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Chronic Atmospheric NO_3^- Deposition Does Not Induce NO_3^- Use by *Acer saccharum* Marsh.

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Abstract

The ability of an ecosystem to retain anthropogenic nitrogen (N) deposition is dependent upon plant and soil sinks for N, the strengths of which may be altered by chronic atmospheric N deposition. Sugar maple (*Acer saccharum* Marsh.), the dominant overstory tree in northern hardwood forests of the Lake States region, has a limited capacity to take up and assimilate NO_3^-. However, it is uncertain whether long-term exposure to NO_3^- deposition might induce NO_3^- uptake by this ecologically important overstory tree. Here, we investigate whether 10 years of experimental NO_3^- deposition (30 kg N ha^{-1} y^{-1}) could induce NO_3^- uptake and assimilation in overstory sugar maple (approximately 90 years old), which would enable this species to function as a direct sink for atmospheric NO_3^- deposition. Kinetic parameters for NH_4^+ and NO_3^- uptake in fine roots, as well as leaf and root NO_3^- reductase activity, were measured under conditions of ambient and experimental NO_3^- deposition in four sugar maple-dominated stands spanning the geographic distribution of northern hardwood forests in the Upper Lake States. Chronic NO_3^- deposition did not alter the V_{max} or K_m for NO_3^- and NH_4^+ uptake nor did it influence NO_3^- reductase activity in leaves and fine roots. Moreover, the mean V_{max} for NH_4^+ uptake (5.15 μmol ^{15}N g^{-1} h^{-1}) was eight times greater than the V_{max} for NO_3^- uptake (0.63 μmol ^{15}N g^{-1} h^{-1}), indicating a much greater physiological capacity for NH_4^+ uptake in this species. Additionally, NO_3^- reductase activity was lower than most values for woody plants previously reported in the literature, further indicating a low physiological potential for NO_3^- assimilation in sugar maple. Our results demonstrate that chronic NO_3^- deposition has not induced the physiological capacity for NO_3^- uptake and assimilation by sugar maple, making this dominant species an unlikely direct sink for anthropogenic NO_3^- deposition.

Key words: *Acer saccharum*; NO_3^- deposition; NO_3^- reductase; N uptake; forest N cycling; northern hardwood forest.

Introduction

Humans have increased global atmospheric N deposition from 32 to 103 Tg N y^{-1} over the past 150 years, and this rate is likely to reach 195 Tg N y^{-1} by 2050 (Galloway and others 2004). Atmospheric deposition of this magnitude has the potential to induce N saturation, a condition in which the biological availability of N in terrestrial ecosystems exceeds the metabolic needs of plants and soil microorganisms (Aber and others 1989;

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Aber 1992). Although plant growth in many terrestrial ecosystems is limited by soil N availability (Pastor and others 1984; Zak and others 1989; Vitousek and Howarth 1991), chronic atmospheric N deposition could eventually surpass plant demand in previously N-limited ecosystems, thereby creating the potential for N loss to ground and surface waters (Pregitzer and others 2004) as well as to the atmosphere via denitrification. The ability of plants to reduce the loss of anthropogenic N deposition from terrestrial ecosystems depends on the magnitude of atmospheric deposition, the extent to which N presently limits plant growth, and physiological capacity of plants to use the forms of anthropogenic N deposited from the atmosphere (Rothstein and others 1996).

In eastern North America, NO_3^- is a dominant component of atmospheric N deposition, and tree species broadly vary in their physiological capacity to take up and assimilate this form of N (BassiriRad and others 1993; Templer and Dawson 2004). For example, high soil NO_3^- concentrations can induce NO_3^- uptake and assimilation in some tree species (Hoff and others 1992; Oaks 1994), whereas uptake by other tree species is unresponsive to soil NO_3^- concentration. For example, red maple, (*Acer rubrum* L.) exhibited 50% greater rates of foliar NO_3^- reductase activity under conditions of high NO_3^- availability (Downs and others 1993), suggesting that NO_3^- assimilation can be induced by the presence of this ion in soil. In contrast, NO_3^- uptake and assimilation in sugar maple (*Acer saccharum* Marsh.) seedlings were unresponsive to high soil NO_3^- concentrations (Rothstein and others 1996). Consequently, temperate tree taxa should differ in their capacity to use anthropogenic NO_3^-, depending on the degree to which NO_3^- deposition induces the uptake of this ion from soil. Because atmospheric NO_3^- deposition is expected to increase across much of eastern North America (Galloway and others 2004), these observations suggest that some temperate trees would directly take up anthropogenic NO_3^- from soil, whereas others will not.

In the Upper Lakes States region, northern hardwood forests receive atmospheric N deposition at the rates of 7–12 kg N ha^{-1} y^{-1}, with NO_3^- comprising approximately 65% of this input (MacDonald and others 1992). Sugar maple (*A. saccharum* Marsh.) dominates the overstory of this common ecosystem (Curtis 1959; MacDonald and others 1991), and their fine roots have a low physiological capacity to take up NO_3^- from soil (Rothstein and others 1996; Templer and Dawson 2004). Moreover, experimental evidence demonstrates that elevated atmospheric NO_3^- deposition does not influence the turnover or biomass of sugar maple fine roots (Burton and others 2000, 2004). Therefore, if elevated NO_3^- deposition does not physiologically induce NO_3^- uptake by the fine roots of this species, then plant uptake in northern hardwood forests is unlikely to directly sequester atmospheric NO_3^- deposition as it increases over the next century. Our objective was to determine whether NO_3^- uptake and assimilation in mature, overstory sugar maple could be induced by elevated atmospheric NO_3^- deposition (ambient plus 30 kg NO_3^--N ha^{-1} y^{-1}). To address our objective, we used ^{15}N to determine the kinetic parameters for NH_4^+ and NO_3^- uptake by overstory sugar maple exposed to a decade of experimental NO_3^- deposition. We also measured rates of NO_3^- reduction in leaf and root tissue to gain further insight into the influence of elevated atmospheric NO_3^- deposition on the physiology of N use by this species.

Materials and Methods

Study Sites

Our study was conducted in four northern hardwood forest stands which lie along a 500-km north-south gradient in lower and upper Michigan (Figure 1); the location of these stands spans the north-south geographic range of northern hardwood forests in the Upper Great Lakes. All study sites have similar floristic composition, stand age, basal area, land-use history, soil type, and soil texture (Burton and others 1991; MacDonald and others 1991), but differ in temperature and rates of ambient atmospheric N deposition (Table 1). Both N deposition rate and mean annual temperature decrease from south to north among the sites, whereas soil N availability is lower in the northern- and southern-most sites (Zogg and others 1996). These even-aged stands are approximately 90-year-old, second-growth northern hardwood forests whose basal area is dominated by sugar maple (Table 1; MacDonald and others 1991). Overstory associates include *Quercus rubra*, *Fraxinus americana*, *Betula alleghaniensis*, and *Prunus serotina*. All stands lack a well-developed understory; groundflora species are sparse. Soils are sandy typic Haplorthods of the Kalkaska series.

In each stand (n = 4), there are six 50-m × 50-m plots, which are composed of a 30-m × 30-m main plot plus a 10-m wide treated buffer surrounding the main plot; all soil samples were collected from within the 10-m treated buffer. Three 50-m × 50-m plots within each stand receive

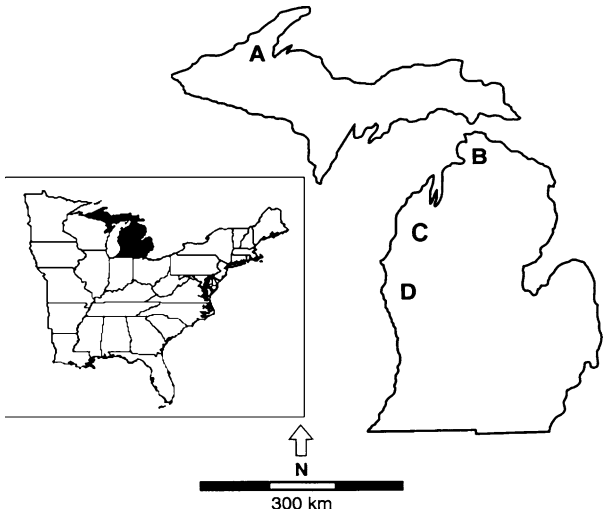

Figure 1. The geographic distribution of the study sites in lower and upper Michigan. In each stand, three plots receive ambient atmospheric N deposition and three plots receive ambient plus 30 kg NO_3^--N ha^{-1} y^{-1}. These treatments have been in place since 1994 in the four sugar maple-dominated northern hardwood stands.

ambient levels of atmospheric N deposition, whereas the other three 50-m × 50-m plots receive ambient plus 30 kg N ha^{-1} y^{-1} applied as $NaNO_3$. This approximates rates of NO_3^- deposition expected to occur across portions of eastern North America and Europe before the end of this century (Galloway and others 2004). Nitrate is added as dry pellets broadcast over the forest floor in six equal increments during the growing season (late April/early May to September). Experimental NO_3^- deposition began in spring 1994 and has continued to the present.

Sample Collection

Measurement of NO_3^- and NH_4^+ uptake in fine roots, as well as NO_3^- reductase activity (NRA) in leaves and fine roots, was made using methods similar to those of Rothstein and others (1996), who studied N uptake in these same forest stands. Fine roots were collected using a 5-cm diameter PVC corer extending 10-cm deep into the mineral soil (A and E horizons), a depth encompassing approximately 30% of all fine roots in this study (Hendrick and Pregitzer 1996). In each plot receiving ambient and experimental NO_3^- deposition, 12 cores were collected within 2 m of randomly chosen *A. saccharum* that were greater than 10 cm diameter at breast height (DBH); these individuals resided in the overstory canopy. Sun-lit leaves, those likely to have the greatest NO_3^- reductase activity (NRA; Smirnoff and Stewart 1985), were obtained with use of a 12-gauge shotgun loaded with steel shot. Leaves were removed from accessible branches on randomly selected overstory trees (DBH ≥ 10 cm) within each plot. Soil and leaf samples were placed on ice prior to laboratory analyses, which occurred within 24 h of field collection. In June and September 2004, root NO_3^- and NH_4^+ uptake, and leaf and root NRA, were measured in plots receiving ambient (n = 3) and experimental (n = 3) NO_3^- deposition in each stand.

Nitrogen Uptake Kinetics

The kinetic parameters for NO_3^- and NH_4^+ uptake in fine roots were determined by measuring the rates of $^{15}NO_3^-$ and $^{15}NH_4^+$ uptake. Using this approach, we have detected significant differences in the kinetic parameters for NH_4^+ and NO_3^- uptake by *Populus tremuloides*, which were growing in N-poor and N-rich soil (*sensu* Rothstein and others 1996); and others have used a similar approach to determine the influence of atmospheric N deposition on N uptake in a variety of broadleaf and evergreen tree species (Högberg and others 1998). Non-woody, very fine roots were collected (<1-mm dia.), gently rinsed free of soil with deionized H_2O, and cut in about 1-cm lengths. The selection of soil cores near sugar maple stems, the high basal area of sugar maple within these stands (>71%, Table 1), and the post collection removal of ectomycorrhizal roots ensured that these root samples were from sugar maple trees. On each sampling date, root segments were composited on a plot basis for each ambient and experimental NO_3^- deposition plot. Thirteen subsamples of fine roots (about 100-mg) from each plot were rinsed three times with 25 ml of 0.5 mM $CaSO_4$. Each sample was assayed for either $^{15}NO_3^-$ or $^{15}NH_4^+$ uptake by suspending the tissue in 50 ml of a nutrient solution consisting of 0.5 mM $CaSO_4$; 1% sucrose; and a 0, 1, 10, 100, 250, 500, or 1,000 μmol of either $K^{15}NO_3$ or $^{15}NH_4Cl$ (both atom 90 ^{15}N; BassiriRad and others 1993). One subsample was used for each of the six ^{15}N concentrations of $^{15}NO_3^+$ and $^{15}NH_4^-$; one subsample was used to determine the background ^{15}N concentration. The root subsamples were incubated in the ^{15}N solutions for 30 min at 25°C, rinsed with 0.5 mM $CaSO_4$ (three 50-ml aliquots), oven dried at 75°C for at least 24 h, and ground with a mortar and pestle. The N concentration (mg N g^{-1}) and δ ^{15}N of each root subsample was determined using a Finnigan Delta Plus isotope ratio mass spectrometer with a Conflo II interface (Thermo Finnagin, San Jose, California,

Table 1. Climatic, Floristic, and Edaphic Characteristics of Four Northern Hardwood Stands Located along a Latitudinal Gradient in Michigan, USA

Ecosystem property	Site A	Site B	Site C	Site D
I. Climate				
Latitude (N)	46°52′	45°33′	44°23′	43°40′
Longitude (W)	88°53′	84°51′	85°50′	86°09′
Mean annual precipitation, 1994–2001 (mm)[1]	821	828	856	793
Mean annual temperature, 1994–2001 (°C)[2]	4.8	6.1	6.9	7.6
Wet plus dry NO_3^-N (g m^{-2} y^{-1})[3]	0.38	0.58	0.78	0.76
Wet plus dry total N (g m^{-2} y^{-1})[3]	0.68	0.91	1.17	1.18
II. Overstory				
Overstory age, 2004	97	91	92	96
Total basal area, 2001(m^2 ha^{-1})	35	33	33	36
Sugar maple basal area, 2001 (% of total)	91	86	79	71
III. Forest floor and soil				
Forest floor (Oe + Oa) C:N ratio[4]	16.7	18.4	20.6	17.9
Net N mineralization (mg N g $soil^{-1}$)[5]	0.29b	0.46a	0.48a	0.32b
pH of A + E soil horizons[6]	4.6–5.1	4.6–5.3	4.4–4.5	4.3–5.2
pH of upper B soil horizons[6]	4.7–5.7	4.8–6.3	5.2–6.9	5.0–5.7

[1] *Precipitation amounts collected in open areas within 5 km of each site.*

[2] *Air temperature was measured at 2 m within all plots at all sites, using thermistors that were read every 30 min throughout the year.*

[3] *Atmospheric deposition data from MacDonald and others (1992).*

[4] *Forest floor C:N data are calculated from MacDonald and others (1991).*

[5] *N mineralization data from Zogg and others (1996) for the top 10 cm of soil and organic matter below the litter (O_i) layer measured using the buried bag technique. Sites indicated by different letters had significantly different rates ($P < 0.05$).*

[6] *Soil pH values from N.W. MacDonald, unpublished data, from samples collected as described in MacDonald and others (1991) for the ambient plots.*

USA). The atom percent ^{15}N of the root suspended in the blank (that is, no ^{15}N) uptake solution was subtracted from the atom percent ^{15}N of each root sample suspended in a $^{15}NO_3^-$ or $^{15}NH_4^+$ uptake solution. Uptake rates of $^{15}NO_3^-$ and $^{15}NH_4^+$ were reported as μmol $^{15}NO_3^-$ or $^{15}NH_4^+$ per gram root per hour (μmol $^{15}NO_3^-$ or $^{15}NH_4^+$ g^{-1} h^{-1}). The $^{15}NO_3^-$ or $^{15}NH_4^+$ uptake rates across the range of substrate concentration were used to estimate maximum velocity (V_{max}) of uptake and substrate affinity (K_m) using the Michaelis-Menton equation [$v = (V_{max}S)/(K_m + S)$] and non-linear regression.

Nitrate Reductase Activity in Roots and Leaves

Fine root and leaf NRA were determined using an in vivo NO_2^- production assay (Jaworski 1971). Within 24 h of field collection, disks of intervein leaf tissue were cut using a hole punch (6-mm diameter). On each sampling date, all leaf disks and root segments from each plot were composited separately in each plot, and NRA was determined on five replicate subsamples of each tissue to estimate a mean NRA rate for leaves and roots in that plot. Two hundred and fifty milligrams of fresh leaf or root tissue was suspended in 7.5 ml of the optimized assay medium, composed of 0.016 M NaH_2PO_4, 0.084 M Na_2HPO_4, 0.04 M KNO_3, 5% $CH_3(CH_2)_2OH$ (*n*-propanol), and 0.5 mg/ml chloramphenicol (pH 7.5). The plant tissue suspended in this medium was vacuum infiltrated and incubated at room temperature (23°C) for 1 h in the dark. Nitrite production was measured colormetrically at 20-min intervals by removing 1.0-ml aliquots. To develop color, 0.3 ml of 1% sulfanilamide and 0.3 ml of 0.02% *N*-(1-naphthyl)ethylenediamine was added to each 1.0-ml aliquot. This solution was incubated for 20 min, diluted with 2.4-ml deonized H_2O and absorbance was measured at 520 nm.

Fine root and leaf NO_3^- NRA was calculated as the slope of the linear regression of NO_2^- production over time, expressed as nmol NO_2^- per g fresh tissue per hour (nmol NO_2^- g^{-1} h^{-1}). Only replicates with r^2 greater than 0.80 were used to calculate a mean NRA for each plot receiving ambient and experimental NO_3^- deposition. On average, we rejected one replicate assay out of five for leaf tissue in each plot and two out of five replicate root NRA assays in each plot; this was true in plots receiving ambient and experimental NO_3^- deposition.

Statistical Analyses

Our sampling scheme enabled us to determine kinetic parameters for NO_3^- and NH_4^+ uptake in each plot receiving ambient and experimental NO_3^- deposition on the two sampling dates. We used an ANOVA for a randomized complete block design replicated in time to test the effect of experimental N deposition on V_{max} and K_m for NH_4^+ and NO_3^- uptake. This model enabled us to test the null hypothesis that NO_3^- deposition had no effect on the kinetic parameters for NO_3^- uptake as well as NH_4^+ uptake in mature sugar maple trees. We used the same ANOVA to test the null hypothesis that NO_3^- deposition had no effect on NRA. In this design, stands are blocks, N deposition is the treatment, and there were two sampling dates. This model included an interaction term between N deposition and time. Tukey's HSD multiple comparison procedure was conducted when there was a significant main or interaction effect. Significance was accepted for all statistical tests at $\alpha = 0.05$.

Results

Nitrogen Uptake

The Michaelis-Menton equation described the uptake of $^{15}NH_4^+$ and $^{15}NO_3^-$ very well. The r^2 values for this non-linear model of $^{15}NO_3^-$ uptake ranged from 0.739 to 0.992 with a mean of 0.910, whereas the r^2 values for $^{15}NH_4^+$ uptake ranged from 0.841 to 0.999 with a mean of 0.983. Experimental NO_3^- deposition did not have a significant effect on the uptake of either NH_4^+ or NO_3^-. For example, there was no significant main effect of experimental NO_3^- deposition on the maximum velocity (V_{max}) or the half-saturation constant (K_m) for either NH_4^+ or NO_3^- uptake (Table 2). The uptake curves, based on the mean kinetic values for the two N deposition treatments are illustrated in Figure 2, demonstrating that uptake kinetics were almost identical in roots in the ambient and experimental NO_3^- deposition treatments. Averaged across NO_3^- deposition treatment, the mean V_{max} for NH_4^+ uptake was eight times greater than the mean V^{max} for NO_3^- uptake. Moreover, the half-saturation constant (K_m) for NH_4^+ uptake was 16 times greater than the mean K_m for NO_3^- uptake.

Sampling date had a significant main effect on the kinetic parameters for N uptake, wherein the mean V_{max} for NH_4^+ uptake in September was significantly greater than that measured in June (6.8 μmol $^{15}NH_4^+$ g^{-1} h^{-1} vs. 3.5 μmol $^{15}NH_4^+$ g^{-1} h^{-1}, $P = 0.001$). This contrasts with a significantly greater K_m for NH_4^+ uptake in June than in September (1,093 μM $^{15}NH_4^+$ vs. 537 μM $^{15}NH_4^+$, respectively; $P = 0.008$). The mean K_m for NO_3^- uptake in June was significantly greater than in September (71.3 μM $^{15}NO_3^-$ vs. 31.4 μM $^{15}NO_3^-$, respectively; $P = 007$), although there was no difference between the V_{max} for NO_3^- uptake in June and September.

There was no significant interaction between sampling date and N deposition treatment for any kinetic parameter of NH_4^+ or NO_3^- uptake in sugar maple roots (Table 2). However, significant differences in the kinetic parameters occurred among stands for both NH_4^+ and NO_3^- uptake, but these differences were relatively small in magnitude and were not related to patterns of soil N availability or to any other characteristics of soil physical or chemical properties (Table 1).

Table 2. Michaelis-Menten Kinetic Parameters for NH_4^+ and NO_3^- Uptake by *A. saccharum* Roots Exposed to Ambient and Experimental NO_3^- Deposition Treatments. Also displayed is mean NRA for leaves and roots for ambient and experimental NO_3^-deposition treatments

	Ambient			NO_3^- Deposition			Significance *P*		
	June	September	Mean	June	September	Mean	N addition	Date	Interaction
V_{max} (μmol ^{15}N g^{-1} h^{-1})									
NH_4^+	3.5 (0.49)	6.6 (0.76)	5.0 (0.54)	3.5 (0.53)	7.1 (1.8)	5.3 (0.97)	0.799	****	0.208
NO_3^-	0.64 (0.08)	0.62 (0.05)	0.63 (0.05)	0.59 (0.08)	0.68 (0.09)	0.63 (0.06)	0.908	0.404	0.106
K_m (μM ^{15}N)									
NH_4^+	497 (106)	1,039 (189)	768 (120)	576 (130)	1,147 (339)	861 (187)	0.639	***	0.308
NO_3^-	79.8 (25)	33.3 (4.1)	56.6 (13)	62.9 (19)	29.5 (3.9)	46.2 (10)	0.459	***	0.639
NRA (nmol NO_2 g^{-1} h^{-1})									
Root	24.0 (15)	31.2 (4.3)	27.7 (7.5)	22.8 (22)	26.2 (2.3)	24.6 (10)	0.85	0.521	0.866
Leaf	104.9 (9.4)	79.5 (5)	92.2 (5.9)	102.4 (12)	83.5 (7.3)	92.9 (7.5)	0.922	***	0.668

*P values are for the ANOVA model used to test significant main effects and interaction of N deposition and sampling date. Significance is denoted as * P < 0.10, ** P < 0.05, *** P < 0.01, **** P < 0.001. One standard error is shown in parentheses.*

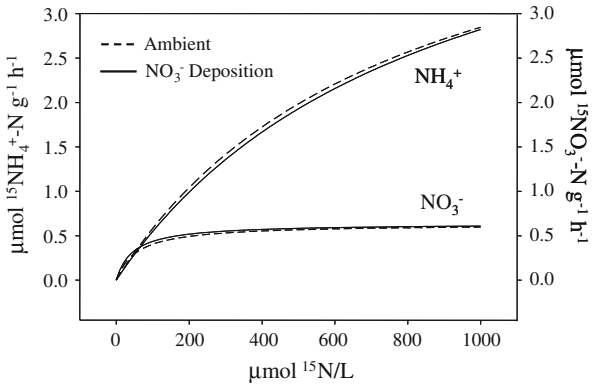

Figure 2. The mean velocity of N uptake by *A. saccharum* roots as a function of substrate N concentration in the ambient and experimental NO_3^- deposition treatment. Kinetic parameters were averaged across stands and dates to derive mean V_{max} and K_m for each treatment. Dashed lines indicated the ambient N deposition, whereas the solid line indicates the experimental NO_3^- deposition treatment. The N substrate ($^{15}NO_3^-$ or $^{15}NH_4^+$) is indicated on the figure.

Nitrate Reductase Activity

Experimental NO_3^- deposition did not have a significant effect on NRA in sugar maple leaves or fine roots, given that there was no significant effect of experimental N deposition on NRA in either tissue (Table 2). There was also no effect of site on NRA in roots or leaves (data not shown). However, leaf NRA differed significantly between the June and September sampling dates (103.6 nmol NO_2^- g^{-1} h^{-1} vs. 81.5 nmol NO_2^- g^{-1} h^{-1}, respectively; $P = 0.006$). A significant site by sampling date interaction ($P = 0.011$) occurred due to higher leaf NRA in Sites A and D in June, relative to the September sampling. There were no main or interactive effects of N deposition treatment, site, or sampling date on sugar maple root NRA. In short, experimental NO_3^- deposition has not induced NO_3^- assimilation in sugar maple leaves or roots.

Discussion

Atmospheric N deposition has the potential to alter the retention and loss of N in terrestrial ecosystems by modifying both plant and microbial sinks. In northern hardwood forests, sugar maple relies on NH_4^+ as its main source of N from soil, and this species has a limited capacity to take up and assimilate NO_3^-, even when seedlings are exposed to high soil NO_3^- (Rothstein and others 1996). Nonetheless, it is plausible that chronic NO_3^- deposition could induce NO_3^- use by mature sugar maple trees whose sun-lit canopies could provide a greater source of reductant for NO_3^- assimilation (Hoff and others 1992; Oaks 1994). After 10 years of experimental NO_3^- deposition, the uptake and assimilation of NO_3^- by overstory sugar maple remained low and did not differ between the ambient and experimental NO_3^- deposition treatments. Thus, low rates of NO_3^- use by sugar maple confirm that this dominant overstory species is not a direct sink for anthropogenic NO_3^- deposition in northern hardwood forests and will likely remain as such as N deposition continues to increase over the next several decades (Galloway and others 2004).

The physiological capacity of sugar maple in Lake States northern hardwood forests to take up NO_3^- after 10 years of chronic experimental NO_3^- deposition was low relative to NH_4^+ uptake. In sugar maple roots, the V_{max} for NO_3^- uptake was only 12% of the V_{max} for NH_4^+ uptake, clearly indicating that sugar maple has a greater capacity to take up NH_4^+. Previous studies have also demonstrated greater NH_4^+ uptake than NO_3^- uptake in sugar maple roots (Rothstein and others 1996; BassiriRad and others 1999; Templer and Dawson 2004). In this study, we determined the maximum rates of NH_4^+ and NO_3^- uptake were 5.15 μmol NH_4^+ g^{-1} h^{-1} and 0.63 μmol NO_3^- g^{-1} h^{-1}, respectively. Rothstein and others (1996) found a much higher V_{max} for NH_4^+ uptake (33 μmol NH_4^+ g^{-1} h^{-1}) and a similar V_{max} for NO_3^- uptake (1 μmol NO_3^- g^{-1} h^{-1}) in the same stands using similar methodology. However, Rothstein and others (1996) used sugar maple seedlings grown in a greenhouse, whereas we studied mature field-grown trees, which may account for the aforementioned differences. BassiriRad and others (1999) also found maximum rates of NH_4^+ (20 μmol NH_4^+ g^{-1} h^{-1}) and NO_3^- uptake (9 μmol NO_3^- g^{-1} h^{-1}) in sugar maple seedlings that were much higher than our observations. The ratio of maximum NH_4^+–N to NO_3^-N uptake has been found to vary from 2 (BassiriRad and others 1999) to 33 (Rothstein and others 1996). In our experiment, we found an intermediate ratio of 8, similar to the findings of Templer and Dawson (2004) who studied sugar maple seedlings in the Catskill Mountains. Despite these differences, it is clear that sugar maple has a greater capacity to take up and assimilate NH_4^+ than NO_3^-. Moreover, increased soil NO_3^- availability does not induce NO_3^- uptake in this species, regardless of ontogenetic stage.

The rates of both NH_4^+ and NO_3^- uptake we measured are low relative to studies of other plant species. The maximum rate of NH_4^- uptake

(5.15 μmol ^{15}N g^{-1} h^{-1}) was lower than the range of values reported for other tree species (6–75 μmol N g^{-1} h^{-1}; Rygiewicz and Bledsoe 1986; Fredeen and Field 1992; Kamminga-van Wijk and Prins 1993; Rothstein and others 1996; BassiriRad and others 1999; Zerihun and BassiriRad 2001). Similarly, the maximum rate of NO_3^- uptake (0.63 μmol ^{15}N g^{-1} h^{-1}) was lower than what has been measured in herbaceous species (1–20 μmol N g^{-1} h^{-1}; Goyal and Huffaker 1986; BassiriRad and others 1993; BassiriRad and others 1999) and other tree species (1–38 μmol N g^{-1} h^{-1}; Chapin and others 1986; Rygiewicz 1986; Fredeen and Field 1992; Kamminga-van Wijk and Prins 1993; Lajtha 1994; Rothstein and others 1996; BassiriRad and others 1999; Zerihun and BassiriRad 2001).

Chronic NO_3^- deposition did not induce NRA in sugar maple leaves or roots, further suggesting that this dominant overstory tree will not become a direct sink for atmospheric NO_3^- deposition in the future. The mean NRA was 26 nmol NO_2^- g^{-1} h^{-1} in roots and 93 nmol NO_2^- g^{-1} h^{-1} in leaves and did not differ between atmospheric N deposition treatments. Thus, it appears that NO_3^- reduction in sugar maple is not inducible by chronic atmospheric NO_3^- deposition, evidence that is consistent with low rates of root NO_3^- uptake. This response contrasts with other studies in which NRA in other tree species increased with greater NO_3^- availability (Smith and Rice 1983; Högberg and others 1986; Zak and Pregitzer 1988; Downs and others 1993; Truax and others 1994). Rates of NRA reported here were an order of magnitude higher than those observed by Rothstein and others (1996) in the same forest stands; the reason for this discrepancy is not known. Regardless of this difference, rates of NO_3^- reduction measured here are still very low relative to other plants known to use NO_3^-. For example, leaf NRA ranges from 1,400 to 8,750 nmol NO_2^- g^{-1} h^{-1} in the ruderal species (Al Gharbi and Hipkin 1984), from 100 to 3,200 nmol NO_2^- g^{-1} h^{-1} in herbaceous woodland and woodland-edge species (Al Gharbi and Hipkin 1984; Högberg and others 1986; Zak and Pregitzer 1988), and from less than 100 to more than 4,800 nmol NO_2^- g^{-1} h^{-1} in the woody species (Al Gharbi and Hipkin 1984; Downs and others 1993; Truax and others 1994). The rates of NRA in sugar maple are most similar to those in ericaceous plant species (Townsend and Blatt 1966; Havill and others 1974) growing in ecosystems with low soil NO_3^- availability. Relative to these observations, the low rates measured in *A. saccharum* (about 90 nmol NO_2^- g^{-1} h^{-1}) indicate that this species does not use NO_3^- as a major N source.

Although it is somewhat surprising that sugar maple that did not use NO_3^- under the conditions of chronically enhanced NO_3^- availability, this observation is consistent with the flow of $^{15}NO_3^-$ in the plant-soil system of our experiment. In a field study, we have learned that $^{15}NO_3^-$ applied to soil solution was rapidly assimilated by the microbial community (that is, within hours), and after several days, the isotope was released into soil solution as $^{15}NH_4^+$ (Zogg and others 2000). The $^{15}NH_4^+$ was then taken up by fine roots (that is, after weeks; Zogg and others 2000), after which canopy leaves and leaf litter became enriched over time (that is, after 1 year; Zak and others 2004). Over a decade of treatment, this pathway of NO_3^- flow has increased the N concentration of canopy leaves in our experimental NO_3^- deposition treatment (19.2 mg N/g vs. 22.8 mg N/g; ambient versus experimental NO_3^- deposition, respectively; Pregitzer and others 2008). Taken together, these observations are consistent with the contrasting physiological capacity of sugar maple to take up NH_4^+ versus NO_3^-; virtually none of the $^{15}NO_3^-$ applied in the field was recovered in fine roots, the majority of which were composed by sugar maple. More importantly, these observations provide evidence that sugar maple can function as a sink for anthropogenic NO_3^-, but it does so only after anthropogenic NO_3^- has been assimilated by soil microorganisms and released as NH_4^+. Inasmuch, sugar maple is an indirect sink for elevated atmospheric NO_3^- deposition and may function in this manner across its geographic distribution in the Upper Great Lakes region. This pathway represents a plausible mechanism by which atmospheric NO_3^- deposition has increased the N concentration of sugar maple leaves across a large geographic region in the northeastern US (McNeil and others 2007). Furthermore, the limited capacity of sugar maple to directly take up NO_3^- likely facilitates the leaching of anthropogenic NO_3^-, which has significantly increased in our study (Pregitzer and others 2004; Zak and others 2004). These ecosystem-level responses appear to result from the limited physiological capacity of overstory sugar maple to directly take up NO_3^- from soil solution.

Sugar maple-dominated forests often have high net nitrification rates (Pastor and others 1984; Zak and Pregitzer 1990; Templer and others 2003), relative to other forest types in the eastern US. This association could be interpreted to suggest that NO_3^- is the dominant form of soil N used by this species. However, the low physiological capacity of sugar maple to take up and assimilate NO_3^- in our experiment provides evidence that such a notion is

incorrect, at least for sugar maple in the Upper Great Lakes region. It is important to point out that net nitrification in our study is low (0.2 μg NO_3^-N g^{-1} d^{-1}, Zak and others 2006), relative to rates in other sugar maple-dominated forest in our region as well as in the northeastern US (4–8 μg NO_3^-N g^{-1} d^{-1}; Zak and Pregitzer 1990; Templer and others 2003). Quantifying NO_3^- uptake and assimilation in soils with high rates of net nitrification would extend our understanding of N use by this species and the degree to which it may, or may not, broadly function as a direct sink for anthropogenic NO_3^- deposition.

In conclusion, the kinetic parameters for NO_3^- uptake by overstory sugar maple exposed to a decade of elevated NO_3^- deposition (ambient plus 30 kg NO_3^- N ha^{-1} y^{-1}) were comparable to individuals receiving low rates of ambient atmospheric N deposition (about 7–11 kg N ha^{-1} y^{-1}). We also observed no difference in rates of NO_3^- assimilation, as measured by NO_3^- reductase activity in leaves and fine roots. In combination, these results suggest that increases in atmospheric NO_3^- deposition will not induce a greater capacity to take up and assimilate this form of N, rendering sugar maple unable to function as a direct sink for atmospheric NO_3^- deposition as it increases over time. As a consequence, the retention of anthropogenic NO_3^- in sugar maple-dominated northern hardwood forests is contingent on the rapid microbial assimilation of NO_3^-, the release of NH_4^+ via mineralization, and the subsequent assimilation of NH_4^+ by sugar maple roots. Therefore, microbial communities in soil mediate the extent to which plant uptake will retain anthropogenic NO_3^- in northern hardwood forests in the Upper Great Lakes region.

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