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Anthropogenic N deposition and the fate of $^{15}NO_3^-$ in a northern hardwood ecosystem

DONALD R. ZAK[1,2,*], KURT S. PREGITZER[3], WILLIAM E. HOLMES[1], ANDREW J. BURTON[3] and GREGORY P. ZOGG[1,4]

*[1]School of Natural Resources and Environment, University of Michigan, Ann Arbor, MI 48109-1115, USA; [2]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109-1048, USA; [3]School of Forest Resources and Environmental Science, Michigan Technological University, Houghton, MI 49931-1295, USA; [4]Current address: Department of Biological Sciences, University of New England, Biddeford, ME 04005, USA; *Author for correspondence (e-mail: drzak@umich.edu)*

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Abstract. Human activity has substantially increased atmospheric NO_3^- deposition in many regions of the Earth, which could lead to the N saturation of terrestrial ecosystems. Sugar maple (*Acer saccharum* Marsh.) dominated northern hardwood forests in the Upper Great Lakes region may be particularly sensitive to chronic NO_3^- deposition, because relatively moderate experimental increases (three times ambient) have resulted in substantial N leaching over a relatively short duration (5–7 years). Although microbial immobilization is an initial sink (i.e., within 1–2 days) for anthropogenic NO_3^- in this ecosystem, we have an incomplete understanding of the processes controlling the longer-term (i.e., after 1 year) retention and flow of anthropogenic N. Our objectives were to determine: (i) whether chronic NO_3^- additions have altered the N content of major ecosystem pools, and (ii) the longer-term fate of $^{15}NO_3^-$ in plots receiving chronic NO_3^- addition. We addressed these objectives using a field experiment in which three northern hardwood plots receive ambient atmospheric N deposition (ca. 0.9 g N m^{-2} $year^{-1}$) and three plots which receive ambient plus experimental N deposition (3.0 g NO_3^--N m^{-2} $year^{-1}$). Chronic NO_3^- deposition significantly increased the N concentration and content (g N/m^2) of canopy leaves, which contained 72% more N than the control treatment. However, chronic NO_3^- deposition did not significantly alter the biomass, N concentration or N content of any other ecosystem pool. The largest portion of ^{15}N recovered after 1 year occurred in overstory leaves and branches (10%). In contrast, we recovered virtually none of the isotope in soil organic matter (SOM), indicating that SOM was not a sink for anthropogenic NO_3^- over a 1 year duration. Our results indicate that anthropogenic NO_3^- initially assimilated by the microbial community is released into soil solution where it is subsequently taken up by overstory trees and allocated to the canopy. Anthropogenic N appears to be incorporated into SOM only after it is returned to the forest floor and soil via leaf litter fall. Short- and long-term isotope tracing studies provided very different results and illustrate the need to understand the physiological processes controlling the flow of anthropogenic N in terrestrial ecosystems and the specific time steps over which they operate.

Introduction

Throughout many areas of the Earth, human activity has more than doubled the amount of nitrogen (N) entering terrestrial ecosystems via atmospheric deposition

(Galloway 1995; Vitousek et al. 1997). In particular, fossil fuel burning and the subsequent production of N-oxides have increased atmospheric nitrate (NO_3^-) deposition in forest ecosystems throughout the northeastern US and Europe (Hauhs and Wright 1983; Ollinger et al. 1993). The majority of excess NO_3^- entering these forests is retained by plant- and microbially-mediated processes (Tietema et al. 1998; Magill et al. 2000), which substantially reduce the export of anthropogenic NO_3^- to ground- and surface-waters (Goodale et al. 2002; Van Breemen et al. 2002). For example, uptake of this growth-limiting nutrient by plants can retain 25–33% of atmospheric NO_3^- deposition, whereas microbial incorporation of N into forest floor and soil organic matter (SOM) often accounts for most of the remainder (Nadelhoffer et al. 1995; Magill et al. 2000). Nonetheless, the extent to which plants and soil microorganisms retain anthropogenic NO_3^- appears to be a function of soil N availability and ambient levels of N deposition, wherein ecosystem retention is least and export is greatest in forests with rapid rates of N soil cycling that receive substantial amounts of atmospheric deposition (Emmett et al. 1998). Such an observation suggests that anthropogenic NO_3^- deposition could have the most immediate impact on forest ecosystems with high initial soil N availability (Aber et al. 1989, 1998).

Northern hardwood forests in the Upper Lake States region receive moderate rates of atmospheric NO_3^- deposition (0.4–0.8 $g\,N\,m^{-2}\,year^{-1}$; MacDonald et al. 1993), have high rates of net N mineralization (8–12 $g\,N\,m^{-2}\,year^{-1}$; Zak and Pregitzer 1990; Zogg et al. 1996), and thus may be ecosystems in which the physiological capacity of plants and soil microorganisms to retain NO_3^- could be exceeded by atmospheric deposition. Microbial assimilation is an immediate sink (i.e., hours to days) for anthropogenic NO_3^- in this ecosystem, but the rapid turnover of N through the microbial community produces NH_4^+ that is subsequently assimilated by plant roots (i.e., days to months; Zogg et al. 2000). Some experimental evidence suggests that chronic NO_3^- deposition can surpass the ability of soil microorganisms and plants to retain NO_3^- in this ecosystem (Pregitzer et al. 2003). For example, the experimental addition of 3 $g\,NO_3^-\text{-}N\,m^{-2}\,year^{-1}$ did not initially increase N export to groundwater, but annual leaching losses represented 76% of annual experimental additions after 5–7 years (Pregitzer et al. 2003). The magnitude of this loss indicates that the capacity of soil microorganisms and plants to retain NO_3^- has been surpassed by levels of atmospheric N deposition similar to those occurring in some portions of the northeastern US (Fenn et al. 1998). Nevertheless, it is not clear whether these sustained additions have altered ecosystem N pools, nor do we understand the pathway for anthropogenic NO_3^- after its initial turnover through the microbial community. The objectives of our study were to determine: (i) whether chronic N deposition has altered the biomass, N concentration, and N content of major ecosystem pools, and (ii) the pathway of chronic NO_3^- deposition subsequent to its initial assimilation by the soil microbial community. To accomplish this task, we quantified ecosystem N pools in a northern hardwood forest receiving ambient and experimental NO_3^- deposition, and we used ^{15}N to determine the fate of NO_3^- 1 year after it had entered soil solution in plots receiving experimental NO_3^- deposition.

Methods

Study site and experimental design

We quantified N pools and followed the fate of $^{15}NO_3^-$ in a well-studied sugar maple (*Acer saccharum* Marsh.) dominated northern hardwood forest in northern Lower Michigan, USA. (45°33′N, 84°51′W; Zogg et al. 2000). This stand is part of a long-term experiment spanning the geographic range of northern hardwood forests in the Upper Lake States region (Burton et al. 1991a; MacDonald et al. 1993). Mean annual temperature of the study site is 5.6°C, and mean annual precipitation is 870 mm. Soil beneath this stand is a sandy (i.e., 84% sand), typic haplorthod of the Kalkaska series, which has pH of 5.03 in the A horizon and a high degree of base saturation (>80%; MacDonald et al. 1993). Net N mineralization during the growing season is ca. 6.8 g N m^{-2} $year^{-1}$ (calculated from Zogg et al. 1996), and annual wet-plus-dry inorganic N deposition is ca. 0.9 g N m^{-2} $year^{-1}$; the majority (65%) enters as NO_3^- (MacDonald et al. 1993). Currently, this even-aged stand is 88 years old, and sugar maple composes 86% of total overstory biomass. Three 30 m × 30 m plots receive ambient atmospheric deposition. An additional three 30 m × 30 m plots receive ambient deposition plus 3 g NO_3^--N m^{-2} $year^{-1}$, an amount that approaches high deposition regions in the northeastern US and Europe (Bredemeier et al. 1998). In each plot, four porous-cup tension lysimeters were installed at a depth of 75 cm to quantify the leaching loss of N. Experimental NO_3^- additions were initiated in April 1994 by applying six equal increments of $NaNO_3$ during the growing season. These annual additions continue to the present day.

During the 1998 field season, we added 24.0 g of ^{15}N to each N-amended plot by mixing 99% atom excess ^{15}N-$NaNO_3$ with our routine applications in June, July and August. An additional 9.9 g of ^{15}N was added to each plot through our regular application of 30 kg NO_3^--N ha^{-1} $year^{-1}$ ($NaNO_3$ was 0.36646 atom % ^{15}N). One year after isotope addition (i.e., September 1999), we quantified the recovery of ^{15}N in overstory, forest floor, and soil pools of the three N-amended plots. A previous, short-term (i.e., 2 h to 16 weeks in 9 m^2 plots) ^{15}N tracer experiment in this stand indicated that microbial assimilation was the initial sink for anthropogenic NO_3^- (Zogg et al. 2000). However, these observations could not provide us with insight into the fate of NO_3^- over longer periods of time (i.e., after 1 year), and the scale of this experiment was too small to trace ^{15}N into the overstory.

Measurements prior to $^{15}NO_3^-$ labeling

Prior to the addition of $^{15}NO_3^-$, we determined the N and ^{15}N concentration of overstory, forest floor, and soil pools in N-amended plots (September 1997). We also recorded the diameter and species of each overstory tree to estimate the mass of overstory components using allometric equations. Tissue samples were collected from three, widely-spaced dominant overstory trees in each N-amended plot. Canopy leaves and branches (<0.5 cm diameter) were obtained with a shotgun.

Bark and stem wood were removed using a 2.5 cm diameter hole saw inserted (0.8 cm) into each stem at breast height. Structural roots (>10 cm) were sampled using a hole saw as described above. Leaf litter and fine woody debris (<2 mm diameter) composing the forest floor (Oi and Oe horizons) were removed from three, randomly located subplots (900 cm^2). The Oa horizon is absent in this soil, and there is an abrupt transition from the Oe horizon to the A horizon. Following forest floor removal, two surface mineral soil cores (5 cm diameter × 10 cm deep; A and E horizons) were collected in each 900 cm^2 subplot; the two cores were combined in the field. We determined the mass of the soil cores to estimate bulk density. Roots were then removed and sorted into five size classes (<0.5 mm, 0.5–1.0 mm, 1.0–2.0 mm, 2.0–5.0 mm and 5.0–10.0 mm). All plant samples were dried at 70°C to a constant mass and ground for chemical analyses. We determined the concentration of N and ^{15}N of each plant tissue and forest floor sample using an NC2500 elemental analyzer (CE Elantech, Lakewood, NJ) interfaced to a Delta Plus isotope ratio mass spectrometer (Thermo Finnigan, San Jose, CA).

Soil samples were homogenized and processed within 24 h of field collection for determination of extractable NH_4^+ and NO_3^-, microbial biomass N, and soil organic N. A 10-g subsample was extracted with 20 mL of 2 mol/L KCl, and the NH_4^+ and NO_3^- concentrations of the filtered extracts were determined using automated colorimetry (OI Analytical, College Station, TX). Ammonium-N and NO_3^--N were sequentially diffused from each KCl extract onto acid traps in preparation for ^{15}N analysis (Brooks et al. 1989). Microbial biomass N and ^{15}N were determined using the chloroform fumigation–extraction method (Horwath and Paul 1994). A 20-g soil subsample was extracted with 80 mL of 0.5 mol L^{-1} K_2SO_4; a second 20-g subsample was fumigated with $CHCl_3$ for 18 h and extracted with K_2SO_4 as described above. Organic N in the K_2SO_4 extracts was analyzed by alkaline persulfate digestion followed by automated colorimetry (Cabrera and Beare 1993). Nitrate was diffused from each extract and digest onto an acid trap for ^{15}N analysis. We used the difference in amount of N and ^{15}N between fumigated and non-fumigated samples to estimate microbial N and the abundance of ^{15}N in the microbial pool. Subsamples of field-fresh soil were air dried and ground to determine organic C, total N, and ^{15}N abundance as described above. We subtracted the amount of N and ^{15}N in extractable NH_4^+, extractable NO_3^-, and microbial N from that in total soil N to estimate soil organic N.

The N content (g N m^{-2}) of each ecosystem pool was calculated as the product of its mass (g m^{-2}) and N concentration (mg N g^{-1}). Similarly, we estimate the mass of ^{15}N in each ecosystem pool as the product of its ^{15}N abundance (atom % ^{15}N) and N content (g N m^{-2}). This provided us with initial values of ecosystem ^{15}N content; these values were later used to quantify where the added isotope resided 1 year after application.

Overstory branch, stem bark, stem wood and coarse root biomass (g m^{-2}) were estimated using species-specific allometric biomass equations (Whittaker et al. 1974). Canopy leaf mass was estimated from annual litter mass using correction factor of 1.14 to adjust for the change in specific leaf area prior to leaf fall (Burton

et al. 1991b, 1993). The mass of fine roots in soil cores was expressed on an areal basis ($g\,m^{-2}$), and we estimated forest floor biomass from the dry weight of material collected in our 900-cm^2 subplots. Bulk density measured in each plot was used to estimate SOM and N pools from their respective concentrations; we assumed that SOM was $470\,mg\,C\,g^{-1}$ SOM.

Measurements following $^{15}NO_3^-$ labeling

In September 1999, 1 year after completion of the isotope addition, we remeasured the N and ^{15}N concentration of overstory, forest floor and soil pools in N-amended plots using the procedures described above. The diameter of all overstory trees on N-amended plots was re-measured in order to estimate biomass, N content, and ^{15}N content; we also collected samples of forest floor and surface mineral soil. In addition, we estimated the biomass and N content of overstory, forest floor and soil in control plots using the same procedures. This enabled us to determine if our chronic NO_3^- additions had altered the biomass, N concentration, and N content of major ecosystem pools, relative the three plots receiving ambient N deposition.

We quantified leaching loss of NO_3^-, dissolved organic N (DON), and ^{15}N in these forms in both control and N-amended plots. Soil solution was collected from each lysimeter on a 2-week interval during fall 1998 (August – November), spring (April–June) 1999 and autumn (August–November) 1999. Lysimeters are dry during mid-growing season at this site due to high rates of transpiration (D.R. Zak, personal observation). A tension of 0.05 MPa was placed on each lysimeter after evacuation and samples were pooled by plot. Prior to any analysis, we passed all lysimeter water through a 0.45 μ filter membrane. Ammonium and NO_3^- concentrations were quantified by automated colorimetry as described above. DON was determined by alkaline persulfate digestion and automated colorimetry. We calculated DON as NO_3^- concentration in the digests minus the inorganic N in undigested soil solution. The ^{15}N abundance of NH_4^+, NO_3^-, and DON was determined using a sequential diffusion/digestion procedure. Ammonium and NO_3^- were successively diffused from each solution onto separate acid traps as described above. The DON remaining in solution was converted to NO_3^- by alkaline persulfate digestion, and the NO_3^- in the digestate was diffused onto an acid trap as described above. These samples were analyzed for atom % ^{15}N by isotope ratio mass spectrometry. To estimate the leaching losses of N from control ($g\,N\,m^{-2}$) and N-amended plots ($g\,N\,m^{-2}$ and $g\,^{15}N\,m^{-2}$), we used the BROOK model of Federer and Lash (1983) to calculate an annual water balance. We summed monthly estimates of leaching for the year following $^{15}NO_3^-$ addition to estimate N and ^{15}N export.

Statistical analyses

We used a one-way analysis of variance (ANOVA) to determine whether our experimental N deposition treatments had altered the biomass, N concentration, and N

content of ecosystem pools. The means of overstory components, forest floor and surface mineral soil pools were compared between control and N-amended plots using a protected Fisher's LSD procedure. In N-amended plots, we used ^{15}N abundance (atom % ^{15}N) to calculate the $\delta^{15}N$ of each ecosystem pool prior to and following the addition of ^{15}N. We then used a one-way ANOVA to compare $\delta^{15}N$ of each ecosystem pool before and after isotope addition. The amount of ^{15}N label residing in each ecosystem pool was calculated as the difference in ^{15}N content (mg $^{15}N\,m^{-2}$) 1 year after isotope addition and its initial ^{15}N content. We expressed this value as the percent of applied ^{15}N (i.e., 33.9 g $^{15}N^{-1}$ plot or 37.67 mg $^{15}N\,m^{-2}$) recovered in each ecosystem pool. Percent recovery data were log transformed to meet the assumptions of normality, and we used a one-way ANOVA to compare the isotope recovery among ecosystem pools in N-amended plots. This enabled us to determine which pool represented the greatest sink for added $^{15}NO_3^-$ in plots receiving experimental N deposition; mean recoveries were compared using a Fisher's protected LSD procedure. Significance for all statistical analyses was accepted at $\alpha = 0.05$.

Results

Ecosystem biomass, N concentration, and N content

Experimental NO_3^- deposition did not have a significant effect on total overstory biomass (g m^{-2}), nor did it have a significant effect on any component of overstory biomass (Table 1, compare control v.s. NO_3^-). This was true even though total overstory biomass and its components were consistently greater in N-amended plots. The N concentration (mg N g^{-1}) of canopy leaves in plots receiving experimental NO_3^- deposition was 58% greater than that in the control treatment; this difference was significant (Table 1). Nonetheless, we found no effect of NO_3^- deposition on the N concentration of any other overstory component (Table 1). Experimental NO_3^- deposition resulted in a significant, 72% increase in the N content (g N/m^2) of canopy leaves, but again we found no effect of NO_3^- deposition on the N content of other overstory components (Table 1). This also was true for total overstory N content, which averaged 34.5 g N m^{-2} in control plots and 39.3 g N m^{-2} in plots receiving NO_3^- (Table 1).

Forest floor biomass, N concentration and N content did not differ between control and N-amended plots (Table 2). However, we observed a significant increase in SOM content, wherein plots receiving NO_3^- deposition had a mean SOM content that was 55% greater than that of the control (Table 2). Although the N concentration and N content of SOM were greater in N-amended plots, these values were not statistically different from those in the control treatment (Table 2). The N concentration and content of microbial biomass also was greater in plots receiving NO_3^- deposition, but again these values were not significantly different from the control (Table 2). The concentration and content of extractable NH_4^+ (0–10 cm) was significantly greater in N-amended plots, but there was no effect of experimental

Table 1. Overstory biomass, N concentration and N content in a northern hardwood forest receiving experimental NO_3^- deposition treatments from 1993 to 1999. For each overstory component, control and NO_3^- treatment means ($n = 3$) with the same letter are not significantly different at $\alpha = 0.05$. Standard deviations are in parentheses. Biomass values are dry weight (70°C) and include ash content.

Ecosystem component	Biomass ($g\,m^{-2}$)		N concentration ($mg\,N\,g^{-1}$)		N content ($g\,N\,m^{-2}$)	
	Control	NO_3^-	Control	NO_3^-	Control	NO_3^-
Leaves	434a (20.0)	478a (42.2)	7.4a (1.87)	11.7b (1.75)	3.25a (0.760)	5.60b (0.727)
Branches	477a (146.4)	637a (151.7)	10.5a (0.23)	10.4a (3.91)	5.05a (1.578)	6.34a (1.507)
Stem wood	10274a (352.1)	10469a (2721.6)	0.9a (0.08)	1.0a (0.07)	9.37a (1.189)	10.02a (2.071)
Stem bark	634a (55.3)	671a (125.2)	4.5a (0.55)	4.4a (0.23)	2.83a (0.104)	2.97a (0.481)
Roots						
Structural						
>10.0 mm	2645a (86.6)	2774a (645.8)	1.9a (0.23)	1.7a (0.18)	5.09a (0.767)	4.73a (1.547)
10.0–5.0 mm	235a (37.3)	260a (22.9)	6.0a (0.61)	7.2a (2.03)	1.43a (0.346)	1.86a (0.401)
5.0–2.0 mm	160a (48.7)	185a (25.3)	5.1a (0.68)	6.8a (0.94)	0.81a (0.223)	1.26a (0.172)
2.0–1.0 mm	100a (22.0)	127a (46.5)	6.8a (0.79)	6.4a (0.89)	0.67a (0.084)	0.84a (0.408)
1.0–0.5 mm	86a (23.4)	102a (34.9)	7.5a (0.92)	6.9a (1.95)	0.63a (0.140)	0.68a (0.150)
<0.5 mm	436a (105.2)	427a (68.2)	12.3a (0.67)	11.7a (1.95)	5.40a (1.392)	4.96a (0.862)
Total overstory	15482a (143.1)	16133a (353.1)	–	–	34.54a (2.697)	39.28a (2.027)

Table 2. Biomass, N concentration and N content of forest floor and soil in a northern hardwood forest receiving experimental NO_3^- deposition treatments from 1993 to 1999. For each forest floor and soil pool, control and NO_3^- treatment means ($n = 3$) with the same letter are not significantly different at $\alpha = 0.05$. Standard deviations are in parentheses. Forest floor biomass has not been corrected for ash content.

Ecosystem component	Biomass ($g\,m^{-2}$)		N concentration*($mg\,N\,g^{-1}$)		N content**($g\,N\,m^{-2}$)	
	Control	NO_3^-	Control	NO_3^-	Control	NO_3^-
Forest floor	891a (310.6)	575a (149.7)	18.7a (1.44)	16.5a (0.31)	16.87a (6.840)	9.51a (2.633)
Mineral soil						
Organic matter	5098a (761.1)	7887b (952.1)	1.77a (0.357)	2.25a (0.229)	191.33a (39.213)	249.42a (16.652)
Microbial N	–	–	177.9a (35.61)	191.9a (48.88)	17.78a (3.561)	19.19a (4.886)
Extractable NH_4^+	–	–	1.74a (0.267)	2.89b (0.843)	0.19a (0.042)	0.32b (0.081)
Extractable NO_3^-	–	–	0.80a (0.163)	0.56a (0.427)	0.09a (0.022)	0.06a (0.049)
Leached NO_3^-	–	–	0.29a (0.025)	4.47b (2.695)	0.07a (0.005)	0.98b (0.583)
Leached DON	–	–	0.15a (0.014)	0.27b (7.527)	0.031a (0.004)	0.056b (0.023)

*Forest floor concentration is $mg\,N\,g^{-1}$ forest floor, and SOM N concentration is $mg\,N\,g^{-1}$ soil. Units for microbial N, extractable NH_4^+-N, and extractable NO_3^--N are $\mu g\,N\,g^{-1}$; leached NO_3^- and DON concentrations are $\mu g\,N\,mL^{-1}$.

**Leached NO_3^- and DON are in units of $g\,N\,m^{-2}\,year^{-1}$.

Table 3. The $\delta^{15}N$ of ecosystem components in plots receiving chronic NO_3^- deposition treatments prior to and following the addition of $^{15}NO_3^-$ -N. We added $^{15}NO_3^-$-N to each plot over the 1998 growing season. Values were measured prior to leaf senescence in autumn 1997 (before) and in 1999 (after); standard deviations are in parentheses.

Ecosystem component	$\delta^{15}N$ (‰)		*P*
	Before	After	
Overstory			
Leaves	−1.99 (2.144)	103.04 (71.032)	0.063
Branches	−2.62 (0.312)	65.89 (50.093)	0.077
Stem wood	0.11 (1.078)	15.18 (1.413)	<0.0001
Stem bark	−2.10 (1.372)	15.45 (1.520)	<0.0001
Roots			
Structural >10.0 mm	−0.61 (1.243)	31.28 (3.475)	<0.0001
10.0–5.0 mm	0.71 (0.366)	9.21 (4.717)	<0.0001
5.0–2.0 mm	2.48 (1.233)	34.76 (22.871)	0.071
2.0–1.0 mm	2.14 (1.138)	31.61 (23.362)	0.094
1.0–0.5 mm	4.87 (2.898)	35.83 (13.937)	0.019
<0.5 mm	5.49 (3.159)	28.25 (6.912)	0.006
Forest floor	1.43 (3.361)	22.60 (14.876)	0.074
Mineral soil			
Organic N	3.94 (0.526)	4.11 (0.324)	0.642
Extractable NH_4^+	−0.12 (2.034)	7.63 (1.949)	0.009
Extractable NO_3^-	−17.63 (9.707)	−6.10 (2.929)	0.120
Microbial N	4.34 (1.435)	7.44 (1.629)	0.069
Leached NO_3^-	−3.92 (3.138)	89.82 (57.911)	0.049
Leached DON	0.39 (2.878)	23.04 (7.527)	0.008

NO_3^- deposition on the concentration or content of extractable NO_3^- (Table 2). The concentration of NO_3^- in lysimeter samples (75 cm depth) was 15 times greater in plots receiving experimental NO_3^- deposition (Table 2); this difference was significant. The leaching loss of NO_3^- from control plots was 0.07 g N m^{-2} $year^{-1}$, which was significantly less than the loss from N-amended plots (0.98 g N m^{-2} $year^{-1}$). DON concentrations in N-amended plots were twice those in control plots, and this difference was significant (Table 2). Annual DON loss differed significantly between control and N-amended plots, with plots receiving experimental NO_3^- deposition exhibiting a loss almost twice that of the control treatment (Table 2). The export of NH_4^+ was undetectable in soil solution collected at 75 cm.

Fate of $^{15}NO_3^-$ in N-amended plots

The addition of $^{15}NO_3^-$ to N-amended plots substantially increased the $\delta^{15}N$ of plant components, forest floor, and leached N, but not microbial N or soil organic N. The most pronounced changes occurred in leaves (+105‰), branches (+68‰),

Table 4. Recovery of added N in ecosystem components for plots receiving chronic NO_3^- deposition. Values were mean recoveries for three ^{15}N labeled plots; standard deviations are in parentheses.

Ecosystem component	Recovery (% applied ^{15}N)
Overstory	
Leaves	5.25 (2.819)
Branches	4.06 (2.938)
Stem wood	0.91 (0.218)
Stem bark	0.28 (0.064)
Roots Structural >10.0 mm	1.27 (0.401)
10.0–5.0 mm	0.28 (0.136)
5.0–2.0 mm	0.32 (0.312)
2.0–1.0 mm	0.21 (0.154)
1.0–0.5 mm	0.18 (0.139)
<0.5 mm	0.88 (0.387)
Total overstory	13.51 (5.096)
Forest floor	2.54 (2.200)
Mineral soil	
Organic N	0.00 (0.000)
Extractable NH_4^+	0.00 (0.000)
Extractable NO_3^-	0.00 (0.000)
Microbial N	0.01 (0.024)
Leached NO_3^-	1.11 (0.746)
Leached DON	0.27 (0.242)
Total recovery	17.45 (7.016)

and leached NO_3^- (+94‰, Table 3). Increases in $\delta^{15}N$ were significant ($P \leq 0.05$) or marginally significant ($P \leq 0.10$) for all pools except soil organic N and extractable NO_3^--N (Table 3). It is important to point out that change in $\delta^{15}N$ is determined by the initial mass of N and ^{15}N in a particular pool, and the mass of label entering that pool. For example, a substantial amount of ^{15}N label could move into a relatively large pool, producing only a small change in $\delta^{15}N$. Conversely, a modest amount of ^{15}N label could move into a small pool and produce a large change in $\delta^{15}N$. Therefore, the $\delta^{15}N$ increase in Table 3 need to be considered within the context of ecosystem pool size (Tables 1 and 2).

Using changes in $\delta^{15}N$ and pool size, we determined the mass of ^{15}N label that had moved into each ecosystem pool in plots receiving experimental NO_3^- deposition (Table 4). The recovery of ^{15}N label in our experiment was relatively low, averaging only 17.5% of the applied isotope (Table 4). The majority (13.5%) of the added isotope was located in overstory trees, which, by far, represented the largest sink for ^{15}N in this ecosystem (Table 4); it was significantly greater than forest floor or soil pools. Leaves (5.25%) and branches (4.06%) contained the majority of isotope recovered in overstory trees (Table 4). Conversely, we recovered virtually none of the ^{15}N label in soil organic N, extractable NH_4^+, extractable NO_3^-, or

microbial N (Table 4). Leaching of NO_3^- (1.11%) and DON (0.27%) represented only a small proportion of the isotope we added to the N-amended plots.

Discussion

Northern hardwood forests in the Upper Lake States region appear to be particularly sensitive to chronic NO_3^- deposition, because the capacity of plants and soil microorganisms to retain N can be rapidly exceeded (i.e., within 5–7 years) by moderate increases in atmospheric N deposition (i.e., three times ambient; Pregitzer et al. 2003) that already occur in other temperate regions. Although these additions have lead to substantial rates of N leaching, they have had only a minor influence on the amount of N stored in overstory, forest floor and surface mineral soil. In our study, 6 years of chronic NO_3^- addition significantly increased the biomass, N concentration and N content of canopy leaves, but did not significantly alter the concentration or content of N in any other ecosystem pool. This change in canopy N was consistent with the results of our ecosystem-level isotope tracer experiment, which provided insight into the pathway NO_3^--N has taken in our N-amended plots. Our ^{15}N tracer results indicate that plant uptake and the internal allocation of N to canopy leaves and branches was the most substantial sink for NO_3^- 1 year following isotope addition. These observations differ substantially from similar experiments in other temperate forests where large amounts of anthropogenic N are incorporated into SOM (Tietema et al. 1998; Nadelhoffer et al. 1999), and they differ substantially from our own short-term isotope tracing experiment (Zogg et al. 2000).

Our previous short-term field ^{15}N labeling experiment in this same stand demonstrated that $^{15}NO_3^-$ was rapidly (i.e., within minutes) assimilated by the soil microbial community and, within a few days, was released into soil solution as $^{15}NH_4^+$; the labeled NH_4^+ was then assimilated by plants after several weeks (Zogg et al. 2000). Within hours after isotope addition, a substantial amount of ^{15}N also moved into forest floor and SOM, suggesting that microbial immobilization was an initial sink for anthropogenic NO_3^- (Zogg et al. 2000). However, our ecosystem labeling experiment suggests that NO_3^- initially immobilized into SOM was released at time steps longer than a month and shorter than a year, because we recovered none of the isotope in soil organic N after 1 year (Table 4). We also recovered very little of the isotope in microbial biomass, further indicating that the microbial community was only a short-term sink for anthropogenic NO_3^-. If anthropogenic N is to accumulate in SOM in this ecosystem, it apparently does so following assimilation by overstory trees, the internal allocation of N to leaves, and transport to the soil via litterfall. Thus, the flow of NO_3^- in our study clearly differs from forests in the northeastern US, in which a large portion of the added isotope was recovered in forest floor and SOM (Nadelhoffer et al. 1995, 1999; Magill et al. 1997, 2000). This contrast reinforces the importance of understanding the time steps and mechanisms that control the cycling of N in forest ecosystems, because our short- and long-term labeling experiments reveal very different fates for anthropogenic NO_3^- that are time dependent. Together, they indicate that plant and

microbial processes retaining N operate on different time scales in this northern hardwood forest, and they likely do so in other forest ecosystems.

Although SOM was not a ^{15}N sink after 1 year, it may become an important sink for anthropogenic N over a longer time frame. In our experiment, canopy leaves were highly enriched with ^{15}N 1 year after application, and the N contained in them will eventually enter forest floor and mineral soil. These highly enriched canopy leaves had not yet been shed at the time of our sampling (September 1999), but labeled leaves produced the prior autumn likely contributed some ^{15}N to forest floor (2.54%). Over a 4-year period, Nadelhoffer et al. (1995) applied $^{15}NO_3^-$ to a northern hardwood forest in New England and recovered 50% of the applied isotope in forest floor and surface mineral soil, concluding that microbial immobilization was responsible for the movement of isotope into this pool. This idea is consistent with many short-term ^{15}N labeling experiments (i.e., 1–2 days), which often demonstrate that microbial communities can assimilate substantial amounts of NO_3^- (Schimel and Firestone 1989; Davidson et al. 1992), not unlike the results of our short-term labeling experiment in this stand (Zogg et al. 2000). Given the substantial enrichment of canopy leaves observed by Nadelhoffer et al. (1995), it is possible that much of the ^{15}N residing in SOM originated from NO_3^- that was initially cycled through the microbial community, taken up by plants, and then returned in litterfall to the forest floor and mineral soil where microbial activity then incorporated ^{15}N into SOM. Thus, differences in our results and those obtained by others may be due to the time-dependent flow of ^{15}N among ecosystem components. Although the initial immobilization of NO_3^- by the microbial community is a short-term (i.e., days) sink, the microbial incorporation of N assimilated by plants into SOM appears to be a longer-term sink (i.e., years) that is ultimately responsible for the incorporation of anthropogenic N into SOM.

One could argue that analytical error might not allow us to detect the movement of ^{15}N into the large, heterogeneous pool of N in SOM; however, several pieces of evidence indicate this was not the case. We added 33.9 g ^{15}N to each N-amended plot, which increased the $\delta^{15}N$ of all ecosystem pools, except soil organic N (Table 3). Given our analytical precision (0.11 ‰ $\delta^{15}N$) and the mass of N in SOM, we could 'miss' only ca. 90 mg ^{15}N in each plot due to analytical error. This represents 0.4% of the added isotope, indicating that only a small amount of ^{15}N could be masked by analytical error. If 30–80% of the added ^{15}N had moved into SOM, as it did in other experiments (Tietema et al. 1998; Magill et al. 2000), then the $\delta^{15}N$ of SOM in our experiment would range from 6.8 ± 0.21 ‰ to 24.3 ± 1.90 ‰. These estimates are significantly greater than the value we measured after isotope addition (4.1 ‰; *t*-test), and they are well outside the bounds of our analytical error. This analysis further suggests that SOM was not a sink for ^{15}N 1 year following the application of $^{15}NO_3^-$ in this northern hardwood forest.

The recovery of isotope in our experiment was substantially lower than that in other ecosystem-level ^{15}N labeling experiments, a result that may have arisen from a combination of factors. For example, we recovered only 17 ± 7.0% of the added isotope, whereas recovery ranged from 65 to 105% in other studies (Tietema et al. 1998; Magill et al. 2000). In our experiment, we established, treated, and labeled

plots in a mature northern hardwood forest stand. Overstory trees outside the labeled plot undoubtedly had roots extending into this area, and thus may have assimilated some of the added isotope. Because we only sampled overstory trees within the labeled plot, we did not account for any ^{15}N that resided in adjacent overstory trees. This amount could be substantial, given that the majority recovered isotope occurred in overstory trees. Moreover, unrecovered isotope could reside in soil at a depth between 10 and 75 cm, a layer that we did not analyze which lies between our collection of surface mineral soil (0–10 cm) and soil solution (75 cm).

It is plausible that our means of estimating leaching losses may have not provided us with an accurate assessment of ^{15}N export, which contribute to our relatively low recovery of isotope. We calculated a water balance on a monthly time step and collected soil solution samples at 2-week intervals during spring and autumn. This technique assumes that our tension lysimeters provide time-integrated samples of NO_3^- and DON concentrations in water moving below the rooting zone, and that our water balance calculations accurately represent the volume of water moving below this point. It is unlikely that lysimeters proportionately sampled soil water over time, and it was clear from our water balance model that water moved below the rooting zone during December and January when we did not collect soil solution. It also is conceivable that summer rainfall could have leached isotope from soil at a time when we did not collect soil water samples.

Denitrification could have been another factor contributing to the low recovery of ^{15}N, but this process could only account for a small proportion of the unrecovered isotope in our experiment. Laboratory experiments using soil from this ecosystem demonstrate that NO_3^- additions can increase denitrification by an order of magnitude (Merill and Zak 1992). Even if our field NO_3^- additions increased the mean daily field rate ($11 \pm 6.6\,\mu g\,N\,m^{-2}\,day^{-1}$; Merrill and Zak 1992) by an order of magnitude over the entire growing season (115 day), less than 0.02% of the isotope we added (i.e., 2–6 mg ^{15}N) could have been lost to denitrification. In making this estimate, we assumed that $\delta^{15}N$ of NO_3^- in soil solution was constant and equivalent to the annual average $\delta^{15}N$ of NO_3^- that had leached below the rooting zone (80‰; Table 3). Although denitrification can display high spatial and temporal variability (Groffman and Tiedje 1989), our analysis suggests this process was not an important pathway for N loss in N-amended plots and could not have contributed to our low isotope recovery.

In summary, chronic NO_3^- deposition at rates that currently occur in portions of the northeastern US did not substantially alter the N concentration or content of most ecosystem pools. However, we observed a significant increase in both the N concentration and content of canopy leaves in N-amended treatment, which contained 72% more N than the control treatment. Our $^{15}NO_3^-$ labeling experiment in N-amended plots also indicated that leaves and branches in the overstory canopy were the greatest sinks of ^{15}N 1 year following isotope addition. Our results suggest that NO_3^- entering soil from atmospheric deposition is initially assimilated by the microbial community, rapidly released as NH_4^+ via microbial turnover, and is then taken up by overstory trees where it is allocated to canopy leaves and branches. Because we found virtually none of the applied isotope in surface SOM after 1 year,

we conclude that the eventual movement of ^{15}N into SOM could only occur after ^{15}N contained in canopy leaves is returned to soil in litterfall and subsequently processed by the soil food web into organic matter. Although the rapid, initial immobilization of NO_3^- by the soil microbial community is only a temporary sink (Zogg et al. 2000), the microbial processing of anthropogenic N in litter and the subsequent formation of SOM is likely to be a substantial sink which operates on longer time steps (i.e., years). Several pieces of evidence suggest that SOM may be accumulating in N-amended plots, supporting the idea that it represents a long-term sink for anthropogenic NO_3^-. Plots receiving chronic NO_3^- additions contained significantly greater amounts of organic matter and had higher N contents, albeit this increase in N content was not significant. Understanding the physiological processes controlling the cycling of anthropogenic NO_3^- between plants and soil microorganisms, and the specific time steps at which they operate, lie at the heart of understanding the mechanisms of N retention and loss in forest ecosystems.

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