# Preliminary estimates of foliar absorption of $^{15}N$-labeled nitric acid vapor ($HNO_3$) by mature eastern white pine (*Pinus strobus*)

JAMES M. VOSE[1] AND WAYNE T. SWANK

*USDA Forest Service Coweeta Hydrologic Laboratory, 999 Coweeta Lab Road, Otto, NC 28763, U.S.A.*

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We used a direct approach to quantify foliar N absorption by exposing foliage of mature eastern white pine (*Pinus strobus* L.) to $^{15}N$-labeled nitric acid vapor ($HNO_3$). Foliage on terminal portions of branches in a 31-year-old white pine plantation was enclosed in 9.0-L teflon film branch cuvettes and exposed to 10, 50, and 100 ppb $H^{15}NO_3$ for 12–30 h. Foliar absorption rates ranged from 0.026 $\mu g\ ^{15}N \cdot g^{-1} \cdot h^{-1}$ at 10 ppb to 0.267 $\mu g\ ^{15}N \cdot g^{-1} \cdot h^{-1}$ at 100 ppb. Extrapolation to the entire canopy resulted in an estimated absorption of 0.30–0.50 kg $N \cdot ha^{-1} \cdot year^{-1}$ at ambient $HNO_3$ concentrations. In contrast, canopy input–output estimates for the same forest stand indicated a depletion of 2.3 kg $N \cdot ha^{-1} \cdot year^{-1}$ by the forest canopy.

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L'absorption foliaire de N a été quantifiée directement en exposant le feuillage de Pins blancs de l'est (*Pinus strobus* L.) matures aux vapeurs d'acide nitrique ($HNO_3$) marqué avec du $^{15}N$. La portion terminale du feuillage sur des branches de Pins blancs de l'est dans une plantation de 31 ans fut enfermée dans une cuvette de teflon de 9,0 L et exposée à 10, 50 et 100 ppb $H^{15}NO_3$ pendant 12 à 30 h. Les taux d'absorption foliaire allaient de 0,026 $\mu g\ ^{15}N \cdot g^{-1} \cdot h^{-1}$ à 10 ppb jusqu'à 0,267 $\mu g\ ^{15}N \cdot g^{-1} \cdot h^{-1}$ à 100 ppb. Une extrapolation pour l'ensemble du couvert a permis d'évaluer l'absorption à 0,30 à 0,50 kg $N \cdot ha^{-1} \cdot an^{-1}$ aux concentrations ambiantes de $HNO_3$. Par contre, des estimés «d'input-output» pour le même peuplement indiquent une perte de 2,3 kg $N \cdot ha^{-1} \cdot an^{-1}$ via le couvert.

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## Introduction

Increased atmospheric deposition of N to forest ecosystems in the form of gases, precipitation, and particulates has been hypothesized to alter tree physiology and nutrient cycling processes (McLaughlin 1985; Nihlgard 1985; Aber et al. 1989). An important component of the fate of these N inputs is absorption by the foliage; however, the magnitude of foliar absorption of atmospheric N inputs in mature forests is largely unknown. We used a direct approach to quantify foliar N absorption by exposing foliage of mature eastern white pine (*Pinus strobus* L.) to $^{15}N$-labeled $HNO_3$. Our objectives were (1) to quantify $^{15}N$ absorption by white pine foliage across a range of $H^{15}NO_3$ concentrations and (2) to evaluate the significance of $^{15}N$ absorption relative to N nutrition and cycling.

## Methods

Foliage was exposed to known $H^{15}NO_3$ (99% enrichment) concentrations in FEP teflon film branch cuvettes (9.0 L volume) placed at the ends of branches in the upper canopy of a 31-year-old white pine plantation (Coweeta Hydrologic Laboratory, NC). Branches were approximately 10 m long and were sampled from three different trees. These trees were representative of dominant and codominant trees in the stand. Access to the upper canopy was facilitated by a 31-m "walk-up" tower used for atmospheric deposition monitoring. The $H^{15}NO_3$ was generated from certified, precalibrated gas permeation tubes that contained $H^{15}NO_3$ in liquid phase (KIN-TEC Laboratories, Texas City, TX). When heated to a specified calibration temperature, $H^{15}NO_3$ diffused through the teflon wall of the permeation tube at a constant rate. Permeation tubes of variable size and calibration temperature were required to generate $H^{15}NO_3$ concentrations of 10, 50, and 100 ppb when mixed with $HNO_3$ free air at a flow rate of 9.0 $L \cdot min^{-1}$. Ambient $HNO_3$ was removed by passing air through a nylon filter (Gelman Nylasorb) prior to mixing with the permeating $H^{15}NO_3$. All tubing in the delivery system was composed of TFE teflon.

Three treatment regimes were imposed to represent a range of exposure conditions. First, foliage was subjected to 50 ppb $H^{15}NO_3$ from 09:00 to 16:00 on August 25, 1988, and from 10:00 to 15:00 on August 26, 1988 (7 and 5 h on days 1 and 2, respectively; the cuvette was removed from the branch between exposures). In the second exposure, foliage was subjected to 10 ppb $H^{15}NO_3$ from 10:00 on September 7, 1988, to 15:00 on September 8, 1988 (30 continuous hours), and in the third exposure foliage was subjected to 100 ppb $H^{15}NO_3$ from 10:00 on September 15, 1988, to 15:00 on September 16, 1988 (30 continuous hours). Exposures were conducted on rainless, sunny days. Monthly precipitation was 93 mm (34% below average) in August and 116 mm (8% below average) in September. In the 2 weeks preceding each exposure period, rainfall was 57, 63, and 70 mm for the three exposure periods, respectively. Scanning electron microscopy of needle surfaces immediately following exposures (needles were immediately fixed using procedures in Sabatini et al. 1963) showed that stomates were open at the end of all exposures (J.M. Vose and W.T. Swank, unpublished data). After exposures, foliage was (1) immediately removed and rinsed vigorously with 1000 mL deionized water spray, as previous research by Marshall and Cadle (1989) has shown that rinsing with deionized water effectively removes adsorbed N, (2) separated by age-class (i.e., foliage produced the past year and foliage produced the current year), (3) dried at 60°C for 48 h and weighed to the nearest 0.01 g, and (4) ground to 100 $\mu m$ using a

[1]Author to whom all correspondence should be addressed.

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TABLE 1. Foliar $^{15}N$ absorption by age-class (current (C), past (P)) and exposure level

| Level (ppb) | Dosage (ppb h)[a] | Age-class | Total $^{15}N$ (%) | Excess $^{15}N$ (%) | Total N (%) | Total N (g) | Total excess $^{15}N$ (μg) |
|---|---|---|---|---|---|---|---|
| 10 | 300 | C | 0.374 25 (0.002) | 0.007 23 | 1.058 (0.02) | 0.055 | 3.977 |
| | | P | 0.383 67 (0.003) | 0.016 65 | 1.097 (0.002) | 0.044 | 7.326 |
| 50 | 600 | C | 0.378 44 (0.0002) | 0.011 42 | 1.189 (0.001) | 0.086 | 9.821 |
| | | P | 0.374 81 (0.0004) | 0.007 79 | 1.017 (0.005) | 0.086 | 6.699 |
| 100 | 3000 | C | 0.425 94 (0.0001) | 0.058 92 | 1.159 (0.008) | 0.053 | 31.228 |
| | | P | 0.443 34 (0.003) | 0.076 32 | 1.043 (0.02) | 0.044 | 33.581 |

NOTE: Total N was estimated by multiplying foliar percent total N by the dry weight of exposed foliage. Total excess $^{15}N$ was estimated by multiplying percent excess $^{15}N$ by total N. SE is a measure of analytical error and is given in parentheses.
[a]Concentration in ppb × exposure duration in hours.

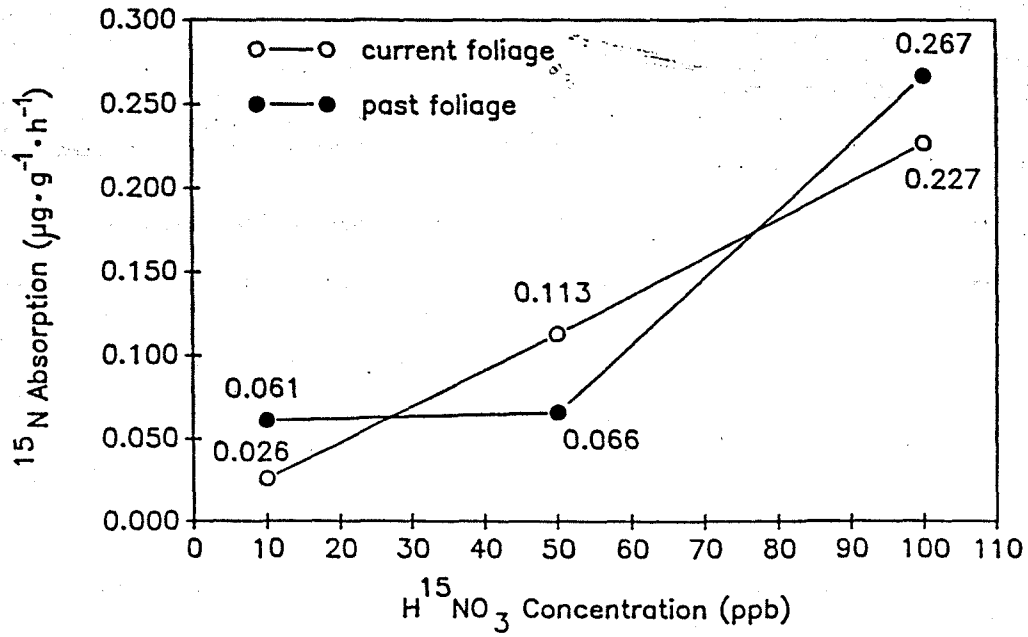


FIG. 1. Nitrogen-15 absorption rate by concentration and foliage age-class. Nitrogen-15 uptake rate was determined by dividing total excess $^{15}N$ (μg) by the dry weight (g) of exposed foliage and by the duration of exposure (h).

ball mill. Subsamples ($n$ = 2) of ground tissue were then analyzed for total N and at.% $^{15}N$ using mass spectrometry (analyzed by ISO-TEC Labs, Europa Scientific Instruments, Miamisburg, OH). Excess $^{15}N$ was determined by subtracting total $^{15}N$ of exposed foliage from total $^{15}N$ of unexposed foliage. Absorption of $^{15}N$ is expressed on a foliage dry weight basis.

Canopy absorption estimates extrapolated from $H^{15}NO_3$ experiments were compared with canopy $NO_3$-N inputs and outputs derived from 2 years of data (1986–1987) as part of the *Integrated Forest Study on the Effects of Atmospheric Deposition* (Johnson and Lindberg 1986). Data were collected using standard measurement protocols (Lindberg and Lovett 1985; Lindberg et al. 1986; Swank and Reynolds 1987).

## Results and discussion

Excess $^{15}N$ was detected after all exposures. Excess at.% $^{15}N$ values ranged from 0.007 23% in current foliage after the 10 ppb exposure, to 0.076 32% in past foliage after the 100 ppb exposure (Table 1). Absorption rates of $^{15}N$ ($\mu g \cdot g^{-1} \cdot h^{-1}$) increased with concentration (Fig. 1), and this pattern was consistent across foliage age-classes. Absorption rate was linearly related to concentration in current foliage, but curvilinearly related to concentration in past foliage. Linear regression combining absorption rates for both foliage age-classes resulted in an $r^2$ of 0.96 (no-intercept model; $F$ = 134.51; $P > F$ = 0.0001; mean square error = 0.0010; $n$ = 6), where the slope of the regression was 0.002 35. Our absorption rates are similar to simulated cloud water $^{15}N$ absorption rates by red spruce foliage (Bowden et al. 1989), which ranged from 0.079 to 0.288 $\mu g \cdot g^{-1} \cdot h^{-1}$.

To obtain a measure of the magnitude and importance of these absorption estimates at the stand level, we extrapolated the uptake data presented in Fig. 1 to the entire forest canopy based on four assumptions: (1) canopy biomass = 9500 $kg \cdot ha^{-1}$ from April to October and 5300 $kg \cdot ha^{-1}$ from November to March (determined following procedures in Swank and Schreuder 1973); (2) from April to October, 50% of the canopy is current foliage and 50% is past foliage; from November to March, 100% of the canopy is past foliage (Swank and Schreuder 1974); (3) foliage is exposed to $HNO_3$ for 365 days $\cdot$ $year^{-1}$; and (4) uptake rates during November to March are reduced by 25% to adjust for lower stomatal conductance in winter. Extrapolated estimates of potential canopy absorption were

TABLE 2. $NO_3$-N flux to the forest canopy

| Canopy inputs and outputs | Flux ($kg \cdot ha^{-1} \cdot year^{-1}$) |
|---|---|
| Inputs | |
| Dry | |
| $HNO_3$ | 2.7 |
| Fine and coarse particles | 0.3 |
| Wet | |
| Rain and snowfall | 2.0 |
| Total | 5.0 |
| Outputs | |
| Throughfall + stem flow | 2.7 |
| Total | 2.7 |
| Net canopy effect (outputs − inputs) | −2.3 |

3.0, 5.3, and 15.9 kg $N \cdot ha^{-1} \cdot year^{-1}$ for the 10, 50, and 100 ppb exposures, respectively. However, ambient $HNO_3$ concentrations at our study site are typically 1–3 ppb. To obtain a first approximation of canopy absorption under ambient $HNO_3$ concentrations, we used the regression between foliar absorption rate and concentration (i.e., $^{15}N$ absorption rate = 0.002 35 × $H^{15}NO_3$ concentration), combined with the assumptions 1, 3, and 4, and estimated N absorption at ambient levels to be approximately 0.2–0.5 kg $N \cdot ha^{-1} \cdot year^{-1}$. In contrast, we calculated a deposition (2-year average) of 2.7 $kg \cdot ha^{-1} \cdot year^{-1}$ $NO_3$-N (from $HNO_3$) to the forest canopy (Table 2). Thus, our absorption estimates indicate that approximately 10–20% of the $NO_3$-N deposited from $HNO_3$ is absorbed by the foliage. We recognize that the results obtained by these extrapolations are dependent on the validity of the experimental technique and assumptions used to scale-up to the stand. However, we feel that these extrapolations must be made in order to evaluate the stand-level significance of the foliar absorption data.

Our quantitative extrapolations are much lower than indicated by studies of mass $NO_3$-N balances (input–output) for the same forest stand (Table 2). For example, total $NO_3$-N input (wet and dry) to the canopy was 5.0 $kg \cdot ha^{-1} \cdot year^{-1}$ (54% of which was contributed by $HNO_3$), whereas throughfall and stem flow outputs were 2.7 $kg \cdot ha^{-1} \cdot year^{-1}$. These figures indicate a net canopy retention of 2.3 $kg \cdot ha^{-1} \cdot year^{-1}$. However, included in this net canopy retention amount are foliar absorption of both wet and dry $NO_3$-N; adsorption (i.e., not rinsed off by rainfall) of $NO_3$-N by foliage, branches, and stems; and utilization and transformation of $NO_3$-N by microflora and microfauna in the canopy. Hence, canopy retention of $NO_3$-N derived from mass balance studies (Lovett and Lindberg 1984; Lindberg et al. 1986) represents the integrated effect of canopy exchange processes and may not be appropriate for quantifying the specific fate (e.g., foliar absorption) of various forms of N within the canopy.

Assuming our approach to quantifying canopy absorption provides a reasonable estimate of absorption, N absorbed through the foliage from $HNO_3$ appears to be a minor component relative to total annual N uptake by our white pine forest. Annual N uptake is approximately 80 kg $N \cdot ha^{-1}$ (W.T. Swank, unpublished data), of which less than 1% (i.e., 0.2–0.5 kg $N \cdot ha^{-1}$) is contributed by foliar absorption of N from $HNO_3$. However, N may also be absorbed from $NO_2$ (Norby et al. 1989) or $NH_4$ and $NO_3$ in cloud water and rainwater (Bowden et al. 1989). Thus, the cumulative effects of foliar N absorption from a variety of N sources may be more significant nutritionally and physiologically.

Constraints imposed by working in mature forests prevented us from replicating treatments in these initial experiments. Hence, results reported in this paper should be considered as preliminary estimates. We have since devised an exposure system that treats two separate branches simultaneously. Thus, to expand on these preliminary findings, we have conducted short-term, replicated field exposures on red spruce (*Picea rubens* Sarg.) (tissue currently being analyzed for $^{15}N$). In addition, we will conduct long-term, replicated field exposures at near ambient $HNO_3$ concentrations (i.e., 5 ppb) on white pine in summer 1990.

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