



Nitrogen uptake by four tree species of the Catskill Mountains, New York: Implications for forest N dynamics

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Received 27 June 2003. Accepted in revised form 5 November 2003

Key words: ammonium, forest nitrogen cycling, nitrate, plant uptake

Abstract

Watersheds of the Catskill Mountains, New York have marked differences in nitrogen (N) dynamics among dominant tree species stands. Our objectives were to study how tree species vary in N uptake to better understand the basis for the observed variation in these forested watersheds. We conducted a ¹⁵N tracer greenhouse study to determine NH₄⁺ and NO₃⁻ uptake of American beech (*Fagus grandifolia* Ehrh.), eastern hemlock (*Tsuga Canadensis* L.), red oak (*Quercus rubra* L.) and sugar maple (*Acer saccharum* Marsh.) seedlings. Seedlings and their native soil were collected in November 1997, over-wintered and allowed to break dormancy in spring 1998. Half of the seedlings of each tree species received ¹⁵NH₄-NO₃ to examine NH₄⁺ uptake and the other half received NH₄-¹⁵NO₃ to examine NO₃⁻ uptake. Plants were harvested 4 days following ¹⁵N addition. Tree species varied in their preference for NH₄⁺ and NO₃⁻. Sugar maple and eastern hemlock seedlings took up more NH₄⁺ than NO₃⁻ per unit plant biomass, while beech was the only species to take up more NO₃⁻ than NH₄⁺. Red oak took up more NH₄⁺ than NO₃⁻ into roots, stems and leaves, but the difference between the two forms of N was not statistically significant. These results demonstrate that tree species of the Catskill Mountains vary in their capacity to take up NH₄⁺ and NO₃⁻. Coupled with stand-level studies of N dynamics, this variation can help explain some of the patterns of forested watershed N retention and loss in the Catskill Mountains shown in our field investigations (Templer, 2001; Templer et al., in press *Ecosystems*).

Introduction

A complete understanding of forest N cycling requires that we understand how much N plants take up, which form of inorganic N they are likely to assimilate and how forest tree species may differ in their N dynamics. These plant attributes are important to understand because they can have implications for the amount of N retained by a forested watershed. Most of the N deposited onto forests is retained within the forest floor (Nadelhoffer et al., 1999; Templer, 2001; Templer et al., in press *Ecosystems*). However, forest N

saturation can occur when N availability is in excess of microbial and plant demand, resulting in some N loss from the forest through either nutrient leaching and/or gaseous N losses (Agren and Bosatta, 1988; Aber et al., 1989; Rennenberg et al., 1998).

The Catskill Mountains receive among the highest rates of N deposition in the northeastern United States (Ollinger et al., 1993; Stoddard, 1994) and many of its watersheds are beginning to show signs of N saturation (Lovett et al., 2000). Forest N retention within the Catskill Mountains varies among stands dominated by different tree species, with forests dominated by red oak having significantly greater N retention than those dominated by sugar maple (Templer, 2001;

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Templer et al., in press *Ecosystems*). This pattern can be partially explained by variation in soil microbial activity and N transformations in stands where tree species differ. For example, rates of net nitrification and N leaching are significantly higher within sugar maple stands than other tree species. The larger production and loss of NO_3^- among sugar maple stands may help explain why those stands retain less N than red oak stands (Templer, 2001; Lovett et al., in press *Biogeochemistry*; Templer et al., in press *Ecosystems*). Nitrate is relatively mobile compared to other forms of N, such as NH_4^+ , and can therefore be more easily leached from soils to groundwater and surface waters. We hypothesized that in addition to differences in microbial processing of N in soils associated with different tree species, variation in NH_4^+ and NO_3^- uptake rates among dominant tree species could also influence forest N retention or loss.

Plant species differ in their capacity to take up NH_4^+ or NO_3^- as their primary N source in part because of physiological trade-offs in taking up either form of N (Horsley, 1988; Stewart et al., 1992; Crabtree and Bazazz, 1993). While NO_3^- is readily available because of its high solubility in soil water, plants must use energy to reduce the NO_3^- prior to incorporation into their amino acids. With this constraint, it may be more efficient for plants to take up NH_4^+ because it can be immediately incorporated into amino acids. However, NH_4^+ is not always available to plants for uptake because it is tightly associated with soil exchange sites and does not move readily through the soil in solution.

The objectives of this research were to compare NH_4^+ and NO_3^- uptake by dominant tree species of the Catskill Mountains, including American beech (*Fagus grandifolia* Ehrh.), eastern hemlock (*Tsuga canadensis* L.), red oak (*Quercus rubra* L.) and sugar maple (*Acer saccharum* Marsh). There are a variety of methods one can employ to measure rates of N uptake by plants, with costs and benefits associated with each of them (e.g., see review by BassiriRad et al., 1999). For example, many researchers have excised roots from intact plants and immediately placed them into a solution containing a known amount of dissolved ^{15}N (e.g., Rothstein et al., 1996). After a specific amount of time, the roots are removed from the solution and the plant tissue is analyzed for ^{15}N concentration. One of the shortcomings of this method is that it could potentially underestimate plant uptake of N since it eliminates the regular transpiration stream of the plant or can deplete the carbohydrate reserves of the roots (Bloom and Caldwell, 1988; BassiriRad et al., 1999). Another

method maintains the connection of the root to the rest of the plant while the root is placed in a solution containing a known concentration of N (BassiriRad et al., 1999). The difference in N concentration before and after the root is placed in the solution is used to calculate how much N the plant roots took up. This method has its advantage in that the entire plant remains intact throughout the experimental period. One drawback to this method is that the root is placed in a water solution, containing only N, which eliminates the surrounding rhizosphere (e.g., the microbial community and soil medium) that the root usually interacts with. The soil solution could therefore be without other important ions, such as phosphorus and potassium. One way researchers avoid this problem is by including other inorganic nutrients, which are present within natural soil water, in their experimental solution (Fredeen and Field, 1992; Lovett and Tobiessen, 1993; Rennenberg et al., 1996; Gessler et al., 1998; Rennenerg et al., 1998). In this study, we applied a method for measuring plant uptake of N while plant roots were still attached and remained in contact with their native rhizosphere, including soil and associated microbial community.

Materials and methods

We conducted a greenhouse study in 1998 to examine N uptake by American beech (*Fagus grandifolia* Ehrh.), eastern hemlock (*Tsuga canadensis* L.), red oak (*Quercus rubra* L.) and sugar maple (*Acer saccharum* Marsh.) seedlings. We collected 14 seedlings of each species, and their native soil (Oe and Oa horizons), which were randomly selected from mixed species stands of the Kanape Brook watershed in the Catskill Mountains, NY in November 1997. Seedlings retained their own soil and were kept moist and cool while being transported from the field. To maintain the dormancy normally experienced in the field (L. Staats, personal communication), we over-wintered them at 2.2 °C. The plants were moved into a greenhouse unit at Cornell University (25–28 °C) on April 3, 1998 to break dormancy. Pots of all seedlings were arranged randomly and were moved within the greenhouse regularly to reduce the effects of environmental variation. Light levels reached 450 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ during the day, which is sufficient to drive photosynthesis for all of the tree species examined and to ensure that light was not a limiting factor in the experiment (Larcher, 1995). A majority of the leaves reached full

expansion by May 5. On that day we added 99 atom% enriched $^{15}\text{N-NH}_4$ (5 mL of 12 mg N/L as $^{15}\text{NH}_4\text{-NO}_3$) to six of the seedlings of hemlock, oak and maple, and 99 atom% enriched $^{15}\text{N-NO}_3$ (5 mL of 12 mg N/L as $\text{NH}_4\text{-}^{15}\text{NO}_3$) to six others to examine uptake of NH_4^+ and NO_3^- , respectively. Two individuals of each tree species served as controls. They did not receive any N and thus we were able to determine the natural abundance ^{15}N signature of each tree species. The same experimental design was used for beech except ^{15}N tracer was added to only 10 individuals because 2 died during the winter.

Plants were harvested 4 days following ^{15}N addition. The experimental period was limited to 4 days to minimize the impact of microbial activity on N transformations of NH_4^+ and NO_3^- in the soil, but was long enough time to detect enriched ^{15}N in the plant biomass. The roots, stem and leaves of each seedling were dried at 65 °C for at least 48 hours and ground with liquid N_2 with a mortar and pestle. Samples were analyzed for carbon (C), N and ^{15}N on a Europa ANCA-SL elemental analyzer that was coupled to a Europa 20–20 isotope ratio mass spectrometer (IRMS) at the Boyce Thompson - Cornell University Isotope Facility in Ithaca, NY.

Soil was sub-sampled from 4 pots of each tree species (within same ^{15}N treatment; 10 g fresh soil each) at the beginning (immediately following ^{15}N addition) and end of the experimental period. We measured soil NH_4^+ and NO_3^- concentrations to learn how N pools changed during the experimental period. The soil was removed with a small diameter soil corer to diminish the chance of disturbing the seedling roots, while obtaining a representative sample. Inorganic N concentration was determined after extracting 10 g of fresh soil with 40 mL 2 M KCl, shaking for one hour and filtering the extractant through a Whatman 42 filter. Concentrations of NH_4^+ and NO_3^- in soil extractions were analyzed on an Alpkem Flow Solution III Autoanalyzer. The ^{15}N in soil extractants was determined at the end of the experiment by concentrating the ^{15}N on a filter with a diffusion technique (Stark and Hart, 1996). Samples were analyzed for ^{15}N on a Europa 20-20 IRMS. We determined soil water content by drying 5 grams of each soil sample for 48 hours at 65 °C.

Calculating plant N uptake

We calculated plant N uptake as:

$$N_{\text{up}} = B^{-1} * \frac{(N_{\text{plt}})(\text{atom}\%^{15}\text{N}_{\text{plt}}) - (N_{\text{plt}})(\text{atom}\%^{15}\text{N}_{\text{NA}})}{\text{atom}\%^{15}\text{N}_{\text{S}}}, \quad (1)$$

where N_{up} is the amount of N taken up by the plant; B = biomass of the plant at the end of the experiment; $\text{atom}\%^{15}\text{N}_{\text{plt}}$ = atom percent ^{15}N of the plant at the end of the experiment; N_{plt} = mass of N in the plant at the end of the experiment; $\text{atom}\%^{15}\text{N}_{\text{NA}}$ = atom percent ^{15}N of the reference plants (seedlings that did not receive ^{15}N tracer); $\text{atom}\%^{15}\text{N}_{\text{S}}$ = atom ^{15}N of soil NO_3^- or NH_4^+ at the end of the experiment. We calculated uptake of N per unit plant biomass to get a measure of plant demand for N that takes into account differences in plant biomass among tree species. We also calculated uptake of N per total soil NH_4 and NO_3 , to take into account differences in availability of the two forms of N.

Statistical analyses

We conducted one-way analyses of variance (ANOVA) using SAS JMP software (Version 3.2.5, 1999) with tree species as the main effect to examine plant biomass, plant C, N and C:N, as well as soil NH_4^+ and NO_3^- . To examine preference for NH_4^+ or NO_3^- , we conducted an ANOVA within each tree species with form of N (NH_4^+ vs NO_3^-) as the main effect. All of the data were log-transformed prior to statistical analysis. A Kruskal-Wallis test was performed if the data were not normally distributed after the log transformation.

Results

Plant uptake of nitrogen

Per unit plant biomass, total plant uptake of $\text{NH}_4\text{-N}$ was significantly greater than uptake of $\text{NO}_3\text{-N}$ in eastern hemlock ($p = 0.020$) and sugar maple seedlings ($p = 0.0006$; Figures 1 and 2). There was a significantly greater amount of N derived from uptake of $^{15}\text{NH}_4^+$ compared to $^{15}\text{NO}_3^-$ in all plant parts of sugar maple, but only the roots ($p = 0.0027$) and stems ($p = 0.038$) of eastern hemlock (Figure 1). While all plant parts of red oak contained more N derived from $^{15}\text{NH}_4^+$ than $^{15}\text{NO}_3^-$ (Figures 1 and 2) there was no significant difference between these two forms of N. In contrast to the other three tree species, beech took up more N as $^{15}\text{NO}_3^-$ than $^{15}\text{NH}_4^+$ ($p = 0.082$; Figures 1 and 2).

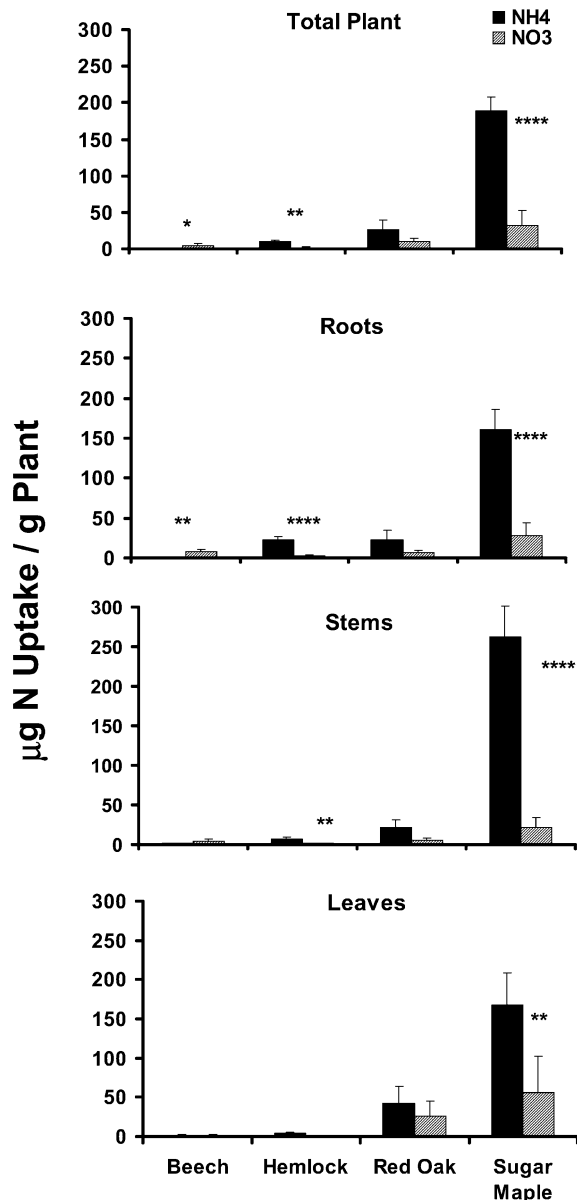


Figure 1. Plant uptake and sequestration of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ per g plant biomass ($n = 4$ for beech and $n = 5$ for hemlock, red oak, and sugar maple seedlings). Within a tree species, differences in $\text{NH}_4\text{-N}$ vs $\text{NO}_3\text{-N}$ uptake are denoted by the following: * $p < 0.10$, ** $p < 0.05$, *** $p < 0.01$, and **** $p < 0.005$.

When total soil $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ were taken into account, beech seedlings still took up a greater proportion of N as $\text{NO}_3\text{-N}$ than $\text{NH}_4\text{-N}$ ($p = 0.022$; Figure 2). Sugar maple seedlings still took up a greater proportion of N as $\text{NH}_4\text{-N}$ than $\text{NO}_3\text{-N}$ ($p = 0.0005$). Hemlock and red oak seedlings both took up greater

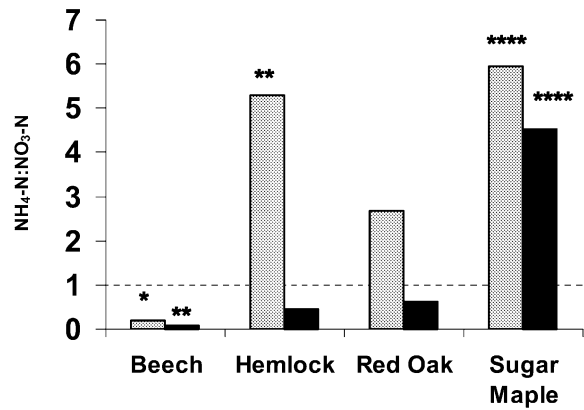


Figure 2. Stippled bars show ratio of total plant uptake of $\text{NH}_4\text{-N}:\text{NO}_3\text{-N}$ per g plant biomass ($n = 4$ for beech and $n = 5$ for hemlock, red oak and sugar maple seedlings). Solid black bars show ratio of total plant uptake of $\text{NH}_4\text{-N}:\text{NO}_3\text{-N}$ per g plant biomass per total $\mu\text{g NH}_4\text{-N}/\text{NO}_3\text{-N}$ in pot. Within a tree species, differences in NH_4 vs NO_3 uptake are denoted by the following: * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$, and **** $p < 0.005$. Dotted line denotes equal $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ uptake. Error bars cannot be calculated since values represent ratio of two groups.

amounts of NO_3^- than NH_4^+ , but the difference was not significant.

In addition to variation in composition of N uptake, tree species also varied in how much N they took up per unit plant biomass. For example, sugar maple took up over 6 times more $\text{NH}_4\text{-N}$ than eastern hemlock and red oak, and over 180-times more $\text{NH}_4\text{-N}$ than beech seedlings ($p < 0.05$; Figure 1). Sugar maple and red oak seedlings had similarly high amounts of $\text{NO}_3\text{-N}$ uptake into their biomass compared to eastern hemlock seedlings ($p < 0.05$).

Plant biomass, carbon and nitrogen

Seedling mass varied dramatically among species. The mass of the roots and leaves of eastern hemlock were over seven times larger than those of the other tree species ($p < 0.0001$; Table 1). Sugar maple had significantly lower root and stem mass than the other tree species ($p < 0.0001$). The sugar maple stems had only one thirteenth the mass of eastern hemlock seedlings. Eastern hemlock had more than double the root:stem ratio of all other tree species ($p = 0.0437$).

In addition to plant biomass, tree species varied in their N and C concentrations. The roots, stems and leaves of sugar maple had significantly higher % N compared to the three other tree species ($p < 0.05$; Table 2). However, sugar maple seedlings had the lowest root and stem %C of the four tree species ($p <$

Table 1. Plant biomass (g) across tree species with standard error ($n = 8$ for beech and $n = 10$ for hemlock, red oak and sugar maple seedlings). Values in parentheses are p -values comparing tree species within each variable. Different letters above values represent statistically significant differences at $p < 0.05$.

Tree Species	Roots	Stems	Leaves	Root:Stem Ratio
Beech	0.60 ^a ± 0.25	0.55 ^a ± 0.14	0.11 ^a ± 0.04	1.16 ^a ± 0.15
Hemlock	4.61 ^b ± 2.09	1.21 ^a ± 0.39	1.91 ^b ± 0.57	3.49 ^b ± 1.07
Red Oak	0.61 ^a ± 0.20	0.41 ^a ± 0.06	0.24 ^c ± 0.08	1.45 ^a ± 0.38
Sugar Maple	0.11 ^c ± 0.02 (<0.0001)	0.09 ^b ± 0.02 (<0.0001)	0.07 ^a ± 0.01 (<0.0001)	1.20 ^a ± 0.09 (0.0437)

0.05; Table 2). The C:N ratio of the roots, stems and leaves of sugar maple was significantly lower than the other tree species ($p < 0.05$; Table 2).

Soil nitrogen

Soil concentrations of NH_4^+ did not vary significantly across tree species at day 0 ($p = 0.29$; Table 3), although there was a trend whereby red oak and sugar maple had more than two times larger soil NH_4^+ concentrations compared to beech and eastern hemlock (power=0.2312). At day 4, sugar maple had larger soil pools of NH_4^+ than the other tree species, but the trend was not significant ($p = 0.23$; Table 3). There was a trend for greater NO_3^- in sugar maple and red oak soils than eastern hemlock soils at day 0 ($p = 0.0939$; Table 3). Day 4 NO_3^- pools were greatest in sugar maple soils ($p = 0.0014$; Table 3).

The net change in soil NH_4^+ and NO_3^- concentration over the 4-day experimental period did not vary across tree species ($p > 0.05$; Table 3). There was no significant net accumulation of NH_4^+ or NO_3^- during the four-day experimental period across all tree species ($p > 0.05$; Table 3). The ratio of NH_4^+ to NO_3^- did not vary significantly among tree species at day 0, but was significantly greater in eastern hemlock soils at day 4 compared to soils of red oak and sugar maple ($p = 0.014$; Table 3). Averaged across tree species, the ratio of NH_4^+ to NO_3^- did not differ between the beginning and end of the experimental period ($p = 0.169$; Table 3).

Discussion

Our results demonstrate that tree seedlings of four dominant species of the Catskill Mountains, NY vary in their capacity to take up NH_4^+ and NO_3^- . Sugar

maple seedlings took up on average 6 times more N as NH_4^+ than NO_3^- into their biomass. When the total amount of soil NH_4^+ and NO_3^- were taken into account, these seedlings still took up approximately 5 times more NH_4^+ than NO_3^- into their biomass. The similarity in NH_4^+ preference by sugar maple seedlings of this study and excised roots of adult sugar maple trees of another study (Rothstein et al., 1996) suggests that we can use the seedlings of this study as a model system for adult tree NH_4^+ or NO_3^- preference in the Catskill Mountains, NY. Eastern hemlock seedlings shared a similar pattern with sugar maple; they took up approximately five-times more NH_4^+ than NO_3^- , but the difference was not significant once differences in soil NH_4^+ and NO_3^- availability were taken into account. There was no significant difference in NH_4^+ than NO_3^- uptake by red oak seedlings. Beech seedlings took up approximately five-times more N as NO_3^- than NH_4^+ . Once differences in soil NO_3^- and NH_4^+ concentrations were taken into account, the ratio of NO_3^- to NH_4^+ uptake by beech seedlings increased to ten.

In this study, we applied a method for measuring plant uptake of N while the plants were intact and remained in contact with their native surroundings, including soil and associated microbial community. We believe that this helped to minimize disturbance of the plants' normal physiology. We removed the plants from their normal environment, but sought to minimize disturbance effects by (1) over-wintering the plants in a greenhouse before we began the experiment to allow the plant and its associated soil to re-equilibrate and (2) measuring plant uptake of N while the roots were still attached and therefore plants were still intact. Unlike other studies that look at plant uptake of N at a variety of concentrations, we determined plant uptake at a N concentration we considered to

Table 2. Plant carbon, nitrogen and C:N ratio across tree species with standard error ($n = 8$ for beech and $n = 10$ for hemlock, red oak and sugar maple seedlings). Values in parentheses are p -values comparing tree species within each variable. Different letters above values represent statistically significant differences at $p < 0.05$.

Tree Species	Roots			Stem			Leaves		
	%Carbon	%Nitrogen	C:N	%Carbon	%Nitrogen	C:N	%Carbon	%Nitrogen	C:N
Beech	45.51 ^a ± 0.53	1.02 ^a ± 0.11	47.86 ^{ab} ± 5.76	47.49 ^a ± 0.43	0.96 ^a ± 0.11	54.71 ± 6.12	46.22 ± 0.75	2.27 ± 0.22	21.73 ± 2.34
Hemlock	44.44 ^a ± 0.71	0.81 ^a ± 0.08	57.45 ^a ± 4.47	48.93 ^b ± 0.13	0.60 ^b ± 0.05	85.96 ^b ± 5.98	47.54 ± 0.23	1.10 ^b ± 0.09	47.35 ^b ± 4.45
Red Oak	44.85 ^a ± 0.31	1.21 ^a ± 0.19	44.50 ^b ± 5.07	47.22 ^a ± 0.31	0.83 ^a ± 0.07	59.70 ^a ± 3.83	46.03 ± 0.42	2.10 ^a ± 0.10	22.28 ^a ± 0.93
Sugar Maple	40.01 ^b ± 1.49	1.81 ^b ± 0.16	23.69 ^c ± 2.16	44.27 ^c ± 0.75	1.59 ^c ± 0.18	32.01 ^c ± 4.38	46.70 ± 0.24	3.15 ^c ± 0.18	15.23 ^c ± 0.80
Maple	(0.0442)	(0.0014)	(0.0005)	(<0.0001)	(<0.0001)	(<0.0001)	(0.0533)	(<0.0001)	(<0.0001)

Table 3. Soil properties across tree species with standard error ($n = 4$ for each tree species). Moisture was determined by the following calculation: (wet-dry mass)/dry mass. NH_4^+ , NO_3^- , DIN ($\text{NH}_4^+ + \text{NO}_3^-$) at day 0 (immediately following ^{15}N addition) and day 4 (time of seedling harvest). Different letters above values represent statistically significant differences. Values in single parentheses are p -values comparing tree species within each variable. Values in double parentheses are p -values comparing tree species for trehanae in each variable over the four-day experimental period.

Tree Species	Moisture	Day 0		Day 0		Day 4		Day 4	
		$\mu\text{g NH}_4\text{-N/ g Soil}$	$\mu\text{g NO}_3\text{-N/ g Soil}$	$\mu\text{g DIN/ g Soil}$	$\text{NH}_4\text{-N:NO}_3\text{-N}$	$\mu\text{g NH}_4\text{-N/ g Soil}$	$\mu\text{g NO}_3\text{-N/ g Soil}$	$\mu\text{g DIN/ g Soil}$	$\text{NH}_4\text{-N:NO}_3\text{-N}$
Beech	0.41 ^a ± 0.05	46.5 ± 17.1	21.5 ^{ab} ± 11.8	68.0 ^a ± 9.9	5.5 ± 3.9	53.10 ± 13.9	4.8 ^a ± 1.4	57.9 ± 13.7	22.1 ^{ab} ± 10.7
Hemlock	0.50 ^{ab} ± 0.06	70.2 ± 6.3	8.3 ^a ± 4.1	78.5 ^a ± 10.2	14.2 ± 4.5	58.90 ± 9.8	0.6 ^a ± 0.2	59.4 ± 10.4	112.7 ^a ± 26.5
Red Oak	0.59 ^b ± 0.04	139.0 ± 32.8	34.4 ^b ± 14.0	173.4 ^b ± 24.6	7.0 ± 2.5	102.00 ± 27.2	17.4 ^b ± 6.7	119.4 ± 32.9	10.4 ^b ± 4.4
Sugar Maple	0.60 ^b ± 0.04	156.5 ± 127.0	39.3 ^b ± 9.7	195.7 ^{ab} ± 136.7	3.4 ± 2.4	144.50 ± 47.8	41.8 ^b ± 17.5	189.0 ± 114.8	3.2 ^b ± 1.1
	(0.0240)	(0.2943)	(0.0939)	(0.0853)	(0.3843)	(0.2279)	(0.0014)	(0.2538)	(0.0136)
						((0.5920))	((0.8501))	((0.6737))	

be large enough to encompass the maximum rate of plant uptake, or V_{max} , of the plant species we were interested in. While our study does not describe all of the possible kinetic rates of N uptake, we are able to make comparisons of N uptake among different plant species of the Catskill Mountains, NY.

Many factors could influence the amount of N taken up by a plant. For example, plant biomass varied significantly among tree species, with eastern hemlock seedlings having larger root and leaf mass compared to the other tree species, which is possibly attributable to differences in age between the seedlings examined. We do not try to interpret the differences among species in the amount of N uptake because initial variation in seedling mass among tree species could influence these differences. Because we could not control for initial plant size at the beginning of the experiment, we weighted N uptake by plant biomass to correct for any variation in biomass between tree species. We focus our discussion on the form of N (e.g., NH_4^+ vs. NO_3^-) taken up by plants.

We are aware of the possibility that microbial transformation of N in soil during the 4-day experiment could confuse our distinction between NH_4^+ and NO_3^- uptake by plants. For example, if the added $^{15}NH_4$ were nitrified and then taken up as NO_3^- , it would appear to us as NH_4^+ uptake in this experiment. We chose an experimental period of 4 days as a compromise; a shorter period would minimize N transformation in the soil and a longer period would enhance our ability to detect uptake in the plants. We do not believe that microbial transformations were a significant problem in this experiment. For instance, if nitrification was a significant problem we would expect to see it most in the sugar maple soils, which have the highest net nitrification rates associated with it than any of these species (Templer et al., 2003; Lovett et al., in press *Biogeochemistry*). If the added $^{15}NH_4^+$ were being nitrified and taken up as NO_3^- , we would expect to observe an equally vigorous uptake of $^{15}NO_3^-$ by sugar maple plants, but we did not. In the opposite extreme, if the $^{15}NO_3^-$ added to beech pots was immobilized by microbes, re-mineralized, and taken up as NH_4^+ , we would expect to observe an equally vigorous uptake of added $^{15}NH_4^+$, but again we did not. This leads us to believe that our experimental procedure accurately assessed the relative uptake of NH_4^+ and NO_3^- by these plants.

On average, the amount of N available to the seedlings of this experiment was approximately five times

greater than that observed in Catskill Mountain forest soils (Templer et al., in press *Ecosystems*). The larger N pools could have resulted from ^{15}N addition or the stimulation of microbial processes during the collection and potting of the soils and plants. Because of this, the inorganic N available for all plant species was less limiting compared to field conditions. However, the relative amounts of N available to the different tree species were similar to field conditions. For example, soil NO_3^- concentrations were consistently greater within sugar maple soils and lower in hemlock soils compared to the other plant species. Also, soil NH_4^+ was consistently greatest in sugar maple soils in both the field and this experiment.

Comparison among tree species

It is not surprising that sugar maple took up more NH_4^+ than NO_3^- given that it is a shade-tolerant species (Godman et al., 1990), and shade tolerance has been associated with greater NH_4^+ than NO_3^- uptake (Fredeen and Field, 1992; Stewart et al., 1992). The sugar maple seedlings of this experiment took up approximately 6 times more NH_4^+ than NO_3^- . This ratio of NH_4 -N: NO_3 -N uptake is intermediate in value between other studies which have examined inorganic N uptake by sugar maple seedlings. For example, BassiriRad et al., (1999) examined intact roots using the N depletion method and found sugar maple roots to take up approximately two times more NH_4^+ than NO_3^- . However, Rothstein et al., (1996) examined excised roots of sugar maple seedlings and found substantially greater ratios of NH_4 -N: NO_3 -N uptake compared to both BassiriRad et al., (1999) and our experiment. The V_{max} and K_m values for NH_4^+ uptake of their sugar maple seedlings were at least 10 times greater than the V_{max} and K_m values for NO_3^- uptake.

Similar to the sugar maple seedlings, it is not surprising that the eastern hemlock seedlings took up more NH_4^+ than NO_3^- given that they too are a shade tolerant species (Martin, 1959) and many conifers tend to reduce NO_3^- within their roots rather than their shoots (Sarjala, 1991). Having NO_3^- reductase primarily located in roots has been associated with larger NH_4^+ than NO_3^- uptake (Gutschick, 1981). However, it appears that the hemlock seedlings in this study were taking up greater amounts of N in the form that was present, rather than discriminating between the two forms. The significant difference in form of N uptake disappears once the amount of NH_4^+ and NO_3^- is taken into account. Therefore, it appears that hemlock does

not have a preference for either form of N, but rather takes up greater amounts of NH_4^+ because its availability exceeds that of NO_3^- . We are unaware of other studies that have examined inorganic N preference by eastern hemlock plants, but studies of western hemlock (*Tsuga heterophylla*) show that they take up NH_4^+ faster than NO_3^- (Knoepp et al., 1993).

The decrease in soil NO_3^- concentration in eastern hemlock soils over time is surprising given that rates of net nitrification are often detectable in soil associated with this tree species (Templer et al., 2003; Lovett et al., in press *Biogeochemistry*). It is not clear what the complete fate of NO_3^- was in the eastern hemlock soils in our experiment. The hemlock seedlings took up less than 2% of the standing NO_3^- pool. Some of the remaining NO_3^- could have been directly incorporated into soil organic matter via abiotic reactions (Johnson, 1992; Magill et al., 1997; Aber et al., 1998; Berntson and Aber, 2000; Dail et al., 2000; Johnson et al., 2000; Fitzhugh et al., 2003; Fitzhugh et al., in press *Global Change Biology*). Soil microbes could have taken up some of the NO_3^- , but they usually take up more NH_4^+ than NO_3^- (Wickramasinghe et al. 1985; Rice and Tiedje, 1989; Recous et al., 1990; Puri and Ashman, 1999), even when NO_3^- is readily available (Puri and Ashman, 1999). Whatever the fate of NO_3^- was in eastern hemlock soils, the seedlings had access to amounts of NO_3^- similar to those observed in the field, but ultimately took up more NH_4^+ .

It is possible that because the sugar maple and eastern hemlock seedlings of this experiment were exposed to high NH_4/NO_3 ratios in soil solution, that they may have been less likely to take up NO_3^- in a short-term experiment like this one because it was not advantageous to produce NO_3^- reductase, which is necessary for assimilating this form of N. However, nitrate reductase is an inducible enzyme at very low external NO_3^- concentrations in many plant species (Tischner, 2000). Induction of NO_3^- reductase activity can occur within hours of exposing plants to NO_3^- for the first time (Min et al., 1998). We therefore believe that four days represents a sufficient time to induce NO_3^- reductase and assert that the significantly greater uptake of NH_4^+ than NO_3^- in eastern hemlock and sugar maple seedlings was indeed reflecting true differences in uptake of different forms of N.

There was no significant difference in form of N uptake by the red oak seedlings. The lack of a strong preference for NO_3^- by red oak seedlings is expected given that they too are a shade tolerant tree species

(Sander, 1990) and may have NO_3^- reductase located in their roots (Gharbi and Hipkin, 1984). Red oak stands tend to have relatively small amounts of N leaching compared to sugar maple stands (Templer, 2001; Templer et al., in press *Ecosystems*), but it is not clear whether uptake of N into plant biomass is a strong retention mechanism.

The fact that beech seedlings tended to have greater NO_3^- than NH_4^+ uptake is surprising given that they are generally considered to be a shade tolerant species (Tubbs and Houston, 1990) and to be associated with low soil NO_3^- concentrations (Lovett and Rueth, 1999; Templer et al., 2003, in press *Ecosystems*). In addition, other tree species of their family (Fagaceae) have NO_3^- reductase located in their roots (Gharbi and Hipkin, 1984). All of these factors would lead one to expect beech seedlings to take up more NH_4^+ since it would be less energetically expensive than taking up NO_3^- and reducing it within their plant tissue. Furthermore, another beech species, *Fagus sylvatica*, has been shown to take up more NH_4^+ than NO_3^- (Gessler et al., 1998). In one experiment, the presence of NH_4^+ significantly reduced NO_3^- uptake, especially at ratios of $\text{NO}_3^-:\text{NH}_4^+$ less than 4. In this study, $\text{NO}_3^-:\text{NH}_4^+$ ratios in beech soils were always less than 4, yet NO_3^- uptake surpassed NH_4^+ uptake. It is possible that the relatively low magnitude of total N uptake by beech seedlings in this experiment was due to these seedlings meeting the bulk of their N demand from stored N reserves. This could have obscured the ratio of NH_4^+ to NO_3^- uptake usually experienced in the field. Additional research examining constraints on NH_4^+ and NO_3^- uptake by beech trees needs to be undertaken.

Implications for forest nitrogen dynamics

Variation in the form of N taken up by plants, in conjunction with previous work examining soil microbial activity among tree species (Templer et al., 2003), may explain some of the variation in N retention and loss among Catskill Mountain watersheds. Forests dominated by sugar maple have greater N leaching and smaller N retention compared to forests dominated by red oak (Templer, 2001; Templer et al., in press *Ecosystems*). This could be due to a combination of processes that occur in the soils and in plant biomass of these forest stands. For example, soils beneath sugar maple stands have relatively high rates of net nitrification (Zak and Pregitzer, 1990; Finzi et al., 1998; Lovett and Rueth, 1999; Templer et al., 2003; Lovett

et al., in press *Biogeochemistry*), yet the ability of sugar maple to take up NO_3^- compared to NH_4^+ is low (Figures 1 and 2; Rothstein et al., 1996; BassiriRad et al., 1999). The combination of a large production of NO_3^- and the lack of uptake by the dominant tree species may explain why forests dominated by sugar maple have a relatively large amount of NO_3^- leaching and smaller N retention compared to other tree species of the Catskill Mountains (Templer, 2001; Lovett et al., 2002; Templer et al., in press *Ecosystems*). Sugar maple does not take up a large amount of the form of N that is most likely to leach from their stands and therefore the plant biomass may not act as an immediate retentive mechanism for the added NO_3^- .

Forest sinks for nitrogen

Atmospheric N deposition to the forests of the Catskills is primarily in the form of NO_3^- (Lovett and Rueth, 1999). The results of this study suggest that, of the species studied, only beech could be a significant sink for the deposited NO_3^- . Nitrate not taken up by plants can be denitrified, and lost in gaseous forms, or lost from the system by leaching. Alternatively, NO_3^- may be taken up by microbes and re-mineralized to NH_4^+ , which would then be available for uptake by most of the species studied here.

Understanding the form of N preferred by dominant tree species may help us to predict the ability of northern hardwood forests to tolerate excess N deposition, especially when other factors such as climate change (Vitousek, 1994) and introduced plant diseases (Houston et al., 1979; Orwig and Foster, 1998) are altering tree species composition. For instance, beech bark disease is responsible for the increasing rate of beech tree mortality throughout the Catskill Mountains (Griffin et al., 2003). The disease is caused by the *Nectria* fungus, which enters the bark of beech trees through fissures produced by an introduced scale insect (*Cryptococcus fagisuga* Lind.). This can eventually girdle and kill the tree (Houston et al., 1979). No matter which species replaces the dying beech, the N economy of the forest could change, because all of the other species take up greater amounts of NH_4^+ than NO_3^- . Thus, such a species shift would lower the ecosystem retention of atmospherically deposited NO_3^- and could lead to either greater N leaching or gas loss. If sugar maple, which is the current dominant species in these forests, replaces beech, the NO_3^- export could accelerate even more because of the high nitrification rate of sugar maple soils (Templer et al.,

2003; Lovett et al., in press *Biogeochemistry*) and the high level of discrimination against NO_3^- uptake. Leaching of N might be further enhanced by sugar maple's ability to mobilize nutrients more efficiently via hydraulic lift (e.g., water loss from roots; Dawson, 1993, 1996, 1998). Longer-term studies need to incorporate soil microbial and plant processing of N simultaneously to better understand how changes in tree species composition could affect forest N retention or loss.

Acknowledgements

Support was provided by the National Science Foundation funded Biogeochemistry and Environmental Change RTG Program at Cornell University and a National Science Foundation funded Human Accelerated Environmental Change graduate fellowship to P. Templer. We appreciate the laboratory assistance provided by Megan Kell and the comments provided by Wendy Chou, Stuart Findlay, Gary Lovett, Megan McGroddy and Whendee Silver on earlier drafts of this manuscript.

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Section editor: M.A. Adams