

RUNNING HEAD: *Atmospheric N Deposition and Soil Organic Matter*

ATMOSPHERIC NO<sub>3</sub><sup>-</sup> DEPOSITION INCREASES SOIL ORGANIC MATTER  
BY SLOWING DECOMPOSITION IN A NORTHERN HARDWOOD ECOSYSTEM

DONALD R. ZAK<sup>1,2</sup>, WILLIAM E. HOLMES<sup>1</sup>, ANDREW J. BURTON<sup>3</sup> KURT S. PREGITZER<sup>4</sup>  
& ALAN F. TALHELM<sup>4</sup>

<sup>1</sup>*School of Natural Resources & Environment, University of Michigan,  
Ann Arbor, Michigan 48109, USA*

<sup>2</sup>*Department of Ecology & Evolutionary Biology, University of Michigan,  
Ann Arbor, Michigan 48109, USA*

<sup>3</sup>*Ecosystem Science Center, School of Forest Resources & Environmental Science,  
Michigan Technological University, Houghton, Michigan 49931, USA*

<sup>4</sup>*Department of Natural Resources & Environmental Science,  
University of Nevada, Reno, Nevada 89512, USA*

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## ABSTRACT

1  
2 Presently, there is uncertainty regarding the degree to which anthropogenic N deposition will  
3 foster C storage in the N-limited forests of the Northern Hemisphere, ecosystems which are  
4 globally important sinks for anthropogenic CO<sub>2</sub>. We constructed organic matter and N budgets  
5 for replicate northern hardwood stands ( $n = 4$ ) which have received ambient (0.7 to 1.2 g N m<sup>-2</sup>  
6 y<sup>-1</sup>) and experimental NO<sub>3</sub><sup>-</sup> deposition (ambient plus 3 g NO<sub>3</sub><sup>-</sup>-N m<sup>-2</sup> y<sup>-1</sup>) for a decade; we also  
7 traced the flow of a <sup>15</sup>NO<sub>3</sub><sup>-</sup> pulse over a 6-year period. Experimental NO<sub>3</sub><sup>-</sup> deposition had no  
8 effect on organic matter or N stored in the standing forest overstory, but it did significantly  
9 increase the N concentration (+19%) and N content (+24%) of canopy leaves. In contrast, a  
10 decade of experimental NO<sub>3</sub><sup>-</sup> deposition significantly increased amounts of organic matter  
11 (+12%) and N (+9%) in forest floor and mineral soil, despite no increase in detritus production.  
12 A greater forest floor (Oe/a) mass under experimental NO<sub>3</sub><sup>-</sup> deposition resulted from slower  
13 decomposition, which is consistent with previously reported declines in lignolytic activity by  
14 microbial communities exposed to experimental NO<sub>3</sub><sup>-</sup> deposition. Tracing <sup>15</sup>NO<sub>3</sub><sup>-</sup> revealed that  
15 N accumulated in soil organic matter by first flowing through soil microorganisms and plants,  
16 and that the shedding of <sup>15</sup>N-labeled leaf litter enriched soil organic matter over a 6-year  
17 duration. Our results demonstrate that atmospheric NO<sub>3</sub><sup>-</sup> deposition exerts a direct and negative  
18 effect on microbial activity in this forest ecosystem, slowing the decomposition of aboveground  
19 litter and leading to the accumulation of forest floor and soil organic matter. To the best of our  
20 knowledge, this mechanism is not represented in simulation models predicting the influence of  
21 anthropogenic N deposition on ecosystem C storage in northern forests.

22 **KEY WORDS:** *Atmospheric N deposition; decomposition; ecosystem N budget; <sup>15</sup>N tracing,*  
23 *northern hardwood forests; SOM accumulation*

## 1 INTRODUCTION

2 Over the past 150 yrs, atmospheric N deposition has increased an order of magnitude (e.g., from  
3 50-100 to 1500-2000 mg N m<sup>-2</sup> y<sup>-1</sup>; Galloway et al. 2004) across large areas of the Northern  
4 Hemisphere, which could foster greater net primary productivity (NPP) in the N-limited forests  
5 of this region. Although such a mechanism could strengthen the terrestrial C sink in the  
6 Northern Hemisphere (Schimel et al. 1995), there remains substantial uncertainty regarding the  
7 extent to which anthropogenic N deposition has contributed to this response (Townsend et al.  
8 1996, Nadelhoffer et al. 1999a, Magnani et al. 2007). Biogeochemical models initially suggested  
9 that atmospheric N deposition could account for an additional 0.1 to 2.3 Pg C annually stored in  
10 temperate and boreal forests (Schindler and Bayley 1993, Townsend et al. 1996, Holland et al.  
11 1997). Empirical approaches also have yielded contrasting results, suggesting that atmospheric  
12 N deposition has a minor as well as substantial influence on C storage in northern forests  
13 (Nadelhoffer et al. 1999a, Magnani et al. 2007, Pregitzer et al. 2007). The uptake of  
14 anthropogenic N by N-limited forest trees and the resulting enhancement of NPP have, up to this  
15 point, been the primary mechanisms thought to increase ecosystem C storage (Nadelhoffer et al.  
16 1999a, Townsend et al. 1996, Currie et al., 2004); however, the deposition of anthropogenic N  
17 could increase C storage in northern forests via other mechanisms.

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

1 accumulation (Carreiro et al. 2000, Frey et al. 2004, Waldrop et al. 2004). Other empirical  
2 evidence demonstrates that the later stages of litter decomposition, which are dominated by  
3 lignin degradation, are slowed in detritus with a high initial N concentration (Berg et al. 1982,  
4 Berg and Matzner 1997, Berg and Meentemeyer 2002). If atmospheric N deposition increases  
5 leaf litter N and elevates inorganic N in soil solution, then it could repress lignolytic activity,  
6 slow organic matter decomposition, and increase soil C storage. To the best of our knowledge,  
7 these specific mechanisms are not considered in any biogeochemical model simulating the  
8 influence of atmospheric N deposition on ecosystem C storage (Townsend et al. 1996, Pepper et  
9 al. 2005, Vetter et al. 2005), and they could have a substantial influence on organic matter  
10 accumulation in the forest floor and mineral soil of forests in the Northern Hemisphere.

11 Over the past decade, we have been studying a series of northern hardwood forest stands  
12 in which experimental  $\text{NO}_3^-$  deposition ( $3 \text{ g N m}^{-2} \text{ y}^{-1}$ ) has altered the biogeochemical cycling of  
13 C and N in a manner consistent with the microbial mechanisms described above. Experimental  
14  $\text{NO}_3^-$  deposition has increased leaf litter N concentration (+15%), reduced lignolytic extracellular  
15 enzyme activity in forest floor (-33%) and mineral soil (-10%), and decreased soil respiration (-  
16 15%; Burton et al. 2004, DeForest et al. 2004, 2005, Pregitzer et al. 2007). Although  
17 aboveground NPP has increased (+10%) under experimental  $\text{NO}_3^-$  deposition, the  
18 aforementioned responses have occurred despite no change in above- or belowground litter  
19 production (Burton et al. 2004, Pregitzer et al. 2007). Moreover, the biomass and respiration of  
20 fine roots, as well as microbial respiration in mineral soil, also have not been altered by  
21 experimental  $\text{NO}_3^-$  deposition (Burton et al. 2004, Zak et al. 2006), indicating that lower  
22 microbial activity in forest floor is likely responsible for declines in soil respiration (Zak et al.  
23 2004). Together, these observations provide evidence of the gradual accumulation of forest floor

1 and soil organic matter in this forest ecosystem (Pregitzer et al. 2007). Additional evidence  
2 suggests that anthropogenic N will accumulate in forest floor and surface mineral soil via a  
3 pathway also not included in some biogeochemical models. Using  $^{15}\text{NO}_3^-$  as a tracer, we have  
4 observed that  $\text{NO}_3^-$  was rapidly (i.e., hours) assimilated by soil microorganisms in forest floor  
5 and, after several days, was released as  $^{15}\text{NH}_4^+$  into soil solution;  $^{15}\text{NH}_4^+$  was then assimilated by  
6 overstory trees (i.e., weeks) where it enriched the canopy after one year (Zogg et al. 2000, Zak et  
7 al. 2004). In contrast to other studies (Nadelhoffer et al. 1999a,b, Magill et al. 1997, 2000), soil  
8 organic matter was not a sink for  $^{15}\text{NO}_3^-$  after one year, and we predicted  $^{15}\text{N}$  would accumulate  
9 in forest floor and soil organic matter only after  $^{15}\text{N}$ -enriched leaf litter was shed by the canopy.

10 Our objective was to determine if experimental  $\text{NO}_3^-$  deposition has altered organic  
11 matter and N stored in northern hardwood forests, especially in forest floor and mineral soil. To  
12 address this objective, we constructed organic matter and N budgets for a long-term study in  
13 which  $\text{NO}_3^-$  deposition has been experimentally manipulated across the north-south geographic  
14 range of northern hardwood forests. Additionally, we followed the flow of tracer  $^{15}\text{NO}_3^-$  in our  
15 experimental  $\text{NO}_3^-$  deposition treatment to determine if forest floor and soil organic matter  
16 became long-term sinks for  $\text{NO}_3^-$  deposition after it flowed through the soil microbial community  
17 and overstory trees.

## 18 METHODS

### 19 *Experimental Design*

20 We investigated the influence of chronic atmospheric  $\text{NO}_3^-$  deposition on the distribution  
21 of organic matter and N in four sugar maple (*Acer saccharum* Marsh)-dominated northern  
22 hardwood stands distributed across lower and upper Michigan, U.S.A (Fig. 1). Their locations  
23 span the north-south geographic range of this forest type, enabling us to generalize our

1 experimental results across this wide-spread and ecologically important ecosystem. Our research  
2 sites are floristically and edaphically similar (Burton et al. 1991), but they differ in climate along  
3 the north-south latitudinal gradient (Table 1). They also span a gradient of atmospheric N  
4 deposition ( $0.68$  to  $1.17$   $\text{g N m}^{-2} \text{y}^{-1}$ ), of which  $\text{NO}_3^-$ -N composes *ca.* 60% of wet-plus-dry  
5 deposition. Located in each site are six 30-m x 30-m plots, each surrounded by a 10-m wide  
6 treated buffer. Three plots in each site receive ambient atmospheric N deposition (Table 1). The  
7 remaining three plots in each site receive ambient N deposition plus  $3$   $\text{g NO}_3^-$ -N  $\text{m}^{-2} \text{y}^{-1}$ , a rate  
8 approaching that expected in large portions of North America by 2050 as well as over other  
9 portions of the Earth (Galloway et al. 2004). The additional  $\text{NO}_3^-$  is delivered during the  
10 growing season in six equal applications ( $0.5$   $\text{g N m}^{-2} \text{month}^{-1}$ ) of solid  $\text{NaNO}_3$  pellets, which are  
11 broadcast over the forest floor. In each plot, four porous-cup ceramic lysimeters were installed  
12 (75 cm depth) to quantify leaching losses of inorganic N and DON (Model 1900, Soilmoisture  
13 Equipment Corp., Santa Barbara, CA). Leaf litter in each plot is collected monthly throughout  
14 the growing season (biweekly during autumn) from four  $0.5$   $\text{m}^2$  litter traps (Pregitzer et al. 2007).  
15 We constructed a detailed organic matter and N budget for each plot receiving ambient ( $n = 3$ )  
16 and experimental ( $n = 3$ ) deposition in all four replicate stands. These data were gathered after  
17 11 years of treatment in September 2004.

### 18 *Field Sampling*

19 In each site ( $n = 4$ ), we identified and measured the diameter of all trees ( $> 5$  cm at breast  
20 height) in each ambient ( $n = 3$ ) and experimental  $\text{NO}_3^-$  ( $n = 3$ ) deposition plot. Tissue samples in  
21 each plot were collected from four widely spaced, dominant overstory trees. For each of these  
22 trees, we used a shotgun to obtain sun-lit canopy leaves and attending branches. Bark and stem  
23 wood were removed with a 2.5-cm-diameter hole saw, which was inserted into each stem at

1 breast height. We also sampled large structural roots near the base of each tree stem using a hole  
2 saw. Fine roots were collected at three random locations in each ambient and experimental N  
3 deposition plot by removing a 10-cm deep soil core (10 cm dia.), which contained Oe, Oa, A and  
4 E horizon material. Soil cores were stored in a freezer until they could be processed. Roots were  
5 carefully removed by hand and sorted into four size classes: 0.5 to 1.0 mm, 1.0 to 2.0 mm, 2.0 to  
6 5.0 mm, and 5.0 to 10 mm (*sensu* Zak et al. 2004); the biomass of fine roots <0.5 mm was  
7 determined by elutriation (Burton et al. 2000). Forest floor samples were collected within a 30-  
8 cm x 30-cm area at 8 random locations in each plot. To determine the depth distribution of  
9 organic matter and N in soil, we used a core (10 cm dia.) extending from the top of the Oe  
10 horizon to a depth of 70 cm. Soil from each core was collected in increments of 0 to 10 cm, 10  
11 to 30 cm, 30 to 50 cm and 50 to 70 cm. The field-fresh mass of each core section was obtained,  
12 and a subsample was oven dried (105 °C); bulk density was estimated using the dry weight and  
13 volume of each core section.

#### 14 *Ecosystem Biomass and N Pools*

15 Canopy leaf mass was estimated from leaf litter fall using a correction factor of 1.14 to  
16 adjust for changes in specific leaf area prior to abscission (Burton et al. 1991, 1993). Species-  
17 specific allometric equations were used to estimate the biomass of overstory branch, stem bark,  
18 stem wood, and structural roots (> 10 cm dia.; Whittaker et al. 1974). The biomass of fine roots  
19 in each size class was expressed on an areal basis ( $\text{g/m}^2$ ) using the oven dry mass (70 °C) of  
20 material recovered from each 10-cm-diameter root core. Forest floor was separated into Oi and  
21 Oe/a horizons, and material from each horizon was oven dried at 70 °C to estimate its biomass.  
22 We used a NC 2500 elemental analyzer (CE Elantech, Lakewood, NJ) interfaced to a Delta Plus  
23 isotope ratio mass spectrometer (Thermo Finnigan, San Jose, CA) to determine the N

1 concentrations of plant tissue, forest floor and soil samples (0 to 70 cm cores). We also used this  
2 instrument to obtain the C concentration of each soil sample, and we estimated soil organic  
3 matter content assuming 470 mg C/g SOM.

4 The N content of plant and forest floor pools (O<sub>i</sub> and O<sub>e/a</sub>) was calculated as the product  
5 of their biomass (g/m<sup>2</sup>) and N concentration (mg N/g). The N content of each soil sample was  
6 calculated as the product of its bulk density (g/cm<sup>3</sup>), depth, and N concentration (μg N/g); soil  
7 organic matter content (g/m<sup>2</sup>) was estimated in a similar manner. The 0 to 10 cm section of our  
8 70-cm core contained O<sub>e/a</sub> material. Using the data from our forest floor sample of the O<sub>e/a</sub>  
9 horizon, we subtracted the biomass and N content of this material from the 0 to 10 cm section of  
10 the 70-cm soil core, to calculate organic matter and N content of the mineral surface horizon (A  
11 and A, E horizons).

12 To determine inorganic N, DON, and microbial N, we collected three 2.5 cm diameter  
13 soil cores (0 to 10 cm) at random locations in each plot (*sensu* Holmes et al. 2003); which were  
14 subsequently composited in each plot. A 12-g field-fresh subsample of root-free soil from each  
15 plot was placed in a 30 mL glass vial containing 20 mL of 2 mol/L KCl. The vials were capped,  
16 placed on a shaker for 20 min, then centrifuged for 15 min at 136 g. Particulate organic matter  
17 and suspended cells were removed by passing the solution through the 0.45 μm filter; the  
18 resulting filtrate contained inorganic N and DON. The same sample was extracted a second time  
19 with a 20-mL aliquot of 2 mol/L KCl. Ammonium-N and NO<sub>3</sub><sup>-</sup>-N concentrations in the filtrates  
20 were determined by automated colorimetry using an OI Analytical Flow Solution 3000  
21 continuous flow analyzer (OI Analytical, College Station, Texas, USA). Following alkaline  
22 persulfate digestion of the filtrate, DON (as NO<sub>3</sub><sup>-</sup>-N) was measured using automated colorimetry  
23 as described above.

1           A second extraction step was performed to separate microbial N and soil organic N. The  
2 KCl-extracted soil and filter remaining in the vials was fumigated with CH<sub>3</sub>Cl for 5 d in a  
3 vacuum desiccator. Residual CH<sub>3</sub>Cl was removed by repeated vacuuming, and 20 mL of  
4 0.25mol/L K<sub>2</sub>SO<sub>4</sub> was added to each vial. Vials were capped, placed on a shaker 30 min, and  
5 centrifuged for 15 min at 136 g. The supernatant was placed in a 120-mL specimen cup; this  
6 extraction was repeated with an additional 20-mL aliquot of K<sub>2</sub>SO<sub>4</sub>. Microbial N was  
7 determined by alkaline persulfate digestion of the K<sub>2</sub>SO<sub>4</sub> solution (Cabrera and Beare 1993),  
8 followed by automated colorimetry for NO<sub>3</sub><sup>-</sup>-N as described above. The soil remaining in the  
9 vial was dried to a constant weight at 60 °C, ground using a SamplePrep 8000mixer/ mill (Spex  
10 Centriprep, Inc. Metuchen, NJ, USA), and analyzed using a CE Instruments NC2500 elemental  
11 analyzer (CE Elantech, Lakewood, NJ) interfaced to a Delta Plus isotope ratio mass spectrometer  
12 (Thermo Finnigan, San Jose, CA). The N content of inorganic N, DON, and microbial N was  
13 calculated as the product of N concentration, bulk density, and sample depth (0 to 10 cm).

14           To estimate amounts of NH<sub>4</sub><sup>+</sup> NO<sub>3</sub><sup>-</sup> and DON leaching from our experiment, we collected  
15 soil solution from the four lysimeters located in each plot receiving ambient and experimental N  
16 deposition. Each lysimeter was evacuated on a 2-week interval during autumn 2003, spring  
17 2004, and autumn 2004. A tension of 0.05 MPa was placed on each lysimeter after evacuation,  
18 and soil solution was composited on a plot basis. Prior to laboratory analyses, we passed each  
19 composite sample through a 0.45 μm filter membrane. Ammonium, NO<sub>3</sub><sup>-</sup> and DON were  
20 analyzed as described above. To estimate leaching losses of these compounds, we calculated a  
21 water balance to estimate the volume of water passing below the rooting zone of each site  
22 (Thornthwaite and Mather 1957). Air temperatures and precipitation, measured continuously at  
23 each site, were used to calculate a water balance for each site. Monthly leaching losses were

1 estimated as the product of mean N concentration ( $\mu\text{gN/mL}$ ) and leaching volume ( $\text{mL/cm}^2$ );  
2 monthly losses were summed to estimate annual losses of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and DON.

### 3 *Experimental $\text{NO}_3^-$ Deposition and Flow of $^{15}\text{N}$*

4 In a previous study, we applied tracer amounts of  $^{15}\text{NO}_3^-$  to follow the flow of  
5 anthropogenic  $\text{NO}_3^-$  through this ecosystem (Zak et al. 2004). In 1998, plots ( $n = 3$ ) receiving  
6 experimental  $\text{NO}_3^-$  deposition in Site B (Fig. 1) were each labeled with 24 g of  $^{15}\text{N}$ . The isotope  
7 was applied by mixing 99% atom excess  $\text{Na}^{15}\text{NO}_3$  with the June, July and August application of  
8  $\text{NaNO}_3^-$ , thereby labeling the forest floor. One year after isotope addition, the primary sink for  
9  $^{15}\text{N}$  was the overstory canopy, whereas no isotope was recovered in surface soil (Zak et al.  
10 2004). Our results indicated that soil organic matter was not an initial sink for anthropogenic N,  
11 at least not over a 1 year time step. We predicted that litterfall from the  $^{15}\text{N}$ -enriched canopy  
12 would eventually enrich mineral soil, making soil a long-term sink for anthropogenic N (Zak et  
13 al. 2004). To test this prediction, we quantified the distribution of  $^{15}\text{N}$  6 years following isotope  
14 addition.

15 Our overall field sampling and analytical approach enabled us to determine the  $^{15}\text{N}$   
16 content ( $\text{mg }^{15}\text{N/m}^2$ ) of plant, forest floor, soil pools and leaching losses. To test our prediction  
17 regarding the flow of anthropogenic  $\text{NO}_3^-$ , we compared  $^{15}\text{N}$  recovery in each ecosystem pool 1  
18 year and 6 years after isotope addition. Our approach for determining the  $^{15}\text{N}$  content of each  
19 ecosystem pool is described in detail by Zak et al. (2004). Prior to isotope addition, we  
20 determined the amount of  $^{15}\text{N}$  in each ecosystem pool (i.e., the product of atom %  $^{15}\text{N}$  and N  
21 content in  $\text{g N/m}^2$ ; see Zak et al. 2004). We used the same sampling and analytical approach to  
22 then determine the  $^{15}\text{N}$  content of each ecosystem pool 1 yr following isotope addition; the  
23 identical approach was used here to quantify the distribution of label  $^{15}\text{N}$  6 yrs after application.

1 The amount of  $^{15}\text{N}$  label residing in each ecosystem pool is the difference between its  $^{15}\text{N}$   
2 content 6 years after isotope addition ( $\text{mg } ^{15}\text{N}/\text{m}^2$ ) and its  $^{15}\text{N}$  content prior to isotope addition.  
3 In this calculation, we also accounted for the amount of  $^{15}\text{N}$  added via our routine application of  
4  $\text{NaNO}_3$  (0.36646 atom %  $^{15}\text{N}$ ). Isotope recovery is expressed as the percent of applied  $^{15}\text{N}$   
5 ( $37.67 \text{ mg } ^{15}\text{N}/\text{m}^2$ ) residing in each ecosystem pool, and we compared recoveries 1 and 6 years  
6 following isotope addition.

### 7 *Statistical Analyses*

8 We used a 2-way ANOVA to determine whether  $\text{NO}_3^-$  deposition treatment ( $df = 1$ ), site  
9 ( $df = 3$ ) or their interaction ( $df = 3$ ) had a significant effect on the biomass, N concentration and  
10 N content of plant, forest floor and soil pools. The interaction term in this model tested the null  
11 hypothesis that replicate stands responded in the same manner to our experimental N deposition  
12 treatment, thus enabling us to determine if we could generalize our results across the geographic  
13 range of sugar maple-dominated northern hardwood forests in the Lake States region. For each  
14 plot, we also calculated forest floor turnover (yrs) as the quotient of forest floor mass ( $\text{Oe}/a$ ) and  
15 aboveground litter fall in order to determine if chronic N deposition slowed forest floor  
16 decomposition, as we had predicted. To test this hypothesis, we also used a 2-way ANOVA  
17 consisting of treatment, site and their interaction. For the 3 plots receiving experimental N  
18 deposition in Site B, we used 1-way ANOVA to compare  $^{15}\text{N}$  recovery in each ecosystem pool 1  
19 y and 6 yrs following isotope addition; these data were log transformed to meet the assumption  
20 of normality. A protected Fishers LSD procedure was used to compare means; significance for  
21 all statistical analyses was accepted at  $\alpha = 0.05$ .

22

## RESULTS

### *Ecosystem Biomass*

Experimental N deposition and site did not interact to influence the biomass of any plant component ( $P = 0.268$  to  $0.924$ ), nor did they interact to influence forest floor biomass ( $O_i$ ,  $P = 0.449$ ;  $O_e/a$ ,  $P = 0.700$ ) or soil organic matter at any depth ( $P = 0.114$  to  $0.437$ ). 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 litterfall (Fig. 2A) or fresh litter ( $O_i$ ) on the forest floor (Table 3 & Fig. 2A). However, the mass of partly decomposed litter ( $O_e/a$ ) 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 ( $O_e/a$ ) in our experimental N deposition treatment. Further evidence for slower decomposition in our experimental  $\text{NO}_3^-$  deposition treatment the significantly slower turnover of partly decomposed litter in the forest floor, relative to the ambient treatment (Fig. 2 C). Although surface mineral soil (0 to 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 to 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 to 5 mm) was influenced by a significant interaction between N deposition and site. However, interaction means for bark N

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

7 Experimental N deposition (main effect) significantly increased N concentrations in  
8 canopy leaves and structural roots, but it had no effect on N concentration in any other plant  
9 tissue (Table 2). Similarly, the N concentration of forest floor (Oi and Oe/a) was not altered our  
10 experimental treatment (Table 3). However, experimental N deposition did elicit a significant  
11 60% increase in the N concentration of surface mineral soil (0 to 10 cm; Table 3); the N  
12 concentration of soil deeper in the profile (i.e., 10 to 70 cm) was not influenced by our  
13 treatments. In the 0 to 10 cm soil depth, microbial N averaged  $39.0 \pm 4.33$  mg N/g in the ambient  
14 treatment and  $38.6 \pm 5.05$  mg N/g under experimental N deposition; these means were not  
15 different. Similarly, experimental N deposition had no influence on extractable  $\text{NH}_4^+$   
16 concentrations in the 0 to 10 cm soil depth ( $8.3 \pm 2.03$  vs  $7.9 \pm 3.72$   $\mu\text{g N/g}$ ; ambient vs.  
17 experimental N deposition). However, the concentration of extractable  $\text{NO}_3^-$  was significantly  
18 higher under experimental N deposition (8.9  $\mu\text{g N/g}$ ), relative to the ambient treatment (2.3  $\mu\text{g}$   
19 N/g). The mean N concentration of leached  $\text{NH}_4^+$  was equivalent between ambient and  
20 experimental N deposition treatments (Table 3), whereas experimental N deposition resulted in a  
21 10-fold increase in the mean N concentration of leached  $\text{NO}_3^-$ -N as well as an 8-fold increase in  
22 the mean leached DON concentration (Table 3).

23

## 1 *Ecosystem N Content*

2           Experimental N deposition significantly increased the N content ( $\text{g N/m}^2$ ) of canopy  
3 leaves, but it did not alter the N content of any other plant component (Table 2). For example,  
4 the N content of canopy leaves increased *ca.* 25% under experimental N deposition, whereas the  
5 N content of other plant components was approximately equivalent (Table 2). Similarly, the N  
6 content of fresh litter (Oi) and partly decomposed litter (Oe/a) in the forest floor increased by 30  
7 to 38% under experimental N deposition, but these increases were not significant (Table 3). In  
8 contrast, experimental N deposition significantly increased the N content of surface mineral soil  
9 (0 to 10 cm) by 34% (Table 3). We found no effect of experimental N deposition on N content  
10 deeper in the soil profile (10 to 70 cm; Table 3). However, when we summed the N content of  
11 forest floor and mineral soil, experimental N deposition significantly increased (*ca.* 10%) the  
12 total N content of these combined ecosystem pools (Table 3). In the 0 to 10 cm soil depth,  
13 experimental N deposition had no effect on the N content of microbial biomass (4.2 vs. 4.1 g  
14  $\text{N/m}^2$ ; ambient vs. experimental N deposition) or extractable  $\text{NH}_4^+$  (0.89 vs. 0.82  $\text{g N/m}^2$ ).  
15 However, the N content of extractable  $\text{NO}_3^-$  in the 0 to 10 cm soil depth was significantly higher  
16 under experimental N deposition (1.59 vs 0.37  $\text{g N/m}^2$ ). Consistent with our previous results  
17 (Pregitzer et al. 2004), experimental N deposition significantly increased the amount of N (g  
18  $\text{N/m}^2$ ) exported via leaching as  $\text{NO}_3^-$  and DON, but it did not alter leaching losses of  $\text{NH}_4^+$   
19 (Table 3). The combined annual leaching loss of  $\text{NO}_3^-$ -N and DON from the experimental  $\text{NO}_3^-$   
20 deposition treatment is an order of magnitude greater than under the ambient treatment;  
21 moreover, it was equivalent to 93% of the  $\text{NO}_3^-$ -N delivered by our treatment in 2004.

22



1 appeared to have slowed organic matter decomposition (Fig. 2) and increased the mass of  
2 partially decomposed litter in the forest floor, resulting in overall accumulation of organic matter  
3 on the soil surface. This response is equivalent to the enhancement of ANPP (+10%) by  
4 experimental  $\text{NO}_3^-$  deposition, which has not resulted in greater overstory biomass or overstory  
5 N content due to an acceleration of tree mortality (Pregitzer et al. 2007). Course woody litter  
6 produced by greater tree mortality would have been missed by our sampling scheme, and over  
7 time, would further increase the accumulation of organic matter in surface soil. The degree to  
8 which experimental  $\text{NO}_3^-$  deposition has increased soil organic matter (*ca.*  $215 \text{ g m}^2 \text{ y}^{-1}$  or  $100 \text{ g}$   
9  $\text{C m}^2 \text{ y}^{-1}$ ) over our decade-long experiment indicates that such a mechanism should be considered  
10 in biogeochemical models simulating the influence of atmospheric N deposition on ecosystem C  
11 storage in northern temperate forests. Moreover, greater rates of NPP (Pregitzer et al. 2007) in  
12 combination with organic matter accumulation in forest floor and surface mineral soil indicate  
13 that atmospheric N deposition will increase C storage in this wide-spread forest ecosystem.

14         Before the end of this century, much of eastern North America will receive atmospheric  
15 N deposition approaching our experimental treatment (Galloway et al. 2004), which could  
16 rapidly increase soil organic matter accumulation across the extent of northern hardwood forests  
17 like those in our study. Our experiment was specifically designed to test the null hypothesis that  
18 replicate stands would respond similarly to elevated atmospheric N deposition, a key test for  
19 projecting our experimental inference across the geographic expanse of this northern hardwood  
20 ecosystem. We were unable to reject this hypothesis for the majority of pools composing our  
21 organic matter and N budgets, indicating that pools were similarly influenced by atmospheric N  
22 deposition across replicate stands. If the stands we studied are representative of others across the  
23 region, then organic matter and N likely will accumulate in forest floor and surface mineral soil

1 as atmospheric N deposition increases over the next century, increasing C storage in this  
2 common forest ecosystem. On the other hand, forests which differ in overstory composition, and  
3 hence litter biochemistry, can respond in an opposing manner (Waldrop et al. 2004, Knorr et al.  
4 2005), restricting the relevance of our results to northern hardwood forests dominated by *Acer*  
5 *saccharum*.

6         Several convergent lines of evidence indicate that experimental  $\text{NO}_3^-$  deposition has  
7 slowed microbial activity and altered decomposition in the forest floor. Foremost, experimental  
8  $\text{NO}_3^-$  deposition has increased the turnover time and forest floor mass (Oe/a) as well as the  
9 production of DOC from this soil horizon (Fig. 2; Pregitzer et al. 2004, Smemo et al., 2007).  
10 Taken together with the higher phenolic concentration of forest floor DOC (Smemo et al. 2007),  
11 these findings suggest experimental  $\text{NO}_3^-$  deposition has fundamentally altered the microbial  
12 metabolism of plant detritus, especially leaf litter. Consistent with our expectations,  
13 experimental  $\text{NO}_3^-$  deposition increased the N concentration of leaf litter (15%; Pregitzer et al.  
14 2007) as well as extractable  $\text{NO}_3^-$  in soil solution (+300%; Table 3), both of which are known to  
15 slow the microbial metabolism of lignin and humus (Fog 1988, Berg and Matzner 1997, Berg  
16 and Mentemeyer 2002). These observations are consistent with declines in phenol oxidase and  
17 peroxidase activity in forest floor and mineral soil (DeForest et al. 2004, 2005), which mediate  
18 the non-specific oxidation of polyphenols contained in both lignin and humus. Experimental  
19  $\text{NO}_3^-$  deposition also has not altered the lignin concentration of leaf litter (140 mg/g) or fine roots  
20 (340 mg/g; J. Eikenberry and K. Pregitzer, *unpublished data*), eliminating the possibility that the  
21 greater forest floor mass in our experimental  $\text{NO}_3^-$  deposition treatment resulted from the  
22 production of more lignified litter. These lines of evidence are support the idea that experimental  
23  $\text{NO}_3^-$  deposition has suppressed and altered the microbial degradation of lignin and humus.

1 Experimental  $\text{NO}_3^-$  deposition has clearly altered microbial activity in the forest floor, a  
2 response that could arise from a shift in the function of lignin degrading microorganisms. White-  
3 rot basidiomycetes are the dominant agents of lignin degradation in soil, and their ability to  
4 produce phenol oxidase and other lignolytic enzymes can be repressed by high inorganic N (Tien  
5 and Tu 1987, Boominathan et al. 1990, Vanderwoude et al. 1993, Li et al. 1994). In contrast to  
6 white-rot fungi, the synthesis of lignolytic enzymes by brown-rot fungi is unresponsive to N  
7 availability (Reddy and D'Souza 1994, D'Souza et al. 1996, Worrall et al. 1997), whereas phenol  
8 oxidase and peroxidase synthesis by soil actinobacteria is upregulated by greater N availability  
9 (Bardner and Crawford 1981, Giroux et al. 1988). By increasing inorganic N concentrations in  
10 soil solution (see Results), chronic N deposition could suppress lignin mineralization by white-  
11 rot fungi, shifting this process to organisms which are unaffected (i.e, brown-rot fungi) or  
12 positively affected (i.e., actinomycetes) by greater inorganic N availability. Unlike white-rot  
13 basidiomycetes which mineralize lignin to  $\text{CO}_2$ , actinobacteria metabolize lignin into soluble  
14 polyphenolics (Mason et al. 1988, Godden et al. 1992, Berrocal et al. 1997). This response could  
15 explain increases in DOC production and phenolic content in the experimental  $\text{NO}_3^-$  deposition  
16 treatment (Table 3; Smemo et al. 2007). Because slow-growing white-rot fungi are poor  
17 competitors for labile organic substrates, a reduction in their ability to metabolize lignin could  
18 decrease their abundance or activity. Recent evidence indicates that the abundance of these  
19 organisms has not been altered in our experiment (Hassett et al. 2007), implying that their  
20 activity has been lowered by  $\text{NO}_3^-$  deposition. Such a response would reduce lignolytic enzyme  
21 activity in forest floor, because both brown-rot basidiomycetes and actinomycetes produce  
22 smaller amounts of these extracellular enzymes than do white-rot basidiomycetes (Ramachandra  
23 et al. 1987, D'Souza et al. 1996). The shift in microbial community composition and activity

1 described above is consistent with the decline in phenol oxidase and peroxidase activity, the  
2 increase in forest floor mass, and the greater production of phenolic DOC induced by  
3 experimental  $\text{NO}_3^-$  (DeForest et al. 2004, Pregitzer et al. 2004, Smemo et al. 2007). It also is  
4 consistent with similar observations in different forest ecosystems receiving experimental N  
5 deposition (Frey et al. 2004) as well as the positive relationship between ambient rates of  
6 atmospheric N deposition (*ca.* 0.5 to 4.5 g N m<sup>-2</sup> y<sup>-1</sup>) and streamwater DOC/DON in forested  
7 watersheds of eastern North America (Brookshire et al. 2007). The shift in microbial community  
8 composition and function we describe above is a plausible mechanism by which  $\text{NO}_3^-$  deposition  
9 has altered soil C cycling in our experiment; it remains a hypothesis to be tested.

10       The N from our experimental  $\text{NO}_3^-$  deposition treatment has accumulated in soil to a  
11 greater extent than in plant biomass, and it has done so via a pathway different from a rapid  
12 immobilization N into forest floor and mineral soil. In our previous study,  $^{15}\text{NO}_3^-$  was  
13 assimilated by the microbial community over a time scale of minutes, and within hours, was  
14 released into soil solution as  $\text{NH}_4^+$  that was subsequently taken up by plant roots over several  
15 weeks (Zogg et al. 2000). Also within hours of application, a substantial amount of  $^{15}\text{N}$  was  
16 incorporated into forest floor and soil organic matter (Zogg et al. 2000). However, virtually no  
17  $^{15}\text{N}$  label was detected in soil organic matter after one year (Zak et al. 2004), indicating it had  
18 been released into soil solution and either assimilated by plants or lost to leaching. This  
19 observation contrasts with other studies in which the majority of tracer  $^{15}\text{N}$  resided in forest floor  
20 and soil organic matter after one year (Nadelhoffer et al. 1999a,b, Magill et al. 1997, 2000). IN  
21 contrast, the overstory canopy contained the greatest amount of  $^{15}\text{N}$  after one year in our study,  
22 suggesting that soil organic matter would become a sink for anthropogenic  $\text{NO}_3^-$  only after the  
23 shedding of  $^{15}\text{N}$ -enriched leaf litter and its subsequent decomposition into humus. The fact that

1  $^{15}\text{N}$  recovery in overstory trees declined after 6 years while isotope recovery in soil organic  
2 matter significantly increased (Table 4), supports the prediction that soil organic matter became a  
3 sink for anthropogenic  $\text{NO}_3^-$  only after it has moved through the microbial community and has  
4 subsequently been assimilated by plants and shed in leaf litter. Interestingly, fine roots contained  
5 small amounts of isotope, further indicating that the  $^{15}\text{N}$  residing in forest floor and organic  
6 matter after 6 years was derived from canopy leaves. This insight provides a potential  
7 mechanism for the accumulation of N in the forest floor and surface mineral soil of our  
8 experimental  $\text{NO}_3^-$  deposition treatment (Table 3).

9         Our  $^{15}\text{N}$  tracer experiment provides insight into the time steps and processes by which  
10 anthropogenic  $\text{NO}_3^-$  was retained in this ecosystem. Although total recovery of applied  $^{15}\text{N}$  was  
11 relatively low, it was similar between years 1 and 6 of this study (17-20%). This observation  
12 suggests that the rapid assimilation of  $^{15}\text{NO}_3^-$  by soil microorganisms set in motion a series of  
13 events which retained *ca.* 20% of anthropogenic N within this ecosystem over a 5-year duration.  
14 Further, leaching of inorganic and organic N annually equals approximately 70-90% of the  $\text{NO}_3^-$   
15 applied in our experimental treatment (Table 3; Pregitzer et al. 2004), which is almost equivalent  
16 to the proportion of isotope tracer we were unable to recover. These observations imply that  
17  $\text{NO}_3^-$  is subject to loss, if it is not initially assimilated by the microbial community and retained  
18 by the sequence of events described above. Over time, it is likely that the slowing of  
19 decomposition by  $\text{NO}_3^-$  deposition, which we document here, will further lead to the  
20 accumulation of N in organic matter contained in forest floor and surface mineral soil. It will be  
21 important to understand the rate and time steps by which N is released from these pools as well  
22 as its subsequent fate (Currie et al. 2004); those processes will control the long-term potential for  
23 anthropogenic N to be sequestered in this northern temperate forest.

## 1 *Summary & Implications*

2           A decade of experimental  $\text{NO}_3^-$  deposition, at a rate approaching that expected in the next  
3 several decades (Galloway et al. 2004), has increased the accumulation of organic matter in  
4 forest floor and soil, a response that was consistent across a large geographic region. Moreover,  
5 the organic matter accumulated in forest floor and soil resulted from a direct slowing of  
6 decomposition by experimental  $\text{NO}_3^-$  deposition, rather than from greater rates of detritus  
7 production. Our previous work demonstrated that  $\text{NO}_3^-$  deposition can lower the activity of key  
8 extracellular enzymes involved in lignin and humus degradation in some litter-decomposing  
9 fungi, which is consistent with the slowing of decomposition and the resultant accumulation of  
10 organic matter in forest floor (Oe/a) and surface soil. Inasmuch, atmospheric  $\text{NO}_3^-$  deposition  
11 appears to have increased C storage in this particular forest ecosystem via a direct effect on the  
12 composition and function of the heterotrophic microbial community; further work is necessary to  
13 evaluate this hypothesis and proposed mechanism. However, the accumulation of organic matter  
14 we document may not occur in forest ecosystems which differ in litter biochemistry, especially  
15 those in which  $\text{NO}_3^-$  deposition could aid microbial cellulose metabolism, increase  
16 decomposition, and decrease organic matter in forest floor and soil (Sinsabaugh et al. 2002,  
17 Waldrop et al. 2004; Knorr et al. 2005). Additionally, the N we have applied has accumulated in  
18 soil organic matter, and it has done so by first flowing through microbial and plant pools, and  
19 then reentering soil via leaf litter. This pathway differs from other studies in which  
20 anthropogenic N is initially immobilized into organic matter, gradually mineralized over time,  
21 and then eventually assimilated by plants (Currie et al. 2004).

22           Our results demonstrate that the manner in which soil microbial communities respond to  
23 anthropogenic N deposition is central to understanding how this environmental change will

1 influence the storage of C and N in northern temperate forests. The representation of these  
2 mechanisms in simulation models predicting the influence of atmospheric N deposition on  
3 ecosystem C storage would better represent the underlying biological processes contributing to  
4 the current sink for anthropogenic CO<sub>2</sub> in northern temperate forests, and perhaps the Northern  
5 Hemisphere. More importantly, the accumulation organic matter in forest floor and surface  
6 mineral soil we document here, in combination with enhanced rates of NPP (Pregitzer et al.  
7 2007), provides evidence that future rates of atmospheric N deposition could increase C storage  
8 in an ecologically and economically important temperate forest with a wide geographic  
9 distribution in eastern North America.

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Table 1. Climatic, floristic and edaphic characteristics of four northern hardwood sites receiving experimental atmospheric  $\text{NO}_3^-$  deposition. Sites are located in lower and upper Michigan, U.S.A., and they have been receiving experimental  $\text{NO}_3^-$  deposition beginning in 1994. Stands are similar in age, plant composition, and soil development, but they differ in temperature and growing season length.

	<b>Site</b>			
	A	B	C	D
<b>I. Location</b>				
Latitude, N	46°52'	45°33'	44°23'	43°40'
Longitude, W	88°53'	84°52'	85°50'	86°09'
<b>II. Climate</b>				
Mean Annual Temperature ( $^{\circ}\text{C}$ )	4.7	6.0	6.9	7.6
Mean Annual Precipitation (mm)	873	871	888	812
Wet + Dry $\text{NO}_3^-$ -N Deposition ( $\text{g m}^{-2} \text{y}^{-1}$ )	0.38	0.58	0.78	0.76
Wet + Dry Total N Deposition ( $\text{g m}^{-2} \text{y}^{-1}$ )	0.68	0.91	1.17	1.18

Table 2. Overstory biomass, N concentration and N content in four northern hardwood stands receiving experimental  $\text{NO}_3^-$  deposition treatments. Values are for 2004, the eleventh year of treatment. For each overstory component, significance between ambient and experimental  $\text{NO}_3^-$  deposition treatment means ( $n = 12$ ) is denoted as  $P < 0.001$ \*\*\*;  $P < 0.01$ \*\*, and  $P < 0.05$ \*. Standard deviations are in parentheses.

Ecosystem Component	Ambient $\text{NO}_3^-$		Ambient $\text{NO}_3^-$		Ambient $\text{NO}_3^-$	
	Biomass g/m <sup>2</sup>		N Concentration mg N/g		N Content g N/m <sup>2</sup>	
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						
Structural > 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 to 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 to 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 to 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 to 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)
<b>Total Overstory</b>	30382 (3391.2)	30147 (5137.0)	--	--	1019.2 (514.81)	835.5 (172.74)

Table 3. Biomass, N concentration and N content of forest floor and soil in four northern hardwood forest stands receiving experimental  $\text{NO}_3^-$  deposition treatments for 10 years. For each forest floor and soil pool, significance between ambient and experimental  $\text{NO}_3^-$  deposition treatment means ( $n = 12$ ) is denoted as  $P < 0.001^{***}$ ;  $P < 0.01^{**}$ , and  $P < 0.05^*$ . Standard deviations are in parentheses.

<b>Ecosystem Component</b>	<b>Ambient Organic Matter g/m<sup>2</sup></b>	<b><math>\text{NO}_3^-</math> g/m<sup>2</sup></b>	<b>Ambient N Concentration<sup>†</sup></b>	<b><math>\text{NO}_3^-</math> N Concentration<sup>†</sup></b>	<b>Ambient N Content<sup>‡</sup> g N/m<sup>2</sup></b>	<b><math>\text{NO}_3^-</math> N Content<sup>‡</sup> g N/m<sup>2</sup></b>
<b>Forest Floor</b>						
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 <sup>**</sup> (1196.9)	15.7 (2.71)	14.6 (4.07)	26.2 (11.42)	33.9 (12.72)
<b>Mineral Soil</b>						
0 to 10 cm <sup>§</sup>	3730 (1303.3)	4406 (2662.8)	1.33 (0.412)	2.13 <sup>*</sup> (1.089)	142.5 (32.21)	190.5 <sup>*</sup> (62.33)
10 to 30 cm	5388 (1238.6)	5402 (1351.4)	0.64a (0.159)	0.62b (0.150)	168.1 (39.33)	170.2 (35.39)
30 to 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 to 70 cm	2396 (570.3)	2864 (1085.4)	0.27 (0.063)	0.26 (0.081)	73.1 (18.41)	80.7 (25.85)
<b>Total Forest Floor &amp; Soil</b>	17557 (2310.3)	19718 <sup>**</sup> (3991.7)	--	--	529.9 (51.42)	578.9 <sup>*</sup> (95.56)
Leached $\text{NH}_4^+$ -N	--	--	0.22 (0.357)	0.19 (0.178)	0.07 (0.124)	0.07 (0.074)
Leached $\text{NO}_3^-$ -N	--	--	0.50 (0.566)	4.8 <sup>**</sup> (1.72)	0.19 (0.226)	1.92 <sup>**</sup> (0.583)
Leached DON	--	--	0.30 (0.117)	2.38 <sup>**</sup> (1.686)	0.09 (0.046)	0.86 <sup>**</sup> (0.811)

<sup>†</sup>Note: Forest floor and soil organic matter N concentration is mg N/g. Units for leached  $\text{NO}_3^-$  and DON concentrations are  $\mu\text{g N/mL}$ .

<sup>‡</sup>Note: Leached  $\text{NO}_3^-$  and DON are in units of  $\text{g N m}^{-2} \text{y}^{-1}$ .

<sup>§</sup>Note: Soil N content includes microbial N, extractable inorganic N and extractable DON.

Table 4. Mean recovery of  $^{15}\text{N}$  in ecosystem pools 1 year and 6 years after application of  $^{15}\text{NO}_3^-$  to the experimental  $\text{NO}_3^-$  deposition treatment in Site B. Total recover after 6 yrs includes soil N from 10-30 cm ( $1.3 \pm 0.27\%$ ), 30 to 50 cm ( $0.5 \pm 0.41\%$ ), and 50 to 70 cm ( $0.1 \pm 0.38\%$ ); these depths were not sampled 1 yr after  $^{15}\text{N}$  addition.

Ecosystem Component	$^{15}\text{N}$ Recovery (% Applied $^{15}\text{N}$ )		P
	After 1 Year	After 6 Years	
<b>I. Overstory</b>			
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)	<b>0.023</b>
Roots			
Structural > 10.0 mm	1.27 (0.401)	0.853 (0.196)	0.178
10.0 to 5.0 mm	0.28 (0.136)	0.00 (0.132)	<b>0.026</b>
5.0 to 2.0 mm	0.32 (0.312)	0.00 (0.040)	0.159
2.0 to 1.0 mm	0.21 (0.154)	0.00 (0.037)	0.056
1.0 to 0.5 mm	0.18 (0.139)	0.00 (0.029)	0.084
< 0.5 mm	0.88 (0.387)	0.00 (0.346)	<b>0.025</b>
<b>Total Overstory</b>	13.51 (5.096)	5.47 (0.336)	<b>0.050</b>
<b>II Forest Floor</b>	2.54 (2.200)	1.89 (0.451)	0.640
<b>III. Soil (0 to 10 cm)</b>			
Organic N	0.00 (0.000)	10.02 (2.860)	<b>0.004</b>
Extractable $\text{NH}_4^+$	0.00 (0.000)	0.08 (0.029)	<b>0.008</b>
Extractable $\text{NO}_3^-$	0.00 (0.000)	0.24 (0.197)	0.102
Microbial N	0.01 (0.024)	0.300 (0.087)	<b>0.005</b>
Leached $\text{NO}_3^-$	1.11 (0.746)	0.00 (0.001)	0.062
Leached DON	0.27 (0.242)	0.00 (0.005)	0.129
<b>IV. Total Recovery</b>	17.45 (7.016)	19.86 (3.436)	0.622

### List of Figures

Fig 1. The distribution of study sites spanning the geographic range of northern hardwood forests in the Upper Lake States Region. 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 m x 50 m) receive ambient atmospheric N deposition and three plots have received an additional  $3 \text{ g NO}_3^- \text{-N m}^{-2} \text{ y}^{-1}$  since 1994.

Fig 2. Aboveground litterfall (Panel A), forest floor (Oe and Oa horizons; Panel B), and forest floor turnover (Panel C) under ambient and experimental atmospheric N deposition.

Experimental N deposition had no significant effect on aboveground litter production, but it significantly increase forest floor mass and turnover time. Values are treatment means in 2004 ( $n = 12$ ) and the error bars are one standard deviation.

Fig. 1

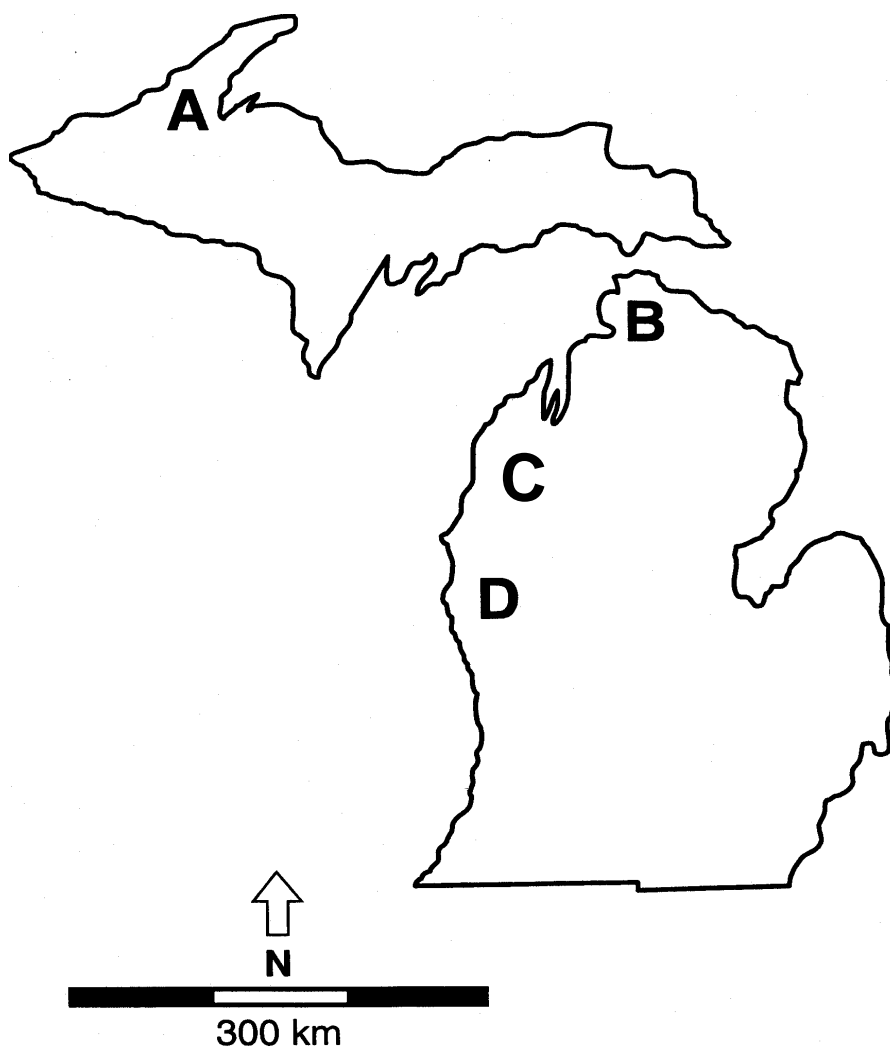


Fig. 2

