Ecological Applications, 18(8), 2008, pp. 2016–2027

SIMULATED ATMOSPHERIC NO_3^- DEPOSITION INCREASES SOIL ORGANIC MATTER BY SLOWING DECOMPOSITION

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Abstract. Presently, there is uncertainty regarding the degree to which anthropogenic N deposition will foster C storage in the N-limited forests of the Northern Hemisphere, ecosystems which are globally important sinks for anthropogenic CO_2. We constructed organic matter and N budgets for replicate northern hardwood stands ($n = 4$) that have received ambient (0.7–1.2 g $N \cdot m^{-2} \cdot yr^{-1}$) and experimental NO_3^- deposition (ambient plus 3 g NO_3^--$N \cdot m^{-2} \cdot yr^{-1}$) for a decade; we also traced the flow of a $^{15}NO_3^-$ pulse over a six-year period. Experimental NO_3^- deposition had no effect on organic matter or N stored in the standing forest overstory, but it did significantly increase the N concentration (+19%) and N content (+24%) of canopy leaves. In contrast, a decade of experimental NO_3^- deposition significantly increased amounts of organic matter (+12%) and N (+9%) in forest floor and mineral soil, despite no increase in detritus production. A greater forest floor (Oe/a) mass under experimental NO_3^- deposition resulted from slower decomposition, which is consistent with previously reported declines in lignolytic activity by microbial communities exposed to experimental NO_3^- deposition. Tracing $^{15}NO_3^-$ revealed that N accumulated in soil organic matter by first flowing through soil microorganisms and plants, and that the shedding of ^{15}N-labeled leaf litter enriched soil organic matter over a six-year duration. Our results demonstrate that atmospheric NO_3^- deposition exerts a direct and negative effect on microbial activity in this forest ecosystem, slowing the decomposition of aboveground litter and leading to the accumulation of forest floor and soil organic matter. To the best of our knowledge, this mechanism is not represented in the majority of simulation models predicting the influence of anthropogenic N deposition on ecosystem C storage in northern forests.

Key words: atmospheric N deposition; decomposition; ecosystem N budget; ^{15}N tracing; northern hardwood forests; SOM accumulation.

Introduction

Over the past 150 years, atmospheric N deposition has increased an order of magnitude (e.g., from 50–100 to 1500–2000 mg $N \cdot m^{-2} \cdot yr^{-1}$; Galloway et al. 2004) across large areas of the Northern Hemisphere, which could foster greater net primary productivity (NPP) in the N-limited forests of this region. Although such a mechanism could strengthen the terrestrial C sink in the Northern Hemisphere (Schimel et al. 1995), there remains substantial uncertainty regarding the extent to which anthropogenic N deposition has contributed to this response (Townsend et al. 1996, Nadelhoffer et al. 1999*a*, Magnani et al. 2007). Biogeochemical models initially suggested that atmospheric N deposition could account for an additional 0.1–2.3 Pg C annually stored in temperate and boreal forests (Schindler and Bayley 1993, Townsend et al. 1996, Holland et al. 1997). Empirical approaches also have yielded contrasting results, suggesting that atmospheric N deposition has a minor as well as substantial influence on C storage in northern forests (Nadelhoffer et al. 1999*a*, Magnani et al. 2007, Pregitzer et al. 2008). The uptake of anthropogenic N by N-limited forest trees and the resulting enhancement of NPP have, up to this point, been the primary mechanisms thought to increase ecosystem C storage (Townsend et al. 1996, Nadelhoffer et al. 1999*a*, Currie et al. 2004); however, the deposition of anthropogenic N could increase C storage in northern forests via other mechanisms.

Forest floor and soil organic matter are the least certain aspects of C storage in northern forests (Goodale et al. 2002), and there are reasons to expect that atmospheric N deposition could influence these pools by means other than greater rates of NPP and detritus production. For unknown reasons, the synthesis of lignolytic enzymes by some litter-decomposing fungi can be repressed by high levels of inorganic N (Boominathan et al. 1990, Vanderwoude et al. 1993, Worral et al. 1997), which can, in turn, slow decomposition and increase soil

Manuscript received 19 October 2007; revised 18 March 2008; accepted 11 April 2008. Corresponding Editor: A. R. Townsend.
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organic matter accumulation (Carreiro et al. 2000, Frey et al. 2004, Waldrop et al. 2004). Other empirical evidence demonstrates that the later stages of litter decomposition, which are dominated by lignin degradation, are slowed in detritus with a high initial N concentration (Berg et al. 1982, Berg and Matzner 1997, Berg and Meentemeyer 2002). If atmospheric-N deposition increases leaf-litter N and elevates inorganic N in soil solution, then it could repress lignolytic activity, slow organic matter decomposition, and increase soil C storage. To the best of our knowledge, these specific mechanisms are not considered by the majority biogeochemical model simulating the influence of atmospheric N deposition on ecosystem C storage (Townsend et al. 1996, Pepper et al. 2005, Vetter et al. 2005), and they could have a substantial influence on organic matter accumulation in the forest floor and mineral soil of forests in the Northern Hemisphere (sensu Moorhead and Sinsabaugh 2006).

Over the past decade, we have been studying a series of northern hardwood forest stands in which experimental NO_3^- deposition (3 g $N \cdot m^{-2} \cdot yr^{-1}$) has altered the biogeochemical cycling of C and N in a manner consistent with the microbial mechanisms described above. Experimental NO_3^- deposition has increased leaf litter N concentration (+15%), reduced lignolytic extracellular enzyme activity in forest floor (−33%) and mineral soil (−10%), and decreased soil respiration (−15%; Burton et al. 2004, DeForest et al. 2004, 2005, Pregitzer et al. 2008). Although aboveground NPP has increased (+10%, primarily woody biomass) under experimental NO_3^- deposition, the aforementioned responses have occurred despite no change in above- or belowground litter production (Burton et al. 2004, Pregitzer et al. 2008). Although arbuscular mycorrhizal biomass has declined (Van Diepen et al. 2007), the biomass and respiration of fine roots, as well as microbial respiration in mineral soil, also have not been altered by experimental NO_3^- deposition (Burton et al. 2004, Zak et al. 2006), indicating that lower microbial activity in the forest floor is likely responsible for declines in soil respiration (Zak et al. 2004). Together, these observations provide evidence of the gradual accumulation of forest floor and soil organic matter in this forest ecosystem (Pregitzer et al. 2008). Additional evidence suggests that anthropogenic N will accumulate in forest-floor and surface mineral soil via a pathway also not included in some biogeochemical models. Using $^{15}NO_3^-$ as a tracer, we have observed that NO_3^- was rapidly (i.e., hours) assimilated by soil microorganisms in forest floor and, after several days, was released as $^{15}NH_4^+$ into soil solution; $^{15}NH_4^+$ was then assimilated by overstory trees (i.e., weeks), where it enriched the canopy after one year (Zogg et al. 2000, Zak et al. 2004). In contrast to other studies (Magill et al. 1997, 2000, Nadelhoffer et al. 1999*a*, *b*), soil organic matter was not a sink for $^{15}NO_3^-$ after one year, and we predicted ^{15}N would accumulate in forest floor and soil organic matter only after ^{15}N-enriched leaf litter was shed by the canopy.

FIG. 1. The distribution of study sites spanning the geographic range of northern hardwood forests in lower and upper Michigan, USA. Individual study sites were selected from a larger pool of candidate sites due to their similarity of floristic and edaphic characteristics. In each study site, three plots (50 × 50 m) received ambient atmospheric N deposition, and three plots received an additional 3 g NO_3^--$N \cdot m^{-2} \cdot yr^{-1}$ since 1994.

Our objective was to determine if experimental NO_3^- deposition has altered organic matter and N stored in northern hardwood forests, especially in forest floor and mineral soil. To address this objective, we constructed organic matter and N budgets for a long-term study in which NO_3^- deposition has been experimentally manipulated across the north–south geographic range of northern hardwood forests. Additionally, we followed the flow of tracer $^{15}NO_3^-$ in our experimental NO_3^- deposition treatment to determine if forest floor and soil organic matter became long-term sinks for NO_3^- deposition after it flowed through the soil microbial community and overstory trees.

METHODS

Experimental design

We investigated the influence of chronic atmospheric NO_3^- deposition on the distribution of organic matter and N in four sugar maple (*Acer saccharum* Marsh)-dominated northern hardwood sites distributed across lower and upper Michigan, USA (Fig. 1). Their locations span the north–south geographic range of northern hardwood forests in the Great Lakes region, which encompasses most of the hemlock–white pine–northern hardwoods biome (Braun 1950). Overstory associates include *Quercus rubra*, *Fraxinus americana*, *Betula alleghaniensis*, and *Prunus serotina*; all stands lack a well developed understory, and ground flora species are sparse. The forest floor is composed of an Oi horizon dominated by sugar maple leaf litter and an Oe horizon

TABLE 1. Climatic, floristic, and edaphic characteristics of four northern hardwood sites receiving experimental atmospheric NO_3^- deposition.

Characteristic	Site			
	A	B	C	D
Location				
Latitude, N	46°52′	45°33′	44°23′	43°40′
Longitude, W	88°53′	84°52′	85°50′	86°09′
Climate				
Mean annual temperature (°C)	4.7	6.0	6.9	7.6
Mean annual precipitation (mm)	873	871	888	812
Wet + dry NO_3^--N deposition ($g \cdot m^{-2} \cdot yr^{-1}$)	0.38	0.58	0.78	0.76
Wet + dry total N deposition ($g \cdot m^{-2} \cdot yr^{-1}$)	0.68	0.91	1.17	1.18

Note: Sites (see Fig. 1) are located in lower and upper Michigan, USA, and they have been receiving experimental NO_3^- deposition since 1994. Stands are similar in age, plant composition, and soil development, but they differ in temperature and growing season length.

in which the fine roots of sugar maple form a dense mat. Mineral soils are sandy (85–90% sand) and are classified as typic Haplorthods of the Kalkaska series.

Although our research sites are floristically and edaphically similar (Burton et al. 1991), they differ in climate along the north–south latitudinal gradient (Table 1). They also span a gradient of atmospheric-N deposition (0.68–1.17 g $N \cdot m^{-2} \cdot yr^{-1}$), of which NO_3^--N composes ~60% of wet-plus-dry deposition. Located in each site are six 30 × 30 m plots, each surrounded by a 10 m wide treated buffer. Three plots in each site receive ambient atmospheric N deposition (Table 1). The remaining three plots in each site receive ambient N deposition plus 3 g NO_3^--$N \cdot m^{-2} \cdot yr^{-1}$, a rate approaching that expected in large portions of North America by 2050 as well as over other portions of the Earth (Galloway et al. 2004). The additional NO_3^- is delivered during the growing season in six equal applications (0.5 g $N \cdot m^{-2} \cdot month^{-1}$) of solid $NaNO_3$ pellets, which are broadcast over the forest floor. We chose Na^+ as a counter ion for our NO_3^- deposition treatment because it is not an essential plant nutrient. In each plot, four porous-cup ceramic lysimeters were installed (75 cm depth) to quantify leaching losses of inorganic N and dissolved organic nitrogen (DON) (Model 1900, Soilmoisture Equipment, Santa Barbara, California, USA). Leaf litter in each plot is collected monthly throughout the growing season (biweekly during autumn) from four 0.5-m^2 litter traps (Pregitzer et al. 2008). We constructed a detailed organic-matter and N budget for each plot receiving ambient ($n = 3$) and experimental ($n = 3$) deposition in all four replicate stands. These data were gathered after 11 years of treatment in September 2004.

Field sampling

In each site ($n = 4$), we identified and measured the diameter of all trees (>5 cm at breast height) in each ambient ($n = 3$) and experimental NO_3^- ($n = 3$) deposition plot. Tissue samples in each plot were collected from four widely spaced, dominant overstory trees. For each of these trees, we used a shotgun to obtain sun-lit canopy leaves and attending branches. Bark and stem wood were removed with a 2.5 cm diameter hole saw, which was inserted into each stem at breast height. We also sampled large structural roots near the base of each tree stem using a hole saw. Fine roots were collected at three random locations in each ambient and experimental N deposition plot by removing a 10 cm deep soil core (10 cm diameter), which contained Oe, Oa, A, and E horizon material. Soil cores were stored in a freezer until they could be processed. Roots were carefully removed by hand and sorted into four size classes: 0.5–1.0 mm, 1.0–2.0 mm, 2.0–5.0 mm, and 5.0–10 mm (sensu Zak et al. 2004); the biomass of fine roots <0.5 mm was determined by elutriation (Burton et al. 2000). Forest floor samples were collected within a 30 × 30 cm area at eight random locations in each plot. To determine the depth distribution of organic matter and N in soil, we used a core (10 cm diameter) extending from the top of the Oe horizon to a depth of 70 cm. Soil from each core was collected in increments of 0–10 cm, 10–30 cm, 30–50 cm, and 50–70 cm. The field-fresh mass of each core section was obtained, and a subsample was oven-dried (105°C); bulk density was estimated using the dry mass and volume of each core section.

Ecosystem biomass and N pools

Canopy leaf mass was estimated from leaf litter fall using a correction factor of 1.14 to adjust for changes in specific leaf area prior to abscission (Burton et al. 1991, 1993). Species-specific allometric equations were used to estimate the biomass of overstory branch, stem bark, stem wood, and structural roots (>10 cm diameter; Whittaker et al. 1974). The biomass of fine roots in each size class was expressed on an areal basis (g/m^2) using the oven-dry mass (70°C) of material recovered from each 10 cm diameter root core. Forest floor was separated into Oi and Oe/a horizons, and material from each horizon was oven dried at 70°C to estimate its biomass. We used a NC 2500 elemental analyzer (CE Elantech, Lakewood, New Jersey, USA) interfaced to a

Delta Plus isotope ratio mass spectrometer (Thermo Finnigan, San Jose, California, USA) to determine the N concentrations of plant tissue, forest floor, and soil samples (0–70 cm cores). We also used this instrument to obtain the C concentration of each soil sample, and we estimated soil organic matter (SOM) content assuming 470 mg C/g SOM.

The N content of plant and forest floor pools (Oi and Oe/a) was calculated as the product of their biomass (g/m^2) and N concentration (mg N/g). The N content of each soil sample was calculated as the product of its bulk density (g/cm^3), depth, and N concentration (μg N/g); soil organic matter content (g/m^2) was estimated in a similar manner. The 0–10 cm section of our 70-cm core contained Oe/a material. Using the data from our forest floor sample of the Oe/a horizon, we subtracted the biomass and N content of this material from the 0–10 cm section of the 70-cm soil core to calculate organic matter and N content of the mineral surface horizon (A and A, E horizons).

We used a sequential extraction procedure to determine inorganic N, DON, and microbial N (sensu Holmes et al. 2003). Three 2.5 cm diameter soil cores (0–10 cm) were collected at random locations in each plot, which we subsequently composited in each plot. A 12 g field-fresh subsample of root-free soil from each plot was placed in a 30-mL glass vial containing 20 mL of 2 mol/L KCl. The vials were capped, placed on a shaker for 20 min, then centrifuged for 15 min at 136*g* (1333.7 m/s^2). Particulate organic matter and suspended cells were removed by passing the solution though the 0.45-μm filter; the resulting filtrate contained inorganic N and DON. The same sample was extracted a second time with a 20-mL aliquot of 2 mol/L KCl. Ammonium-N and NO_3^--N concentrations in the filtrates were determined by automated colorimetry using an OI Analytical Flow Solution 3000 continuous-flow analyzer (OI Analytical, College Station, Texas, USA). Following alkaline persulfate digestion of the filtrate, DON (as NO_3^--N) was measured using automated colorimetry as described.

A second extraction step was performed to separate microbial N and soil organic N (Holmes et al. 2003). The KCl-extracted soil and filter remaining in the vials was fumigated with CH_3Cl for five days in a vacuum desiccator. Residual CH_3Cl was removed by repeated vacuuming, and 20 mL of 0.25 mol/L K_2SO_4 was added to each vial. Vials were capped, placed on a shaker for 30 min, and centrifuged for 15 min at 1333.7 m/s^2. The supernatant was placed in a 120-mL specimen cup; this extraction was repeated with an additional 20-mL aliquot of K_2SO_4. Microbial N was determined by alkaline persulfate digestion of the K_2SO_4 solution (Cabrera and Beare 1993), followed by automated colorimetry for NO_3^--N as described. The soil remaining in the vial was dried to a constant mass at 60°C, ground using a SamplePrep 8000 mill (Spex Centriprep, Metuchen, New Jersey, USA), and analyzed using a CE Instruments NC2500 elemental analyzer (CE Elantech, Lakewood, New Jersey, USA) interfaced to a Delta Plus isotope ratio mass spectrometer (Thermo Finnigan, San Jose, California, USA). The N content of inorganic N, DON, and microbial N was calculated as the product of N concentration, bulk density, and sample depth (0–10 cm).

To estimate amounts of NH_4^+, NO_3^-, and DON leaching from our experiment, we collected soil solution from the four lysimeters located in each plot receiving ambient and experimental N deposition. Each lysimeter was evacuated on a 2-week interval during autumn 2003, spring 2004, and autumn 2004. A tension of 0.05 MPa was placed on each lysimeter after evacuation, and soil solution was composited on a plot basis. Prior to laboratory analyses, we passed each composite sample through a 0.45-μm filter membrane. Ammonium, NO_3^-, and DON were analyzed as described above. To estimate leaching losses of these compounds, we calculated a water balance to estimate the volume of water passing below the rooting zone of each site (Thornthwaite and Mather 1957). Air temperatures and precipitation, measured continuously at each site, were used to calculate a water balance for each site. Monthly leaching losses were estimated as the product of mean N concentration (μg N/mL) and leaching volume (mL/cm^2); monthly losses were summed to estimate annual losses of NH_4^+, NO_3^-, and DON.

Experimental NO_3^- deposition and flow of ^{15}N

In a previous study, we applied tracer amounts of $^{15}NO_3^-$ to follow the flow of anthropogenic NO_3^- through this ecosystem (Zak et al. 2004). In 1998, plots ($n = 3$) receiving experimental NO_3^- deposition in Site B (Fig. 1) were each labeled with 24 g of ^{15}N. The isotope was applied by mixing 99% atom excess $Na^{15}NO_3$ with the June, July, and August application of $NaNO_3^-$, thereby labeling the forest floor. One year after isotope addition, the primary sink for ^{15}N was the overstory canopy, whereas no isotope was recovered in surface soil (Zak et al. 2004). Our results indicated that soil organic matter was not an initial sink for anthropogenic N, at least not over a one-year time step. We predicted that litter fall from the ^{15}N-enriched canopy would eventually enrich mineral soil, making soil a long-term sink for anthropogenic N (Zak et al. 2004). To test this prediction, we quantified the distribution of ^{15}N six years following isotope addition.

Our overall field sampling and analytical approach enabled us to determine the ^{15}N content (mg $^{15}N/m^2$) of plant, forest floor, soil pools, and leaching losses. To test our prediction regarding the flow of anthropogenic NO_3, we compared ^{15}N recovery in each ecosystem pool one year and six years after isotope addition. Our approach for determining the ^{15}N content of each ecosystem pool is described in detail by Zak et al. (2004). Prior to isotope addition, we determined the amount of ^{15}N in each ecosystem pool (i.e., the product of atom $\%^{15}N$ and

TABLE 2. Overstory biomass, N concentration, and N content in four northern hardwood stands receiving experimental NO_3^- deposition treatments.

Ecosystem component	Biomass (g/m^2)		N concentration (mg N/g)		N content (g N/m^2)	
	Ambient	NO_3^-	Ambient	NO_3^-	Ambient	NO_3^-
Leaves	410 (73.1)	429 (66.9)	19.2 (1.65)	22.8*** (1.29)	7.9 (1.72)	9.8*** (1.40)
Branches	5555 (939.5)	5343 (1181.9)	8.9 (0.75)	9.15 (1.03)	597 (292.1)	484 (124.3)
Stem wood	17033 (1755.4)	16948 (2814.7)	0.9 (0.14)	0.9 (0.18)	190 (108.1)	147 (32.2)
Stem bark	2066 (191.7)	2065 (326.1)	5.1 (0.37)	5.2 (0.51)	131.9 (66.1)	106.6 (14.9)
Roots (diameter)						
10.0 mm	4718 (405.8)	4725 (714.9)	1.5 (0.41)	1.8* (0.52)	85 (55.9)	80 (25.7)
10.0–5.0 mm	106 (37.3)	260 (22.9)	3.5 (4.33)	3.7 (3.24)	0.72 (1.725)	0.79 (0.926)
5.0–2.0 mm	79 (54.8)	86 (173.9)	8.1 (3.51)	6.5 (2.54)	0.62 (0.486)	0.54 (0.281)
2.0–1.0 mm	60 (31.9)	62 (35.5)	8.2 (2.12)	7.5 (2.45)	0.49 (0.321)	0.46 (0.263)
1.0–0.5 mm	57 (18.8)	55 (18.9)	8.42 (1.43)	8.34 (2.31)	0.47 (0.146)	0.46 (0.186)
<0.5 mm	298 (76.8)	287 (104.5)	15.6 (2.39)	15.3 (2.53)	4.65 (1.390)	4.30 (1.532)
Total overstory	30382 (3391.2)	30147 (5137.0)			1019.2 (514.81)	835.5 (172.74)

Notes: Values are for 2004, the 11th year of treatment. For each overstory component, significance between ambient and experimental NO_3^- deposition treatment means ($n = 12$, three plots in four replicate stands) is denoted as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Standard deviations are in parentheses.

N content in g N/m^2; see Zak et al. 2004). We used the same sampling and analytical approach to then determine the ^{15}N content of each ecosystem pool one year following isotope addition; the identical approach was used here to quantify the distribution of label ^{15}N six years after application. The amount of ^{15}N label residing in each ecosystem pool is the difference between its ^{15}N content six years after isotope addition (mg $^{15}N/m^2$) and its ^{15}N content prior to isotope addition. In this calculation, we also accounted for the amount of ^{15}N added via our routine application of $NaNO_3$ (0.36646 atom $\%^{15}N$). Isotope recovery is expressed as the percentage of applied ^{15}N (37.67 mg $^{15}N/m^2$) residing in each ecosystem pool, and we compared recoveries one and six years following isotope addition.

Statistical analyses

We used a two-way ANOVA to determine whether NO_3^- deposition treatment (df = 1), site (df = 3), or their interaction (df = 3) had a significant effect on the biomass, N concentration, and N content of plant, forest floor, and soil pools. The interaction term in this model tested the null hypothesis that replicate stands responded in the same manner to our experimental N-deposition treatment, thus enabling us to determine if we could generalize our results across the geographic range of sugar-maple-dominated northern hardwood forests in the Lake States region. For each plot, we also calculated forest floor turnover (years) as the quotient of forest floor mass (Oe/a) and aboveground litter fall in order to determine if chronic N deposition slowed forest floor decomposition, as we had predicted. To test this hypothesis, we also used a two-way ANOVA consisting of treatment, site, and their interaction. For the three plots receiving experimental N deposition in Site B (Fig. 1), we used one-way ANOVA to compare ^{15}N recovery in each ecosystem pool one year and six years following isotope addition; these data were log transformed to meet the assumption of normality. A protected Fishers LSD procedure was used to compare means; significance for all statistical analyses was accepted at $\alpha = 0.05$.

RESULTS

Ecosystem biomass

Experimental N deposition and site did not interact to influence the biomass of any plant component ($P = 0.268$–0.924), nor did they interact to influence forest floor biomass (Oi, $P = 0.449$; Oe/a, $P = 0.700$) or soil organic matter at any depth ($P = 0.114$–0.437). After a decade of treatment, experimental N deposition (main effect) had no effect on the biomass of any plant component (Table 2) nor did it alter aboveground litter fall (Fig. 2A) or fresh litter (Oi) on the forest floor (Table 3, Fig. 2A). However, the mass of partly decomposed litter (Oe/a) increased by 50% under experimental N deposition (Table 3, Fig. 2B). Because aboveground litter production was equivalent under ambient and experimental N deposition (Fig. 2A), slower rates of decomposition likely facilitated the accumulation of partly decomposed litter (Oe/a) in our experimental N deposition treatment. Further evidence for slower decomposition in our experimental NO_3^- deposition treatment was the significantly slower turnover of partly decomposed litter in the forest floor, relative to the ambient treatment (Fig. 2C). Although surface mineral soil (0–10 cm) exposed to experimental N deposition contained 18% more organic matter than the ambient treatment, this difference was not significant (Table 3). Organic matter deeper in the soil profile (10–70 cm) was equivalent between ambient and experimental N deposition treatments (Table 3). However, the amount of organic matter contained in forest floor and mineral soil (i.e., total forest floor and soil to 70 cm) was 12% greater under experimental N deposition (Table 3); this increase was significant.

Nitrogen concentrations

The N concentration of bark and a root fraction (2–5 mm) was influenced by a significant interaction between N deposition and site. However, interaction means for bark N concentration occupied a narrow range of values (4.6–5.6 mg N/g), with N deposition increasing N concentrations in Sites A and C and decreasing them in Sites B and D (Fig. 1). The significant treatment-by-site interaction on fine-root (2–5 mm) N concentration arose from a high N concentration in the ambient treatment of Site D (12.2 mg N/g vs. 3.9–9.3 mg N/g). The interaction of treatment and site had no effect on the N concentration of any other plant components.

Experimental N deposition (main effect) significantly increased N concentrations in canopy leaves and structural roots, but it had no effect on N concentration in any other plant tissue (Table 2). Similarly, the N concentration of forest floor (Oi and Oe/a) was not altered in our experimental treatment (Table 3). However, experimental N deposition did elicit a significant 60% increase in the N concentration of surface mineral soil (0–10 cm; Table 3); the N concentration of soil deeper in the profile (i.e., 10–70 cm) was not influenced by our treatments. In the 0–10 cm soil depth, microbial N averaged 39.0 ± 4.33 mg N/g (mean ± SD) in the ambient treatment and 38.6 ± 5.05 mg N/g (mean ± SD) under experimental N deposition; these means were not different. Similarly, experimental N deposition had no influence on extractable NH_4^+ concentrations in the 0–10 cm soil depth (8.3 ± 2.03 vs. 7.9 ± 3.72 μg N/g [mean ± SD]; ambient vs. experimental N deposition). However, the concentration of extractable NO_3^- was significantly higher under experimental N deposition (8.9 μg N/g) relative to the ambient treatment (2.3 μg N/g). The mean N concentration of leached NH_4^+ was equivalent between ambient and experimental N deposition treatments (Table 3), whereas experimental N deposition resulted in a 10-fold increase in the mean N concentration of leached NO_3^--N as well as an eightfold increase in the mean leached dissolved organic nitrogen (DON) concentration (Table 3).

Ecosystem N content

Experimental N deposition significantly increased the N content (g N/m^2) of canopy leaves, but it did not alter the N content of any other plant component (Table 2). For example, the N content of canopy leaves increased ~25% under experimental N deposition, whereas the N content of other plant components was approximately equivalent (Table 2). Similarly, the N content of fresh litter (Oi) and partly decomposed litter (Oe/a) in the forest floor increased by 30–38% under experimental N deposition, but these increases were not significant (Table 3). In contrast, experimental N deposition significantly increased the N content of surface mineral soil (0–10 cm) by 34% (Table 3). We found no effect of experimental N deposition on N content deeper in the soil profile (10–70 cm; Table 3). However, when we summed the N content of forest floor and mineral soil, experimental N deposition significantly increased (~10%) the total N content of these combined ecosystem pools (Table 3). In the 0–10 cm soil depth, experimental N deposition had no effect on the N content of microbial biomass (4.2 vs. 4.1 g N/m^2; ambient vs. experimental N deposition) or extractable

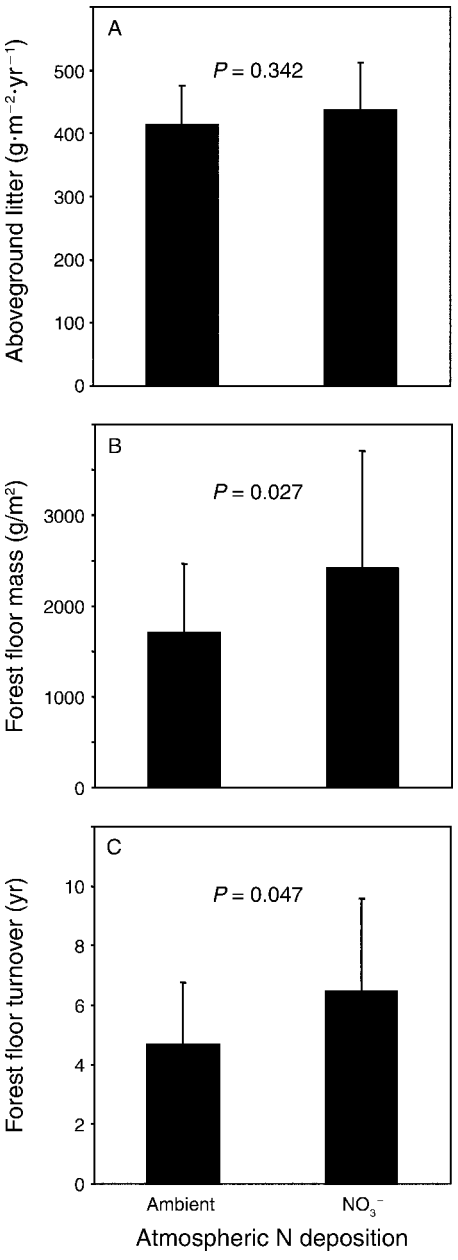

FIG. 2. (A) Aboveground litter fall, (B) forest floor (Oe and Oa horizons), and (C) forest floor turnover under ambient and experimental atmospheric N deposition. Experimental N deposition had no significant effect on aboveground litter production, but it significantly increased forest floor mass and turnover time. Values are treatment means in 2004 ($n = 12$, three plots in four replicate stands), and the error bars are one standard deviation.

TABLE 3. Biomass, N concentration, and N content of forest floor and soil in four northern hardwood forest stands receiving experimental NO_3^- deposition treatments for 10 years.

Ecosystem component	Organic matter (g/m^2)		N concentration (mg N/g)		N content ($g\ N/m^2$)	
	Ambient	NO_3^-	Ambient	NO_3^-	Ambient	NO_3^-
Forest floor						
Oi	51 (56.4)	67 (46.9)	16.1 (2.57)	16.8 (2.30)	0.83 (1.001)	1.15 (0.783)
Oe/Oa	1708 (748.4)	2579** (1196.9)	15.7 (2.71)	14.6 (4.07)	26.2 (11.42)	33.9 (12.72)
Mineral soil§						
0–10 cm	3730 (1303.3)	4406 (2662.8)	1.33 (0.412)	2.13* (1.089)	142.5 (32.21)	190.5* (62.33)
10–30 cm	5388 (1238.6)	5402 (1351.4)	0.64 (0.159)	0.62* (0.150)	168.1 (39.33)	170.2 (35.39)
30–50 cm	4293 (1176.6)	4396 (1499.9)	0.37 (0.074)	0.38 (0.134)	106.1 (22.09)	114.8 (35.96)
50–70 cm	2396 (570.3)	2864 (1085.4)	0.27 (0.063)	0.26 (0.081)	73.1 (18.41)	80.7 (25.85)
Total forest floor and soil	17 557 (2310.3)	19 718** (3991.7)			529.9 (51.42)	578.9* (95.56)
Leached NH_4^+-N			0.22 (0.357)†	0.19 (0.178)†	0.07 (0.124)‡	0.07 (0.074)‡
Leached NO_3^--N			0.50 (0.566)†	4.8** (1.72)†	0.19 (0.226)‡	1.92** (0.583)‡
Leached DON			0.30 (0.117)†	2.38** (1.686)†	0.09 (0.046)‡	0.86** (0.811)‡

Notes: For each forest floor and soil pool, a significant difference between ambient and experimental NO_3^- deposition treatment means ($n = 12$) is denoted as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Standard deviations are in parentheses.

† Units for concentrations are μg N/mL.

‡ Units are $g\ N{\cdot}m^{-2}{\cdot}yr^{-1}$.

§ Soil N content includes microbial N, extractable inorganic N, and extractable DON.

NH_4^+ (0.89 vs. 0.82 $g\ N/m^2$). However, the N content of extractable NO_3^- in the 0–10 cm soil depth was significantly higher under experimental N deposition (1.59 vs. 0.37 $g\ N/m^2$). Consistent with our previous results (Pregitzer et al. 2004), experimental N deposition significantly increased the amount of N ($g\ N/m^2$) exported via leaching as NO_3^- and DON, but it did not alter leaching losses of NH_4^+ (Table 3). The combined annual leaching loss of NO_3^--N and DON from the experimental deposition treatment is an order of magnitude greater than under the ambient treatment; moreover, it was equivalent to 93% of the NO_3^--N delivered by our treatment in 2004.

Experimental NO_3^- deposition and flow of ^{15}N

We compared ^{15}N recovery in the experimental N deposition plots ($n = 3$) of Site B, which revealed that recovery in overstory biomass declined, whereas recovery in soil organic matter increased 1–6 years following isotope addition (Table 4). For example, ^{15}N recovery in each plant component decreased from year 1 to 6 (Table 4), albeit most of these declines were not significant. Declines of largest magnitude occurred in leaves (−50%), branches (−75%), and in fine roots, which contained almost no detectable ^{15}N after six years. Recovery in fresh litter (Oi) did not differ between years 1 and 6, and values were approximately equivalent (Table 4). One year after isotope addition, soil organic matter (e.g., Oe/a and A horizons) was not a sink for ^{15}N whatsoever (0% recovery); however, soil organic matter did become a significant sink for ^{15}N over time, and contained 10% of the added isotope after six years (Table 4). Recovery of ^{15}N in microbial biomass and extractable NH_4^+ also increased significantly from year 1 to 6 (Table 4).

DISCUSSION

The uptake of anthropogenic N by N-limited forest trees and the resulting enhancement of NPP are thought to be the primary mechanisms that could increase C storage within temperate and boreal forests of the Northern Hemisphere, albeit the magnitude of that response is uncertain (Townsend et al. 1996, Nadelhoffer et al. 1999*a*, Currie et al. 2004). We have argued that atmospheric N deposition could increase the accumulation of organic matter in forest floor and mineral soil via a reduction in microbial activity and a slowing of decomposition, rather than by enhanced rates of detritus production. Following a decade of experimental NO_3^- deposition, organic matter and N have both accumulated in forest floor and soil, despite no increase in above- or belowground litter production (Burton et al. 2004, Pregitzer et al. 2008). Rather, declines in lignolytic activity under experimental NO_3^- deposition (DeForest et al. 2004, 2005) appeared to have slowed organic-matter decomposition (Fig. 2) and increased the mass of partially decomposed litter in the forest floor, resulting in overall accumulation of organic matter on the soil surface. This response is equivalent to the enhancement of ANPP (+10%) by experimental NO_3^- deposition, which has not resulted in greater overstory biomass or overstory N content due to an acceleration of tree mortality (Pregitzer et al. 2008). Coarse woody litter produced by greater tree mortality would have been missed by our sampling scheme, and over time, would further increase the accumulation of organic matter in surface soil. The degree to which experimental NO_3^- deposition has increased soil organic matter (SOM; ~215 $g\ SOM{\cdot}m^2{\cdot}yr^{-1}$ or 100 $g\ C{\cdot}m^2{\cdot}yr^{-1}$) over our decade-long experiment indicates that such a mechanism should be considered in biogeochemical models simulating the influence of atmospheric N

TABLE 4. Mean recovery of ^{15}N in ecosystem pools one year and six years after application of $^{15}NO_3^-$ to the experimental NO_3^- deposition treatment in Site B (Fig. 1).

Ecosystem component	^{15}N recovery (percentage of applied ^{15}N)		P
	After 1 year	After 6 years	
Overstory			
Leaves	5.25 (2.819)	2.55 (0.311)	0.175
Branches	4.06 (2.938)	1.55 (0.240)	0.213
Stem wood	0.91 (0.218)	0.68 (0.205)	0.235
Stem bark	0.28 (0.064)	0.07 (0.083)	0.023
Roots			
>10.0 mm	1.27 (0.401)	0.853 (0.196)	0.178
10.0–5.0 mm	0.28 (0.136)	0.00 (0.132)	0.026
5.0–2.0 mm	0.32 (0.312)	0.00 (0.040)	0.159
2.0–1.0 mm	0.21 (0.154)	0.00 (0.037)	0.056
1.0–0.5 mm	0.18 (0.139)	0.00 (0.029)	0.084
<0.5 mm	0.88 (0.387)	0.00 (0.346)	0.025
Total overstory	13.51 (5.096)	5.47 (0.336)	0.050
Forest floor	2.54 (2.200)	1.89 (0.451)	0.640
Soil (0–10 cm)			
Organic N	0.00 (0.000)	10.02 (2.860)	0.004
Extractable NH_4^+	0.00 (0.000)	0.08 (0.029)	0.008
Extractable NO_3^-	0.00 (0.000)	0.24 (0.197)	0.102
Microbial N	0.01 (0.024)	0.300 (0.087)	0.005
Leached NO_3^-	1.11 (0.746)	0.00 (0.001)	0.062
Leached DON	0.27 (0.242)	0.00 (0.005)	0.129
Total recovery	17.4 (7.016)	19.9 (3.436)	0.622

Note: Total recovery after six years includes soil N from 10–30 cm (1.3% ± 0.27%; ±SD), 30–50 cm (0.5% ± 0.41%), and 50–70 cm (0.1% ± 0.38%); these depths were not sampled one year after ^{15}N addition.

deposition on ecosystem C storage in northern temperate forests. Moreover, greater rates of NPP (Pregitzer et al. 2008) in combination with organic matter accumulation in forest floor and surface mineral soil indicate that atmospheric N deposition will increase C storage in this widespread forest ecosystem.

Before the end of this century, portions of eastern North America will receive atmospheric N deposition approaching our experimental treatment (Galloway et al. 2004), which could rapidly increase soil organic matter accumulation across the extent of northern hardwood forests like those in our study. Our experiment was specifically designed to test the null hypothesis that replicate stands would respond similarly to elevated atmospheric N deposition, a key test for projecting our experimental inference across the geographic expanse of this northern hardwood ecosystem. We were unable to reject this hypothesis for the majority of pools composing our organic matter and N budgets, indicating that pools were similarly influenced by atmospheric N deposition across replicate stands. If the stands we studied are representative of others across the region, then organic matter and N likely will accumulate in forest floor and surface mineral soil as atmospheric N deposition increases over the next century, increasing C storage in this common forest ecosystem. On the other hand, forests which differ in overstory composition, and hence litter biochemistry, can respond in an opposing manner (Waldrop et al. 2004, Knorr et al. 2005), restricting the relevance of our results to northern hardwood forests dominated by *Acer saccharum*.

Several convergent lines of evidence indicate that experimental NO_3^- deposition has slowed microbial activity and altered decomposition in the forest floor. Foremost, experimental NO_3^- deposition has increased the turnover time and forest floor mass (Oe/a horizon) as well as the production of dissolved organic nitrogen (DOC) from this soil horizon (Fig. 2; Pregitzer et al. 2004, Smemo et al. 2007). Taken together with the higher phenolic concentration of forest floor dissolved organic carbon (DOC; Smemo et al. 2007), our observations suggest experimental NO_3^- deposition has fundamentally altered the microbial metabolism of plant detritus. Consistent with our expectations, experimental NO_3^- deposition increased the N concentration of leaf litter (+15%; Pregitzer et al. 2008) as well as extractable NO_3^- in soil solution (+300%; Table 3), both of which are known to slow the microbial metabolism of lignin and humus (Fog 1988, Berg and Matzner 1997, Berg and Mentemeyer 2002). These observations are consistent with declines in phenol oxidase and peroxidase activity in forest floor and mineral soil (DeForest et al. 2004, 2005), which mediate the nonspecific oxidation of polyphenols contained in both lignin and humus. Experimental NO_3^- deposition has not altered the lignin concentration of leaf litter (140 mg/g) or fine roots (340–400 mg/g; J. Eikenberry and K. Pregitzer, *unpublished data*), eliminating the possibility that the greater forest floor mass in our experimental NO_3^-

deposition treatment resulted from the production of more lignified litter. These lines of evidence support the idea that experimental NO_3^- deposition has suppressed and altered the manner in which microbial communities in soil degrade lignin and humus in this sugar-maple-dominated ecosystem.

This result might appear unexpected because simulated N deposition can initially accelerate the decay of leaf litter (Carreiro et al. 2000), particularly the decay of sugar-maple leaves, which are low in lignin. However, the forest floor (i.e., Oe/a horizon) in our study is permeated by a dense mat of sugar-maple fine roots (<1 mm diameter), which are a lignin-rich substrate for microbial decay (e.g., 340–400 mg lignin/g; J. Eikenberry and K. Pregitzer, *unpublished data*). Fine-root litter (280 $g \cdot m^{-2} \cdot yr^{-1}$; Pregitzer et al. 2008) composes ~45% of annual detritus production, making fine-root litter an important substrate for microbial metabolism. It is plausible that the accumulation of soil organic matter we observed partly results from the slowing of fine-root litter decay in forest floor and surface mineral soil. It also is plausible that a higher leaf-litter N concentration induced by a decade of experimental NO_3^- deposition has slowed the latter stages of leaf decay, which are dominated by lignin. These observations have come to light after a decade of experimental treatment, and they may not have been captured by shorter term exposure to simulated atmospheric N deposition. Understanding the manner in which experimental NO_3^- deposition has influenced the decay of individual dead leaves and roots may help reveal which of these plant tissues has facilitated organic matter accumulation in forest floor and surface mineral soil.

Experimental NO_3^- deposition clearly altered microbial activity in the forest floor, a response that could arise from a shift in the function of lignin-degrading soil microorganisms. White-rot basidiomycetes are the dominant agents of lignin degradation in the forest floor (Osono 2007), and their ability to produce phenol oxidase and other lignolytic enzymes can be repressed by high inorganic N (Tien and Tu 1987, Boominathan et al. 1990, Vanderwoude et al. 1993, Li et al. 1994). In contrast to some white-rot basidiomycetes, the synthesis of lignolytic enzymes by brown-rot fungi is unresponsive to N availability (Reddy and D'Souza 1994, D'Souza et al. 1996, Worrall et al. 1997), whereas phenol oxidase and peroxidase synthesis by soil actinobacteria can be upregulated by greater N availability (Bardner and Crawford 1981, Giroux et al. 1988). By increasing inorganic-N concentrations in soil solution (see *Results*), chronic N deposition could suppress lignin mineralization by white-rot basidiomycetes, shifting this process to organisms which are unaffected (i.e, brown-rot fungi) or positively affected (i.e., actinomycetes) by greater inorganic N availability. Unlike white-rot basidiomycetes, which mineralize lignin to CO_2, actinobacteria metabolize lignin into soluble polyphenolics (Mason et al. 1988, Godden et al. 1992, Berrocal et al. 1997). This response could explain increases in DOC production and phenolic content in the experimental NO_3^- deposition treatment (Table 3; Smemo et al. 2007). Because slow-growing white-rot basidiomycetes are poor competitors for labile organic substrates, a reduction in their ability to metabolize lignin could decrease their abundance or activity. Such a response would reduce lignolytic enzyme activity in the forest floor because both brown-rot basidiomycetes and actinomycetes produce smaller amounts of these extracellular enzymes than do white-rot basidiomycetes (Ramachandra et al. 1987, D'Souza et al. 1996). The shift in microbial community composition and activity described above is consistent with the decline in phenol oxidase and peroxidase activity, the increase in forest floor mass, and the greater production of phenolic DOC induced by experimental NO_3^- (DeForest et al. 2004, Pregitzer et al. 2004, Smemo et al. 2007). It also is consistent with similar observations in different forest ecosystems receiving experimental N deposition (Frey et al. 2004) as well as the positive relationship between ambient rates of atmospheric N deposition (~0.5–4.5 g $N \cdot m^{-2} \cdot yr^{-1}$) and stream water DOC/dissolved organic nitrogen (DON) in forested watersheds of eastern North America (Brookshire et al. 2007). The shift in microbial community composition and function we describe here is a plausible mechanism by which NO_3^- deposition has altered soil C cycling in our experiment; it remains a hypothesis to be tested.

One could argue that Na^+ additions associated with our NO_3^- deposition treatment could negatively impact microbial activity and slow decomposition in a manner consistent with our observations, especially if this ion accumulated in surface soil. However, several lines of evidence indicate this is not the case. In our experimental NO_3^- deposition treatment, the concentration of Na^+ in soil water (0.45 ± 0.00 mmol [mean ± SD]; W. E. Holmes, *unpublished data*) is two orders of magnitude lower than concentrations known to alter microbial community composition and decrease its biomass, respiration, and extracellular enzyme activity (40 mmol Na^+ as NaCl; Garcia and Hernández 1996). Although exchangeable Na^+ in surface soil (0–10 cm depth) was greater in our experimental NO_3^- deposition treatment (23 ± 2.3 mmol/kg vs. 35 ± 2.4 mmol/kg [mean ± SD]; $P < 0.001$; W. E. Holmes, *unpublished data*), this concentration also was lower than those documented to negatively impact soil microbial activity and litter decomposition (~80 mmol/kg; Li et al. 2006). Furthermore, Na^+ composes a small proportion of the cation exchange capacity in both ambient (3.2% ± 0.80%; mean ± SD) and experimental NO_3^- deposition treatments (3.6% ± 1.75% [mean ± SD]; W. E. Holmes, *unpublished data*); these values are well below those of saline soils in which microbial activity is negatively impacted by Na^+ accumulation (Rietz and Haynes 2003). The lack of Na^+ accumulation in our experimental NO_3^- treatment likely results from the ample

precipitation (812–888 mm/yr) and sandy soils (85–90% sand) of our study sites, in which Na^+ leaching losses are substantial (~40–60% of annual Na^+ additions; W. E. Holmes, *unpublished data*). Inasmuch, we have no evidence that declines in microbial activity, slower rates of litter decomposition, or the accumulation of soil organic matter can be attributed to high levels of Na^+ in surface soil.

The N from our experimental NO_3^- deposition treatment has accumulated in forest floor and surface soil to a greater extent than in plant biomass, and it has done so via a pathway different from a rapid immobilization of N into forest floor and mineral soil. In our previous study, $^{15}NO_3^-$ was assimilated by the microbial community over a time scale of minutes, and within hours, was released into soil solution as NH_4^+ that was subsequently taken up by plant roots over several weeks (Zogg et al. 2000). Also within hours of application, a substantial amount of ^{15}N was incorporated into forest floor and soil organic matter (Zogg et al. 2000). However, virtually no ^{15}N label was detected in soil organic matter after one year (Zak et al. 2004), indicating it had been released into soil solution and either assimilated by plants or lost to leaching. This observation contrasts with other studies in which the majority of tracer ^{15}N resided in forest floor and soil organic matter after one year (Nadelhoffer et al. 1999*a*, *b*, Magill et al. 1997, 2000). In our previous study, the overstory canopy contained the greatest amount of ^{15}N after one year (Zak et al. 2004), suggesting that soil organic matter would become a sink for anthropogenic NO_3^- only after the shedding of ^{15}N-enriched leaf litter and its subsequent decomposition into humus. The fact that ^{15}N recovery in overstory trees declined after six years while isotope recovery in soil organic matter significantly increased (Table 4), supports the prediction that soil organic matter becomes a sink for anthropogenic NO_3^- only after it has moved through the microbial community and has subsequently been assimilated by plants and shed in leaf litter. Interestingly, fine roots contained small amounts of isotope, further indicating that the ^{15}N residing in forest floor and organic matter after six years was derived from canopy leaves. This insight provides a potential mechanism for the accumulation of N in the forest floor and surface mineral soil of our experimental NO_3^- deposition treatment (Table 3).

Our ^{15}N tracer experiment provides insight into the time steps and processes by which anthropogenic NO_3^- was retained in this ecosystem. Although total recovery of applied ^{15}N was relatively low, it was similar between years 1 and 6 of this study (17–20%). This observation suggests that the rapid assimilation of $^{15}NO_3^-$ by soil microorganisms set in motion a series of events which retained ~20% of anthropogenic N within this ecosystem over a five-year duration. Further, leaching of inorganic and organic N annually equals ~70–90% of the NO_3^- applied in our experimental treatment (Table 3; Pregitzer et al. 2004), which is almost equivalent to the proportion of isotope tracer we were unable to recover. These observations imply that NO_3^- is subject to loss if it is not initially assimilated by the microbial community and retained by the sequence of events described here. Over time, it is likely that the slowing of decomposition by NO_3^- deposition, which we document here, will further lead to the accumulation of N in organic matter contained in forest floor and surface mineral soil. It will be important to understand the rate and time steps by which N is released from these pools as well as its subsequent fate (Currie et al. 2004); those processes will control the long-term potential for anthropogenic N to be sequestered in this northern temperate forest.

Summary and implications

A decade of experimental NO_3^- deposition, at a rate approaching that expected in the next several decades (Galloway et al. 2004), has increased the accumulation of organic matter in forest floor and soil, a response that was consistent across a large geographic region. Moreover, the organic matter accumulated in forest floor and soil resulted from a direct slowing of decomposition by experimental NO_3^- deposition, rather than from greater rates of detritus production. Our previous work demonstrated that NO_3^- deposition can lower the activity of key extracellular enzymes involved in lignin and humus degradation in some litter-decomposing fungi, which is consistent with the slowing of decomposition and the resultant accumulation of organic matter in forest floor (Oe/a) and surface soil. Inasmuch, atmospheric NO_3^- deposition appears to have increased C storage in this particular forest ecosystem via a direct effect on the composition and function of the heterotrophic microbial community; further work is necessary to evaluate this hypothesis and proposed mechanism. However, the accumulation of organic matter we document may not occur in forest ecosystems which differ in litter biochemistry, especially those in which NO_3^- deposition could aid microbial cellulose metabolism, increase decomposition, and decrease organic matter in forest floor and soil (Sinsabaugh et al. 2002, Waldrop et al. 2004, Knorr et al. 2005). Additionally, the N we have applied has accumulated in soil organic matter, and it has done so by first flowing through microbial and plant pools, and then reentering soil via leaf litter. This pathway differs from other studies in which anthropogenic N was initially immobilized into organic matter, gradually mineralized over time, and then eventually assimilated by plants (Currie et al. 2004).

Our results demonstrate that the manner in which soil microbial communities respond to anthropogenic N deposition is central to understanding how this environmental change will influence the storage of C and N in northern temperate forests. The representation of these mechanisms in simulation models predicting the influ-

ence of atmospheric N deposition on ecosystem C storage would better represent the underlying biological processes contributing to the current sink for anthropogenic CO_2 in northern temperate forests, and perhaps the northern hemisphere. More importantly, the accumulation of organic matter in forest floor and surface mineral soil we document here, in combination with enhanced rates of net primary productivity (NPP; Pregitzer et al. 2008), provides evidence that future rates of atmospheric N deposition could increase C storage in an ecologically and economically important temperate forest with a wide geographic distribution in eastern North America.

Acknowledgments

Research funding for the Michigan Gradient Study has been provided by National Science Foundation grants DEB-061442, DEB-0315138, DEB-0075397, and DEB-9629842. Kurt Smemo assisted us with field sampling. Bill Currie, Kirsten Hofmockel, and Knute Nadelhoffer shared valuable insights and provided comments on an earlier draft of this manuscript; we sincerely thank them.

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