of phenol oxidase enzyme activity by different microbial communities within these three ecosystems. This fact underscores the current lack of understanding of the important role that microbial communities, and specifically fungal communities, play in controlling soil C cycling. The impact of N deposition on forest ecosystems has traditionally focused on the N cycle, but renewed focus must be pointed at the C cycle, especially microbial mechanisms of C degradation and sequestration.

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