Ecosystems (2007) 10: 1133–1147
DOI: 10.1007/s10021-007-9081-4

ECOSYSTEMS

Changes in Canopy Processes Following Whole-Forest Canopy Nitrogen Fertilization of a Mature Spruce-Hemlock Forest

E. Gaige,[1] D. B. Dail,[1,*] D. Y. Hollinger,[2] E. A. Davidson,[3] I. J. Fernandez,[1] H. Sievering,[4] A. White,[5] and W. Halteman[6]

[1]*Department of Plant, Soil, and Environmental Sciences, University of Maine, 5722 Deering Hall, Orono, Maine 04469-5722, USA;* [2]*US Forest Service, Northern Region Research Station, 271 Mast Road, Durham, New Hampshire 03824, USA;* [3]*Woods Hole Research Center, 149 Woods Hole Road, Falmouth, Massachusetts 02540-1644, USA;* [4]*Geography and Environmental Sciences, University of Colorado-Denver, Campus Box 172, P.O. Box 173364, Denver, Colorado 80217-3364, USA;* [5]*Forest Ecosystem Science, School of Forest Resources, University of Maine, 5755 Nutting Hall, Orono, Maine 04469-5755, USA;* [6]*Math and Statistics, University of Maine, 5752 Neville Hall, Orono, Maine 04469-5752, USA*

ABSTRACT

Most experimental additions of nitrogen to forest ecosystems apply the N to the forest floor, bypassing important processes taking place in the canopy, including canopy retention of N and/or conversion of N from one form to another. To quantify these processes, we carried out a large-scale experiment and determined the fate of nitrogen applied directly to a mature coniferous forest canopy in central Maine (18–20 kg N ha^{-1} y^{-1} as NH_4NO_3 applied as a mist using a helicopter). In 2003 and 2004 we measured NO_3^-, NH_4^+, and total dissolved N (TDN) in canopy throughfall (TF) and stemflow (SF) events after each of two growing season applications. Dissolved organic N (DON) was greater than 80% of the TDN under ambient inputs; however NO_3^- accounted for more than 50% of TF N in the treated plots, followed by NH_4^+ (35%) and DON (15%). Although NO_3^- was slightly more efficiently retained by the canopy under ambient inputs, canopy retention of NH_4^+as a percent of inputs increased markedly under fertilization. Recovery of less than 30% of the fertilizer N in TF suggested that the forest canopy retained more than 70% of the applied N (>80% when corrected for N which bypassed tree surfaces at the time of fertilizer addition). Results from plots receiving ^{15}N enriched NO_3^- and NH_4^+ confirmed bulk N estimations that more NO_3^- than NH_4^+ was washed from the canopy by wet deposition. The isotope data did not show evidence of canopy nitrification, as has been reported in other spruce forests receiving much higher N inputs. Conversions of fertilizer-N to DON were observed in TF for both $^{15}NH_4^+$ and $^{15}NO_3^-$ additions, and occurred within days of the application. Subsequent rain events were not significantly enriched in ^{15}N, suggesting that canopy DON formation was a rapid process related to recent N inputs to the canopy. We speculate that DON may arise from lichen and/or microbial N cycling rather than assimilation and re-release by tree tissues in this forest. Canopy retention of experimentally added N may meet and exceed calculated annual forest tree demand, although we do not know what fraction of retained N was actually physiologically assimilated by the plants. The observed retention and transformation of DIN within the canopy

---

Received 6 July 2006; accepted 10 July 2007; published online 9 August 2007.

Electronic supplementary material: The online version of this article (doi:10.1007/s10021-007-9081-4) contains supplementary material, which is available to authorized users.

**Corresponding author; e-mail:* bryan.dail@maine.edu

demonstrate that the fate and ecosystem consequences of N inputs from atmospheric deposition are likely influenced by forest canopy processes, which should be considered in N addition studies.

Key words: nitrogen deposition; canopy fertilization; canopy N retention; throughfall; stemflow; ^{15}N tracer.

INTRODUCTION

Productivity in many ecosystems tends to be limited by nitrogen (N) availability (Vitousek 1982). Increasing N availability resulting from atmospheric inputs that are derived from fossil fuel consumption and fertilizer use has the potential to overcome N limitation in terrestrial ecosystems (Vitousek and others 1997; Galloway and Cowling 2002) and thus increase sequestration of atmospheric CO_2 in plant biomass (Townsend and others 1996; Seivering and others 2000). Crucial to understanding of the fate of additional N in forest environments has been a series of studies begun in the 1980s to test another consequence of N deposition, the concept of N saturation; a state of N availability meeting and then exceeding ecosystem demand (Aber and others 1989). Most of these studies showed that forests of many types and varying historical N inputs retained a substantial fraction of experimental N inputs, sometimes exceeding 90%, even when additions greatly exceeded background inputs (Magill and others 1997; Tietema and others 1998).

A number of N saturation study sites have been used to assess the impact of added N on ecosystem processes by adding ^{15}N tracer additions to forest ecosystems (nine closed-canopy forests in Europe and North America) (Nadelhoffer and others 1999). Tracer levels of ^{15}N were applied to the forest floor, and then subsequent sampling of the various compartments was used to assess the fate of the added N, which was serving as a proxy for enhanced atmospheric N. In general, less than 5% (but as much as 30%) of the applied N was incorporated into the woody biomass pool. Most of the N was retained in the soil, with little evidence for leaching losses. These tracer studies, summarized by Nadelhoffer and others (1999), suggested that an increase in N deposition may lead to enhanced tree growth, but only slightly so, in temperate forests. Other studies, however, suggest that these tracer studies may have underestimated the impact of N additions by not assessing canopy-N assimilation; a mechanism bypassed by tracer additions to the forest floor (Lawrence and Fernandez 1991; Boyce and others 1996; Calanni and others 1999).

Retention of N by the canopy has been typically attributed to physiological assimilation by trees (Liechty and others 1993), but epiphytes and microbes on foliar surfaces may also contribute appreciably to canopy-N assimilation (Carlisle and others 1966; Friedland and others 1991; Balestrini and Tagliaferri 2001; Papen and others 2002). In a study that compared precipitation and thoughfall (TF) chemistry at sites across North America and Europe, Lovett and Lindberg (1993) estimated that forest canopies retained about 40% of incident N; however, the range was between 10 and 90%. Canopy retention in these studies varied from 1 to 12 kg N ha^{-1} y^{-1} and was highest in spruce and spruce-fir stands. This range of N retention, if actually assimilated by the trees, could satisfy a significant portion of the annual N demands of coniferous forests, which have been estimated to be between 5 and 50 kg N ha^{-1} y^{-1} (Cole and Rapp 1981). Greenhouse investigations of N assimilation by application of $^{15}NH_4{}^{15}NO_3$ to seedling canopies suggested a preference by trees for NH_4^+ (Bowden and others 1989; Eilers and others 1992), and uptake as high as 9 kg N ha^{-1} y^{-1} (Eilers and others 1992). Canopy processes may also convert added inorganic N to other forms of N, including dissolved organic N (DON) which is often not quantified but remains a highly mobile N form. Balestrini and Tagliaferri (2001) reported substantial NH_4^+ retention with a concomitant doubling of NO_3^- concentration in TF. Dry deposition, precipitation DON, canopy conversion of DIN, or release of organic N from needles, leaves, damaged foliage, microbes and other epiphytes, as well as insect frass are all possible sources of DON in TF, and DON is often the major form of N in TF in unpolluted or less polluted forests (Carroll 1980). Although previous studies suggest that canopy-N uptake could impact C sequestration in forests, the impact likely depends on the addition rate, N demand, and the timing and form of N entering different ecosystem components (Seivering and others 2000).

Despite the demonstrated importance of canopy retention and processing of added N, the difficulty of adding N to mature forest canopies has led most researchers to apply N to the forest floor in sprays or as granulated fertilizer (Wright and Rasmussen 1998; Magill and others 1997; Nadelhoffer and others 2002). A notable exception was a Scottish Sitka spruce study where a young (10 yr) mixed stand was exposed to nutrient and acid mists (Chiwa and others 2004; Cape and others 2001). This study used relatively high N addition rates of 48 and 96 kg N ha^{-1} y^{-1}, and found that about one-third of the applied N was retained by the canopy. Here we report on the potential of a mature (~140 years old), coniferous forest canopy to retain moderate amounts of N (~20 kg N ha^{-1} y^{-1}) applied directly to the canopy by helicopter spraying, and quantify the transformation of N from inorganic to organic forms, and discuss possible implications for forest tree N demand. We hypothesized that a large fraction (>30%) of our 18–20 kg N ha^{-1} y^{-1} N addition, applied during the growing season, would be retained by the canopy and that we would observe increases in NO_3^-, NH_4^+ and DON present in throughfall.

MATERIALS AND METHODS

Site Description

The Howland Integrated Forest Study (HIFS) was established in 1987 in east-central Maine (45° 12′ N, 68° 45′ W). The forest is typical of the region's low-elevation transition spruce-fir forests, although the balsam fir (*Abies balsamea* (L.) Mill.) component was reduced to less than 5% of basal area due to spruce-budworm infestation beginning in the early 1970s. Red spruce (*Picea rubens* Sarg.) and eastern hemlock (*Tsuga canadensis* (L.) Carr.) are the current co-dominant species constituting approximately 44 and 26% of the basal area, respectively, with white pine (*Pinus strobus* L.; 9%), eastern white cedar (*Thuja occidentalis* L.; 10%) and red maple (*Acer rubrum* L.; 10%) representing the other species common to the site (Hollinger and others 1999). The mean tree age for the site is 140 years with some trees in excess of 200 years old. Mean live basal area, determined from 48 forest-inventory-and-analysis plots (FIA) (USDA Forest Service 2002) was 56.7 ± 16.5 m^2 ha^{-1} (Hollinger and others 2004). Leaf area index (silhouette method, counting overlapping needles only once) was 6 m^2 m^{-2} and the mean tree height was 20 m (Scott and others 2004).

Epiphytic lichens are present at the site, but have not been quantitatively assessed on an area basis but represent about 3% of biomass present in litterfall collectors (unpublished data). The elevation at Howland is 68 m with flat to hummocky topography. Soils are either Aquic Haplorthods of the moderately well-drained Skerry series or Aeric Haplaquods of the more poorly drained Westbury series, developed from a dense basal till that is characteristic of the region (McLaughlin and others 1996). Annual precipitation averages 1,040 mm with mean temperature of 5.8°C (1987–1996). The summer mean temperature is 19°C, winter mean temperature −8°C with a snow pack of up to 2 m from December to March. Additional information on the HIFS can be found in Fernandez and others (1990), Lawrence and Fernandez (1991), McLaughlin and others (1996) and Hollinger and others (1999).

In 2000 and 2001 the HIFS received 0.65 and 0.8 kg N ha^{-1} y^{-1} as dry N deposition, respectively, and more than 50% of this as HNO_3. Dry deposition was measured weekly with three-stage filter packs. The filter packs contained a Teflon filter for collection of particulate species, a nylon filter for nitric acid and an alkaline cellulose filter for sulfur dioxide collection. Coarse particulate N inputs were not estimated. In addition to dry inputs, the site received 3 and 2 kg N ha^{-1} y^{-1} in these same years as wet-only deposition, mostly as NO_3^-, which was measured using an Aerochem wet only precipitation collector (USEPA 2004). Analysis of foliar chemistry by Fernandez and others (1990), characterized the HIFS site as deficient in N and P. Current year red spruce foliage from 1987 was found to contain 1.03% N and 1986 growth (past year) contained 1.04% N, putting the site into the "acute deficiency" classification developed by Swan (1971). Lawrence and Fernandez (1991) estimated that virtually 100% of ambient wet-only NO_3^- inputs and 90% of ambient wet-only NH_4^+ inputs were retained in the ecosystem (that is, not detected leaking from the solum), indicating that N saturation at the site has yet to occur (sensu Aber and others 1989).

Experimental Design

Aerial applications of dissolved N fertilizer to the canopy were performed over a 21 ha area of forest from 2001 to 2005, although here, we report only an intensively sampled period (both TF and SF) of 2003 and 2004. A contiguous 21 ha area was chosen to minimize edge effects and to permit study of effects of N addition on carbon fluxes by eddy covariance, which will be presented elsewhere. A helicopter sprayed a fine mist of liquid NH_4NO_3 on five different dates during the growing season (May–August). The spray application rate delivered approximately 224 l H_2O ha^{-1} (0.48 M NH_4NO_3) with drop sizes centered at approximately 1,500 µm. The water addition was to the N treated plots only and the rate was of sub-mm amount and not enough to elicit a measurable TF event on a volumetric basis. Each of the five fertilizations delivered 3.6 kg N ha^{-1} as NH_4NO_3 for a total of 18 kg N ha^{-1} y^{-1} above ambient inputs to the canopy. Two flight passes assured coverage of the entire 21 ha area, aided by on-board GPS-based navigational equipment. Spray applications were scheduled in advance such that they were roughly 2 weeks apart spaced over the growing season. We attempted to insure ample contact time between N and the canopy, relying upon weather forecasts to plan application that would likely insure at least 24 h contact before subsequent rain events. Spray drift was minimized by flying low over the canopy and on days of low wind.

The frequency and dose of N applications represents a compromise between competing concerns of simulating a significant increase in N deposition in the most realistic way possible, but at an affordable cost. More frequent applications of smaller doses might have been preferable, but would have been prohibitively expensive. Replicating this large-scale aerial N fertilization experiment would also have been prohibitively expensive. Hence, we can report only on how this particular forest canopy retained the fertilizer-N and do not attempt to infer that similar forests would necessarily respond at similar retention rates. Three 0.3 ha study plots (30 × 100 m), each with 10 throughfall collectors, provide replication of throughfall measurements within the full 21 ha area, thus affording estimates of within-treatment variance (Figure 1). Two of these smaller plots also received ^{15}N label in the fertilizer (one as ammonium and the other as nitrate). Again due to cost, it was not feasible to replicate ^{15}N treatments. Thus the use of isotopic labeling is intended to provide insight into N retention mechanisms by identifying fates of applied N, rather than to provide quantifiable estimates based on a statistically robust replicated design.

The ^{15}N label was applied by an additional helicopter fertilization either as 10% enriched $^{15}NH_4Cl$ (hereafter F+$^{15}NH_4$) or $Na^{15}NO_3$ (F+$^{15}NO_3$ plot). The ^{15}N dose added 0.3 kg N ha^{-1} with each flight, increasing the seasonal load of experimental N to 19.8 kg N ha^{-1} y^{-1} (a final enrichment of 1% ^{15}N) within these two plots during the 2001, 2002, and 2003 seasons. The third intensively sampled fertilized plot (F/^{14}N) was established within the larger fertilized area but did not receive ^{15}N label. Finally, a control plot (C), was established at a distance more than 200 m from the treated area to avoid spray contamination. Each plot contained 10 randomly placed funnel-type throughfall collectors for a total of 40 collectors in these plots. To determine ambient precipitation N inputs to the forest, precipitation was collected from three open areas (former clear-cuts) within 1 km of the N fertilization site. Each plot also contained approximately eight stemflow collectors; collar-type collectors were fashioned out of expanding foam and silicon sealant about 1 m from the forest floor and funneled water to an opaque 10 l bottle. Because bark was not removed to install these collectors, we frequently monitored leakage and SF trapping efficiency.

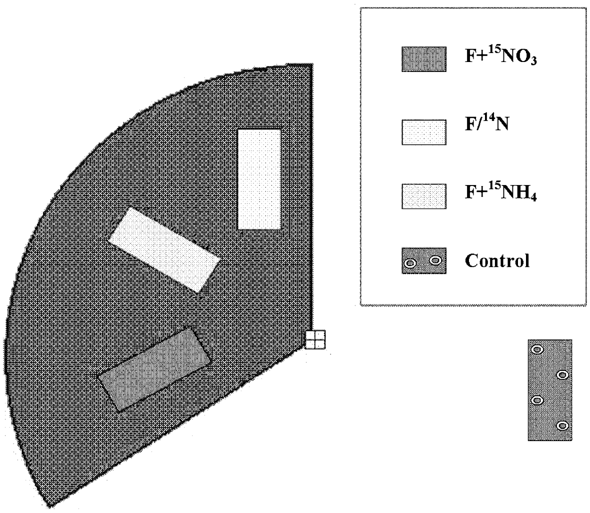

Figure 1. Plot layout: a 21 ha wedge centered on an eddy covariance tower was fertilized with 18 kg N ha^{-1} growing season^{-1}. Three 0.3 ha plots were established within the fertilized area within which throughfall was collected. Two of these smaller plots were fertilized with ^{15}N as either NH_4^+ or NO_3^- bringing total input to these two plots to approximately 20 kg N ha^{-1} growing season^{-1}.

Sampling

The ten TF collectors were permanently placed in each of the four plots, and the collector openings were approximately 1 m from the forest floor and the funnel diameter was 16 cm. Samples were collected on a wet precipitation event basis and immediately refrigerated at 4°C when returned to the lab. Collection volumes (for the calculation of flux) were recorded in the field and solutions were filtered through a pre-washed 0.45 μm filter and then refrigerated to stabilize the samples during the analysis and storage phase. Filtered samples that were not sub sampled for analysis within 48 h were frozen after filtration until they could be further processed. A total of 37 collar-type stemflow collectors (26 in the N treatment areas and 11 in the control area) were constructed in mid summer of 2003. Most of the stemflow collectors were placed on spruce and hemlock although other species were represented. Stemflow collections were made in tandem with the throughfall event-based collections and the samples were handled and analyzed in an identical manner. Flux of stemflow N was calculated at the stand level using stand allometric data. Briefly, the total N delivered to the forest floor in a stemflow event on an area basis, SFN, was a product of the total stemflow N of all trees within a plot and the total basal area of trees in the plot divided by the total basal area of the sampled trees:

$$\text{SFN} = (\text{Total stemflow N of sampled trees}) \times \frac{\text{Total basal area of all trees in plot}}{\text{Total basal area of the sampled trees}} \quad (1)$$

A weakness of this method is that it does not allow an assessment of a confidence interval for the estimate of stemflow N (Lewis 2003), but despite substantial water fluxes in stemflow events, little N was recovered (<2% of inputs) and further assessment of the data did not seem warranted.

A wet precipitation event could constitute more than one discreet rain event if rain fell on contiguous days or if rain did not overcome canopy water storage capacity. In 2003 and 2004, 13 and 14 TF collections were made that were unevenly spaced with respect to the fertilizer applications. The dates (Julian day) of fertilizer sprays and TF collections are shown in Table 1. The event number to the left of the decimal in Table 1 indicates which fertilization (of five) the precipitation event followed and the number to the right of the decimal indicates TF events subsequent to the fertilization. For instance, the TF event on Julian day 167 in 2003 TF was labeled 2.3 as it was the third TF event after the second foliar N application.

Analyses

Inorganic-N ($NH_4^+$and NO_3^-) was quantified using colorimetric analysis by the department of Plant, Soil and Environmental Sciences Soil Analytical Laboratory at The University of Maine. Analysis of NO_3^- and NH_4^+ was determined on an O.I. Alpkem A/E ion analyzer, which measures NH_4^+ by the phenate method and NO_3^- by cadmium reduction. DON concentrations were determined by subtracting inorganic N from total dissolved N (TDN), analyzed with an automated Lachat QuikChem 8000 persulfate digestion followed by the determination of NO_3^- at the Woods Hole Research Center, Woods Hole, MA, USA. Because samples were filtered through 0.45 μm filters, we defined TDN and DON as smaller than this pore diameter.

After NH_4^+, NO_3^- and DON concentrations were quantified, a limited number of TF event samples were analyzed for isotopic enrichment (^{15}N:^{14}N) by isotope ratio mass spectrometry (IRMS). Serial diffusions were employed to concentrate the N from solutions onto acidified paper disks so that they can be analyzed on a solid state isotope ratio mass spectrometer (Brooks and others 1989; Sørensen and Jensen 1991). Appropriate corrections were made for incomplete mass recovery and isotopic discriminations that occurred during the diffusion process (Stark and Hart 1996). The same method of concentration and trapping was performed for TDN on a separate sub sample with persulfate-digested aqueous samples (D'Elia and others 1977; Solórzano and Sharp 1980; Cabrera and Beare 1993; Pizzicannella and others 1995).

The standard calculation of the degree of isotopic enrichment of a sample, known as the δ (delta) ^{15}N value, indicates the per mil (‰) excess ^{15}N in a sample compared to an atmospheric N_2 standard (Shearer and Kohl 1993). For this study, however, the standard was the enrichment of the NH_4^+ and NO_3^- of the unlabeled fertilizer (NH_4^+, 3.66‰; NO_3^-, 1.80‰) for those respective isotope analyses. For the DON isotope analysis, an average enrichment of NH_4^+ and NO_3^- components of the fertilizers were used.

Canopy N retention estimation

Estimation of canopy retention was made using two methods, which we refer to as *inorganic* N retention and *total* N retention. Inorganic N retention refers to the difference in NO_3^- and NH_4^+ dissolved in the helicopter applications and NO_3^- and NH_4^+ present in throughfall. Total N retention refers to the difference in total N into and total N out of the canopy, accounting for DON present in incident precipitation and that which was released from the canopy. We also measured the N which bypassed the canopy entirely at the time of spraying. We placed 4.5 cm diameter paper filters in 40 empty litterfall traps on the day of spraying and then collected them within 3 h after the spray. These filters were then leached with 30 ml of a 0.5 M K_2SO_4 solution, which we then submitted for DIN analysis. The flux of N that bypassed the canopy was estimated for each fertilization event, and these data were then used to adjust initial canopy retention estimates.

To compare measured canopy retention with annual forest tree N requirement, FIA performed in 2001, 2003, and 2005 were used to calculate the average annual live tree biomass accumulation and to partition the whole tree biomass (above and belowground) into foliage and wood components

Table 1. Fertilization Spray Dates and Subsequent Throughfall (TF) Collection Dates

Spray no.	Julian day	Event no.[1]	Precipitation[2] (mm)	TF volume as a percent of incident rainfall (%)
2003				
Spray 1	139	1.0	NA	NA
TF	145	1.1	13.3 ± 2.6	71 ± 31
TF	147	1.2	6.8 ± 0.9	81 ± 33
TF	153	1.3	28.8 ± 7.6	86 ± 30
Spray 2	154	2.0	NA	NA
TF	156	2.1	23.6 ± 5.7	90 ± 28
TF	162	2.2	3.8 ± 1.3	22 ± 22
TF	167	2.3	32.9 ± 4.7	95 ± 37
Spray 3	177	3.0	NA	NA
TF	182	3.1	4.9 ± 2.6	52 ± 38
TF	190	3.2	6.9 ± 1.3	62 ± 34
TF	194	3.3	12.5 ± 2.9	76 ± 32
Spray 4	195	4.0	NA	NA
TF	208	4.1	11.4 ± 1.3	43 ± 31
Spray 5	211	5.0	NA	NA
TF	216	5.1	13.4 ± 1.6	83 ± 32
TF	220	5.2	12.2 ± 0.8	86 ± 31
TF	224	5.3	72.0 ± 3.1	91 ± 35
2004				
TF	140	0.1	18.0 ± 7.8	60 ± 39
TF	148	0.2	26.9 ± 20.4	92 ± 59
Spray 1	148	1.0	NA	NA
TF	155	1.1	17.0 ± 0.2	69 ± 11
Spray 2	156	2.0	NA	NA
TF	162	2.1	4.5 ± 4.1	75 ± 20
TF	173	2.2	13.6 ± 0.2	70 ± 29
Spray 3	175	3.0	NA	NA
TF	181	3.1	4.4 ± 5.1	64 ± 33
TF	185	3.2	27.4 ± 2.8	85 ± 25
Spray 4	189	4.0	NA	NA
TF	194	4.1	49.7 ND	82 ± 23
TF	202	4.2	9.4 ± 6.4	84 ± 45
TF	208	4.3	23.3 ± 5.5	69 ± 30
Spray 5	212	5.0	NA	NA
TF	217	5.1	12.6 ± 0.5	59 ± 32
TF	222	5.2	29.1 ± 1.1	86 ± 27
TF	229	5.3	48.2 ± 2.1	87 ± 31
TF	238	5.4	27.9 ± 0.7	69 ± 28

Cumulative precipitation preceding TF collection and TF volumes as a percent of precipitation inputs.
NA = not applicable.

[1] *Whole numbers (1 to 5) were assigned to the fertilization events and subsequent throughfall events assigned numbers to the right of the decimal according to their proximity to the spray fertilizer event.*

[2] *Precipitation depth was an average of a tipping bucket type automated collector and two open funnel throughfall type collector placed in clear cuts within 1 km of the treated area.*

using the allometric equations of Young and others (1980). After partitioning the net increase in biomass (new growth and death) into foliage and wood compartments, these estimates were multiplied by the respective mean N concentrations of those tissues to calculate an annual forest tree N requirement on an area basis.

Statistical Analyses

The TF-N flux data calculated for this study was highly variable; within-plot variability often exceeded that of between-plot variability. Thoughfall N flux differences over the growing season were analyzed using a multivariate-repeated measures analysis that partitioned variation by treatment, time (event) and collector, nested within treatments. A univariate analysis of variance, though more sensitive and able to discern time by treatment effects, was not possible due to inequality of variance across event dates. Means separation by Tukey's HSD (alpha = 0.05) and *P*-values were calculated for each event. Flux data for NO_3^-, NH_4^+, and DON were transformed by using the natural log for all data, and where necessary, constants were added before the transformation.

Mean N retention by the canopy is presented with associated standard deviations and final uncertainties were propagated by a Monte Carlo method. For the two TF collections that were analyzed for isotopic differences, an analysis of variance was used to examine the differing fates of $^{15}NO_3^-$ and $^{15}NH_4^+$ in the canopy. Delta (δ ^{15}N) values were natural log transformed and a constant was added before the transformation. Comparisons were made among the plots that received $^{15}NO_3^-$ (F+$^{15}NO_3$), $^{15}NH_4^+$ (F+$^{15}NH_4$), only unlabeled fertilizer (F/^{14}N plot), and only ambient N deposition (control, or C plot).

RESULTS AND DISCUSSION

Possible Fates of Experimental N

Of the fertilizer N that was sprayed above the forest canopy, possible fates for the N include: (1) Bypassing the canopy entirely at the time of the application, (2) Wash off during rain events and recovered as NO_3^-, NH_4^+ or DON in throughfall or stemflow. (3) Gaseous loss by volatilization and (4) Retention onto foliar and bark surfaces within the canopy. To investigate interactions among precipitation volume, chemistry, and the fate of dissolved N therein, we measured incident rain and canopy water retention (Table 1). Canopy retention of precipitation was highly variable and often not associated with the amount of incident rainfall, although more than 50% was recovered in TF when the precipitation events was greater than 5 mm. A range of 22–95% of incident rainfall was recovered in throughfall and stemflow, with an average of 70%.

Fertilizer N that was not intercepted by the canopy

The mean experimental N that bypassed the forest canopy completely for the five fertilization events ranged from 0.93 to 2.9 kg N ha^{-1} season^{-1} and was different among plots and between years (Table 2). Thus, 5–to–16% of the experimental N bypassed the canopy entirely at the time of application. We observed that the non-labeled fertilizer plot (F/^{14}N) almost always had a greater flux of canopy bypass-N than the other two plots, despite the F+$^{15}NH_4$ and F+$^{15}NO_3$ plots having received slightly higher doses of N in 2003 (Table 2). The N that bypassed the canopy, as determined by filter papers collected immediately after the spray, would have also fallen into the TF funnels and therefore is included in the TF estimates. The larger amount of experimental N that never contacted the canopy in the F/^{14}N plot may help explain why more N was measured in the TF of that plot (Table 2). We attribute the differences in flux means *between plots* to differing canopy architectures in the three treated plots that would influence canopy retention and resultant TF N flux.

Throughfall and Stemflow N Flux

In both 2003 and 2004, the seasonal flux of inorganic TF N in the treated plots was 15 to 50 times the inorganic N flux in the control plot; the experimental input to the treated plots exceeded ambient deposition by a similar magnitude (Table 2). Despite differences in experimental and ambient inputs, the canopies retained in excess of 60% of inputs. Nitrate was the dominant form of N in TF for the treated plots (Table 2), whereas DON dominated control plot TF N (Table 3).

In 2003, ambient TF NO_3^- and NH_4^+ fluxes were 8 and 7% of the total N in TF, respectively. Largest N form in ambient TF, constituting approximately 85% of total N. These trends were similar in 2004 (Table 3). However, when fertilizer-N was applied to the canopy at the rate of 3 kg N ha^{-1} spray event^{-1}, DIN became the dominant contributor to TF-N despite high canopy N retention. In 2003 and 2004, the percentage of total TF-N as NO_3^- in all treated plots ranged from 46-to-49%. This result is consistent with observations of elevated NO_3^- flux in TF when N deposition is elevated due to human activities (Friedland and others 1991; Balestrini and Tagliaferri 2001).

Significant between-plot differences in DIN fluxes derived from the N treatments dropped off as subsequent precipitation events washed experimental N from the canopy; we present data for 2004 only for simplicity, however both years yielded similar trends (Figure 2). After NO_3^- and NH_4^+ were washed from the canopy, these fluxes approached baseline or ambient conditions. We attribute this temporal pattern to the fertilization dose and rainfall frequency and intensity rather than phenological processes. Although it is possible

Table 2. Flux and Canopy Retention of Ambient and Experimental Nitrate and Ammonium Inputs in Control (Ambient Deposition) and Three Experimental Plots

Year/N form/plot	Input (kg N/ha)	Not intercepted (kg N/ha)[1]	Throughfall (kg N/ha)	N retained (kg N/ha)	Canopy N retention (%)	Canopy N retention contact corrected
2003 Nitrate flux						
Control	0.4	NA	0.07 (0.02)	0.33	83	NA
F/^{14}N	9.4	1.2 (1.4)	3.62 (2.03)	5.78	61	74
F+^{15}NH$_4$	9.4	0.8 (1.0)	2.67 (1.56)	6.73	72	80
F+^{15}NO$_3$	11.2	0.6 (0.7)	2.82 (1.50)	8.38	75	80
2004 Nitrate flux						
Control	0.3	NA	0.09 (0.06)	0.21	70	NA
F/^{14}N	9.3	1.2 (1.4)	3.92 (1.74)	5.38	58	70
F+^{15}NH$_4$	9.3	1.0 (1.8)	2.43 (1.47)	6.87	74	85
F+^{15}NO$_3$	9.3	0.8 (0.4)	3.22 (0.89)	6.08	65	73
2003 Ammonium flux						
Control	0.2	NA	0.08 (0.04)	0.12	60	NA
F/^{14}N	9.2	1.7 (1.9)	2.33 (1.34)	6.87	74	94
F+^{15}NH$_4$	11.0	0.8 (0.9)	1.93 (1.55)	9.07	82	90
F+^{15}NO$_3$	9.2	0.4 (0.4)	1.68 (1.11)	7.52	82	86
2004 Ammonium flux						
Control	0.3	NA	0.09 (0.03)	0.21	70	NA
F/^{14}N	9.3	1.2 (1.4)	2.48 (1.75)	6.82	73	86
F+^{15}NH$_4$	9.3	0.9 (0.8)	1.33 (0.93)	7.97	86	96
F+^{15}NO$_3$	9.3	0.9 (0.3)	1.90 (0.64)	7.40	80	87

NA = not applicable.
[1] *Experimental N inputs only.*

Table 3. Total Throughfall N Flux May–August 2003

	Nitrate flux (kg N ha^{-1})	Ammonium flux (kg N ha^{-1})	DON flux (kg N ha^{-1})	Total N flux (kg N ha^{-1})
2003				
Control plot	0.07 (0.02)	0.08 (0.04)	0.78 (0.25)	0.93
F/^{14}N plot	3.62 (2.03)	2.33 (1.34)	1.51 (0.68)	7.45
F+^{15}NH$_4$	2.67 (1.56)	1.93 (1.55)	1.01 (0.36)	5.68
F+^{15}NO$_3$	2.82 (1.50)	1.68 (1.11)	1.60 (1.25)	6.10
2004				
Control plot	0.09 (0.06)	0.09 (0.03)	0.70 (0.22)	0.87
F/^{14}N plot	3.92 (1.74)	2.48 (1.75)	1.55 (0.48)	7.95
F+^{15}NH$_4$	2.43 (1.47)	1.33 (0.93)	1.30 (0.45)	5.05
F+^{15}NO$_3$	3.22 (0.89)	1.90 (0.64)	1.46 (0.71)	6.58

Major N species (NO_3^-, NH_4^+ Dissolved Organic Nitrogen (DON), and sum (total N flux) shown for an unamended plot (control) and three plots receiving 18 kg N ha^{-1} (F/14N plot) or 19.8 kg N ha^{-1} (F+15NH4 and F+15NO$_3$ plots, 2003 only) additions to the canopy from May to July. Flux of N forms is reported for the growing season and is expressed as a mean ± 1 standard deviation in parentheses.

that the retention efficiency of the canopy is highest when more inorganic N is available, the dose of 3 kg N ha^{-1} with each spraying overwhelmed ambient inputs and may saturate canopy N processes such as N assimilation, redox reactions, and conversion to DON. Our experimental control plot data compared well with previous work done by Lawrence and Fernandez (1991) and McLaughlin and others (1996) at Howland, which showed a high percentage of retention of both NH_4^+ and NO_3^- inputs. Both Lawrence and Fernandez (1993) and Beier and others (1993) have attributed high variability of TF ion fluxes to exchangeable ions reacting within a heterogeneous

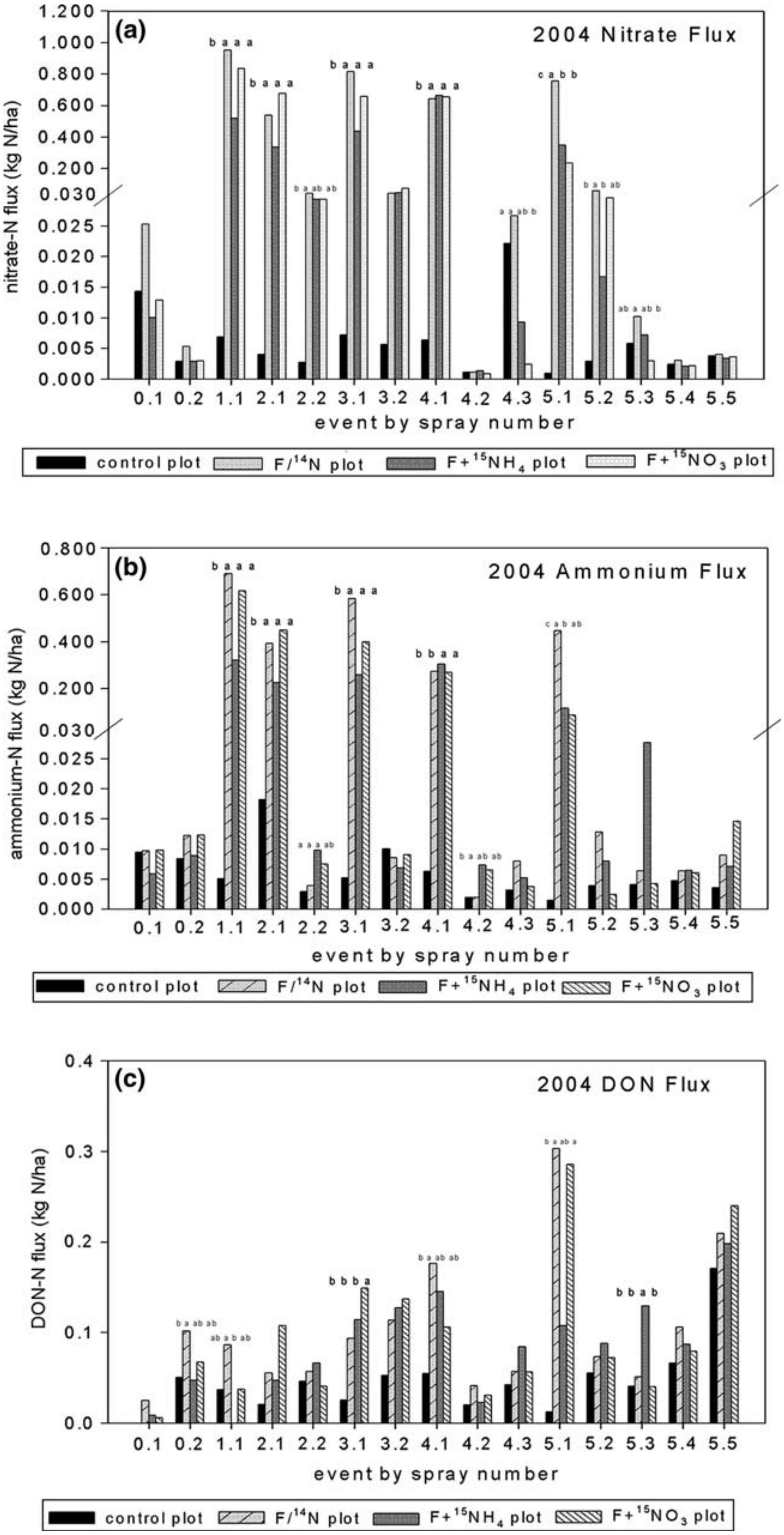

Figure 2. A 2004 flux of NO_3^--N to the forest floor in TF, means separation by Tukey's HSD, alpha = 0.05. Where letters are not shown, means are statistically equal for that event. Y-axis break occurs at 0.030 kg N ha^{-1}. **B** 2004 flux of NH_4^+-N to the forest floor in TF, means separation by Tukey's HSD, alpha = 0.05. Where letters are not shown, means are statistically equal for that event. Y-axis break occurs at 0.030 kg N ha^{-1}. **C** 2004 flux of DON-N to the forest floor in TF, means separation by Tukey's HSD, alpha = 0.05. Where letters are not shown, plot means are statistically equal for that event.

canopy structure and variable liquid funneling patterns created by the canopy architecture. Although N concentrations in stemflow from treated plots was elevated after canopy additions, the flux of experimental N to the forest floor by stemflow was inconsequential and always less than 1% of N addition (data not shown).

Inorganic N Retention

Because the experimental N loading rates were known (18–20 kg N ha^{-1} $season^{-1}$) retention was calculated by subtracting N in TF from the fertilizer loading rate. Because the retention estimates are made by difference, sources of error in input rates or throughfall measurements, as well as unaccounted fluxes, could contribute to uncertainties in the retention estimations.

Conventionally, canopy retention is described simply by flux of N in precipitation less TF N flux, with no consideration of the efficiency of physical interception of N by the canopy. Even in a mature, closed canopy, however, some N deposition would not be intercepted by the canopy and is delivered immediately to the forest floor. Because this flux of N was measured in the addition plots, retention calculations were then corrected by accounting for the flux of NO_3^- and NH_4^+ that bypassed the canopy entirely, yet end up as TF and biased the estimate of uptake efficiency. Hereafter, we refer to the canopy bypass-N corrected retention as ''contact corrected'' canopy retention (Table 2).

In 2003, treated plot NO_3^- retention estimates were 61% (74% contact corrected), 71% (80% contact corrected), and 74% (80% contact corrected) for the F/^{14}N, F+$^{15}NH_4$, and F+$^{15}NO_3$s, respectively (Table 2). Similar NO_3^- retentions were observed in 2004. The uncorrected fertilizer NO_3^- retention was somewhat less than that observed in ambient forest canopy (as a % of inputs), and this suggested that canopy N retention processes were becoming saturated. However, when retention of experimental NO_3^- addition was corrected for that N bypassing the canopy, retention rates approached those of the ambient plot canopy. We lack a means of estimating ambient inputs that bypass the canopy, which could be different than that observed with experimental addition. Retention of experimental NH_4^+ was higher than that for NO_3^- in the treated plots, and averaged 75% (94% contact corrected) for the F/^{14}N plot, 83% (90% contact corrected) for the F+$^{15}NH_4$ plot, and 82% (86% contact corrected) for the F+$^{15}NO_3$ in 2003 (Table 2). Again, these trends continued for the 2004 growing season. Control plot retention of NH_4^+, however, was less than or equal to that for NO_3^-.

To put these high DIN retention estimates in context, in a study that summarized the results of 11 North American and one European site where canopy processing of *ambient* atmospheric N deposition had been measured, Lovett and Lindberg (1993) estimated inorganic N retention (when loss of DON by the canopy was considered) would account for less than 15% of total ecosystem N requirements, despite an average retention estimate of 40% of inputs across the sites. It is often not clearly stated if canopy retention is considered as assimilation or physiologic uptake in many TF studies or what fraction of canopy retained N might be available for plant use. Although foliar assimilation may only provide a small proportion of a tree's nutrition, significant retention of N by forest canopies is reported in much of the existing literature and this N may ultimately, if not immediately, provide support for growth (Carlisle and others 1966; Lawrence and Fernandez 1991; Friedland and others 1991; Potter and others 1991; Lovett and Lindberg 1993; Liechty and others 1993; Lovett and others 1996; McLaughlin and others 1996). Previous work at the Howland Forest site by Lawrence and Fernandez (1991) reported 31% retention of ambient NH_4^+ inputs and 32% of ambient NO_3^- inputs and therefore, our N retention estimates, even when not contact corrected, appear substantially higher than those reported for similar forest types and previous estimates for this site (Lovett and Lindberg 1993; Lawrence and Fernandez 1991). Because we also note significant differences in experimental N retention between closely spaced plots, it could be that our larger estimate of canopy retention is due to differences in canopy architecture or changes in that architecture since the 1991 estimation by Lawrence and Fernandez; our plots were also not at identical locations (>0.5 km apart). Moreover, our method of canopy addition inevitably does not perfectly simulate natural inputs, and this difference may have favored N retention.

Total N Retention

Estimates of retention of total N include DON, an important constituent of throughfall N, if not incident precipitation. Treated plot TDN retention estimates were 64% (80% contact corrected), 76% (84% contact corrected) and 74% (79% contact corrected) for F/^{14}N, F+$^{15}NH_4$, and F+$^{15}NO_3$ in 2003, and similar retention estimates were measured for 2004 (Table 4). By comparison, the total

Table 4. Canopy Retention of Total N (Total N = DIN + DON) Inputs for 2003 and 2004

	N inputs to plot (kg N ha^{-1})	Season flux of TF N (kg N ha^{-1})	Fertilizer not intercepted by canopy (kg N ha^{-1})	N retained as a percent of inputs[1] (%)	N retained as a percent of inputs contact corrected[1] (%)
2003					
Control plot	0.93	1.18	NA	21.2	NA
F plot	18.0	7.45	2.90	63.7	79.9
A plot	19.8	5.68	1.63	76.0	84.2
N plot	19.8	6.10	0.93	73.9	78.6
2004					
Control plot	0.72	0.87	NA	20.8	NA
F plot	18.0	7.95	2.30	60.7	73.4
A plot	18.0	5.05	1.84	76.8	87.0
N plot	18.0	6.58	1.37	68.3	75.9

NA = not applicable.

[1] *Retention of inorganic N inputs only.*

N retention estimated for the ambient plots was about 21% for both 2003 and 2004, much lower than in the treated plots. Percentages of total N retention are lower than estimates of DIN-only retention because the former includes a net flux of DON from the canopy; a form that dominates TF-N chemistry in undisturbed forests.

Not all DON formed in the canopy is likely to have arisen from recent DIN deposition, and DON could arise from damaged leaves and wood as well as from insect honeydew and frass (Coudhury 1988; Kinkel 1997; Stadler and others 1998; Stadler and Michalzik 1998). The leaching of plant exudates, organic material in dust, and metabolic contributions of microbes can raise the DON concentration of TF and some of this might be a function of recent DIN inputs to a canopy (Carlisle and others 1966; Parker 1983). To investigate the temporal fate of DIN inputs to the canopy and the generation of DON within the canopy, we used the more temporally sensitive method of isotope tracers added to the canopy as DIN.

Fate of ^{15}N enriched NH_4^+ and NO_3^-

Despite high variability, a statistically significant increase in δ $^{15}NH_4^+$ was detected in the F+$^{15}NH_4$ (the plot that received 1% ^{15}N as NH_4^+) in the first event after the fertilization, indicating that some of this ^{15}N washed out as TF unchanged (Figure 3A). Similarly, a statistically significant increase in δ $^{15}NO_3^-$ was observed in the F+$^{15}NO_3$ (the plot that received ^{15}N as NO_3^-) in the first event after the fertilization (Figure 3B). Higher enrichment of $^{15}NO_3^-$ in the F+$^{15}NO_3$ plot than enrichment of $^{15}NH_4^+$ in the F+$^{15}NH_4$ plot indicates that, as with the bulk N additions, more NO_3^- than NH_4^+ was washed off the canopy, likely due to preferential retention of NH_4^+.

Gaseous emission of inorganic N (as N_2, N_2O, NH_3, and NO) could potentially decrease TF-N flux and canopy retention estimates. However, gaseous N emission requires that denitrification or nitrification processes be active in forest canopies. Although denitrification is very unlikely within forest canopies (because it requires anaerobic conditions), nitrification has occasionally been observed (Chen and others 1983). Yet, across 17 integrated forest study sites the observed canopy NH_4^+ retention has been attributed to plant adsorption and assimilation and not to nitrification processes (Lovett 1992)—a result which we confirm here with ^{15}N analysis (see below). Thus, canopy gaseous emission was considered to be of no consequence at the Howland Forest.

We did not detect an increase in δ $^{15}NO_3^-$ in TF in the $^{15}NH_4^+$ amended plot, therefore, there was no indication that nitrification was occurring in the canopy as has been reported elsewhere (Papen and others 2002). Chen and others (1983) had also previously reported that consumption of NH_4^+ by the canopy with concomitant increase TF NO_3^- flux might be an indication of canopy nitrification. Lovett and Lindberg (1993), on the other hand, attributed a similar phenomenon to the wash off of hard-to-quantify dry NO_3^- deposition. Potter and others (1991) attempted to do a similar calculation as Chen and others (1983), and reported no indication that canopy nitrification was an important process. Despite having limited ^{15}N data, the increases in bulk NO_3^- in TF as compared to incident precipitation, we argue, were probably due to wash off of dry deposition NO_3^-. Furthermore, the

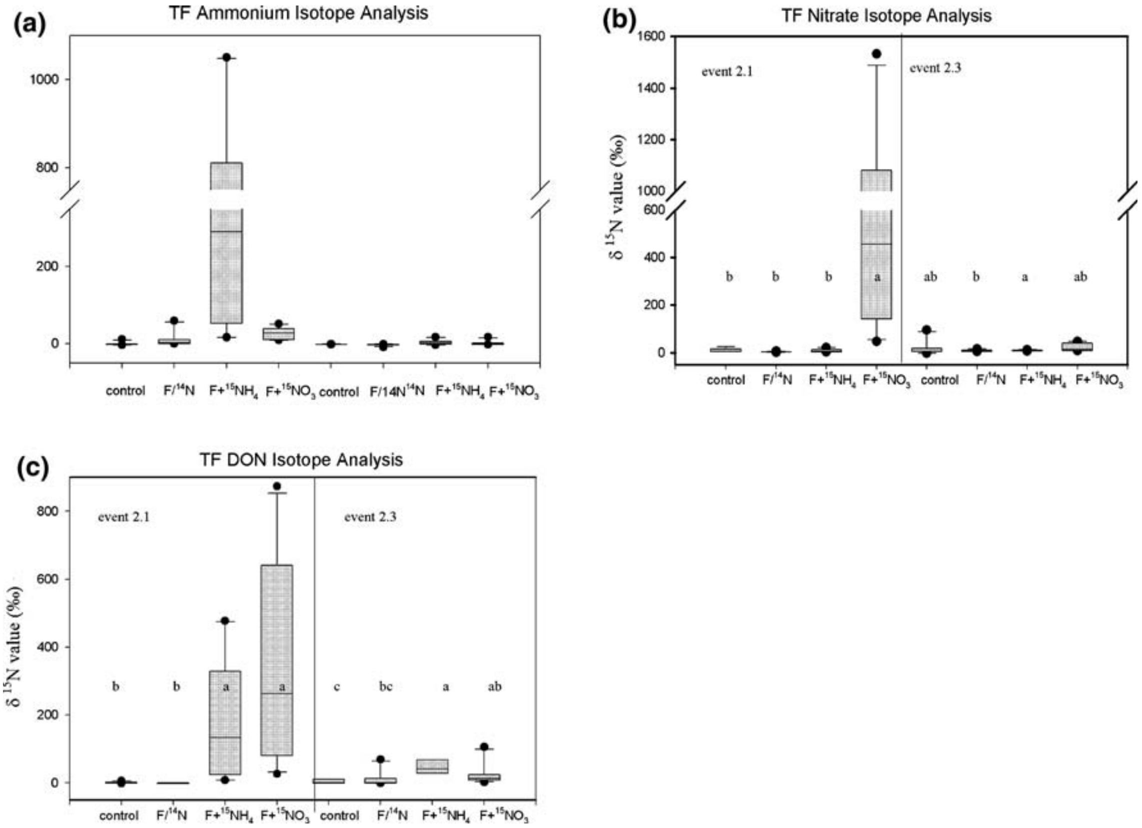

Figure 3. **A** Event 2.1 and 2.3 δ ^{15}N values associated with TF-ammonium. TF enrichments (δ ^{15}N) calculated using an average of nitrate and ammonium fertilizer enrichments. *Box plot* lower and upper hinges indicate 25th and 75th percentile, respectively, and the *line* in the middle is the median delta value Error bars indicate three-fourth of the interquartile range; *solid circles* indicate individual outlier data points. **B** Event 2.1 and 2.3 δ ^{15}N values associated with TF-nitrate. TF enrichments (δ ^{15}N) calculated using an average of nitrate and ammonium fertilizer enrichments. *Box plot* lower and upper hinges indicate 25th and 75th percentile, respectively, and the *line* in the middle is the median delta value Error bars indicate three-fourth of the interquartile range; *solid circles* indicate individual outlier data points. **C** Event 2.1 and 2.3 δ ^{15}N values associated with Throughfall DON. TF enrichments (δ ^{15}N) calculated using an average of nitrate and ammonium fertilizer enrichments. *Box plot* lower and upper hinges indicate 25th and 75th percentile, respectively, and the *line* in the middle is the median delta value Error bars indicate three-fourth of the interquartile range; *solid circles* indicate individual outlier data points.

German forest in which Papen and others (2002) observed the actions of ammonia oxidizing bacteria had received elevated N deposition inputs for several decades (TF flux to the forest floor at ~30 kg N ha^{-1} y^{-1}), and thus a canopy nitrifier community may have developed over time as a result of high NH_4^+ availability.

Isotope analysis of TF-DON confirmed that both NO_3^- and NH_4^+ were converted to soluble organic N in the canopy (Figure 3C). The δ ^{15}N of DON in initial TF events suggested a rapid conversion of some fertilizer N to DON. To a lesser, although still statistically significant degree, the plot receiving labeled NH_4^+ showed ^{15}N enrichments of DON in the third precipitation event after the fertilization (Figure 3C). A strong diminution in the ^{15}N signal by the third spray, however, suggests that export of DON from the canopy may be strongly tied to preceding rain events and the DIN delivered therein. Rapid incorporation into DON could occur via assimilation of DIN by plants, including epiphytes, and re-release as plant, lichen, and microbial exudates, via physical damage to foliage, or via insect mediated release. There was a large amount of variation in the DON TF data that could not be confidently attributed to either temporal or spatial variation.

The high retention we measured is probably not indicative of N retention potential in other forests, particularly those that have received higher, chronic N inputs during all seasons, and as both rain and frozen precipitation. Conversely, chronic N inputs which occur at levels below those that cause physiologic stress may lead to high N retention owing to induction of N assimilatory enzymes in plants. For example, European forests receiving moderate to high N deposition have also shown high canopy N retention (Hansen 1996; Kristensen and others 2004).

Implications of canopy retention for forest N demand

Canopy assimilation of elements in precipitation has been hypothesized to be an important avenue for plants to meet their nutrient demands, however canopy retention could be a physico-chemical and/or a biological phenomenon. Canopy epiphytes (lichens, algae and microorganisms) might assimilate N in precipitation and could lead to an over estimate of the amount of incoming N available for tree growth. Canopy epiphytes were present at this site, but were not quantified; we did observe that lichen biomass collected as litter had become enriched by the ^{15}N additions. Leaching experiments with lichens have shown that they have the potential to alter the chemistry of TF, however at the stand level, they appear to be inconsequential both in terms of altering TF chemistry (Friedland and others 1991), and as sinks for atmospheric N (Tomaszewski and others 2003).

Assuming that mature trees can assimilate N in the same fashion as 10-year old trees, Eilers and others (1992) extrapolated that at the mature stand level, Norway spruce canopies might be capable of assimilating 9 kg N ha^{-1} y^{-1}. This assimilation potential could meet 20-to−100% of annual demand of conifer ecosystems (Cole and Rapp 1981). From forest inventory and analysis taken in 2001 and 2003, coupled with allometric data, we calculated the annual net tree N demand to be 3-to-15 kg N ha^{-1} y^{-1}. Although whole ecosystem demand is probably much higher, (it would include understory plant and soil N demand), we used the mature stand tree N demand to compare with our observed canopy N retention. The fertilized stand retained 60–70% of the 18 kg N annual addition and thus may have assimilated as much as 12 kg N ha^{-1} of the added fertilizer, which would meet and potentially exceed the annual whole-tree N demand. However, we do not know if N retained in the canopy was actually assimilated and available to support new growth. Preliminary results of foliar analysis indicate that less than 5% of a ^{15}N label was recovered in live foliage and wood after 2 years of N addition (Dail and others 2007), but additional studies are needed to monitor the fate of added ^{15}N within the ecosystem.

CONCLUSIONS

Approximately 5–16% of the 18 kg N ha^{-1} y^{-1} applied as a misted spray by helicopter in five doses per year as NH_4NO_3 bypassed the canopy entirely and was immediately delivered to the forest floor. Canopy retention of NO_3^- ranged from 57 to 75% of inputs in 2 years of the study. Ammonium was more strongly retained by the treated plot canopy, with 73–83% of inputs. The difference in retention estimates of DIN forms was attributed to preferential retention of NH_4^+ by the canopy, although ambient NO_3^- was slightly better retained by the control plot canopy. Canopy retention of total N inputs (including DON), were similar, ranging from 60 to 77%. The mean DON export from the canopy via throughfall doubled as a result of fertilizer addition, indicating that some added NO_3^- and NH_4^+ was converted to DON within the canopy. Both DIN and DON in throughfall quickly returned to ‘‘baseline’’ concentrations and were not statistically different from ambient plot TF quantities after added N was washed from the canopy by the second and third precipitation events after fertilization.

Isotope tracer additions showed that NH_4^+ and NO_3^- were both converted to DON at statistically equal probability, although the mean was slightly higher for DON enrichment in the $^{15}NO_3^-$ plot. Significant ^{15}N enrichments of DON were observed in TF from the first event after fertilization, but had largely disappeared by the third TF event. This suggested that for this site, conversion of incident DIN to DON is a rapid process. The amount of experimental N retained by the canopy (~12 kg N ha^{-1}) could meet most or all of the annual net N requirement (3–15 kg N ha^{-1}) of the forest live tree biomass, but we do not know how much of the N retained by the canopy was assimilated by the trees, by epiphytes, or will enter actively cycling pools of N in the ecosystem. Continuing studies of the long-term fate of added N, including the ^{15}N additions, will enable us to assess the potential of the additional N, added as a helicopter spray to the canopy, to fuel growth as it is transferred among forest ecosystem pools.

ACKNOWLEDGMENTS

We thank the International Paper Company, Ltd., and GMO, LLC for providing access to the research site in Howland, Maine. We also thank our technical support staff; Mr. John Lee, Ms. Holly Hughes and Mr. Charles Rodrigues and undergraduates; Mr. David Dunston, Ms. Nell Burger and Ms. Gretchen

Miles; Department of Energy SURE program interns who worked on this project. This research was supported by the Office of Science (BER), U.S. Department of Energy, through the Northeast Regional Center of the National Institute for Global Environmental Change under Cooperative Agreement Nos. DE-FC02-03ER63613, DE-FC-03-90ER61010, and DE-FG02-00ER63002; DOE-NICCR agreement No, DE-FG02-06ER64157 and NSF award No. 0223188. Financial support does not constitute an endorsement by DOE or NSF of the views expressed in this article. We also thank several anonymous reviewers. This is Maine Agriculture and Forestry Experiment Station publication #2964.

REFERENCES

Aber JD, Nadelhoffer KJ, Steudler P, Melillo PM. 1989. Nitrogen saturation in northern forest ecosystems. BioScience 39:378–86.

Balestrini R, Tagliaferri A. 2001. Atmospheric deposition and canopy exchange processes in alpine forest ecosystems (northern Italy). Atmos Environ 35:6421–33.

Beier C, Hansen K, Gundersen P. 1993. Spatial variability of throughfall fluxes in a spruce forest. Environ Pollut 81:257–67.

Bowden RD, Geballe GT, Bowden WB. 1989. Foliar uptake of 15N from simulated cloud water by red spruce (*Picea rubens*) seedlings. Can J For Res 19:382–6.

Boyce RL, Friedland AJ, Chamberlain CP, Poulson SR. 1996. Direct canopy nitrogen uptake from ^{15}N-labeled wet deposition by mature red spruce. Can J For Res 26:1539–47.

Brooks PD, Stark JM, McInteer BB, Preston T. 1989. A diffusion method to prepare soil extracts for automated nitrogen-15 analysis. Soil Sci Soci Am 53:1707–11.

Cabrera ML, Beare MH. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci Soci Am 57:1007–12.

Calanni JE, Berg M, Wood D, Mangis R, Boyce W, Weathers H, Sievering H. 1999. Atmospheric nitrogen deposition at a conifer forest: response of free amino acids in Engelmann spruce needles. Environ Pollut 105:79–89.

Cape JN, Dunster A, Crossley A, Sheppard LJ, Harvey FJ. 2001. Throughfall chemistry in a Sitka spruce plantation in response to six different simulated polluted mist treatments. Water Air Soil Pollut 130:619–24.

Carlisle A, Brown AH, White EJ. 1966. The organic matter and nutrient elements in the precipitation beneath a sessile oak (*Quercus petraea*) canopy. J Ecol 54:87–98.

Carroll GC. 1980. Forest canopies: complex and independent subsystems. In: Waring RH, Ed. Forests: fresh perspectives from ecosystem analysis. Proceedings of the 40th Annual Biology Colloquium. Oregon State University Press. pp 87–107.

Chen CW, Hudson RJ, Gherini SA, Dean JD, Goldstein RA. 1983. Acid rain model: canopy module. J Environ Eng 109:585–603.

Chiwa M, Crossley A, Sheppard LJ, Sakugawa H, Cape JN. 2004. Throughfall chemistry and canopy interactions in a Sitka spruce plantation sprayed with six different simulated polluted mist treatments. Environ Pollut 127:57–64.

Coudhury D. 1988. Herbivory induced changes in leaf-litter resource quality: a neglected aspect of herbivory in ecosystem nutrient dynamics. Oikos 51:389–93.

Cole D, Rapp M. 1981. Elemental cycling in forest ecosystems. In: Reichle D (ed) Dynamic properties of forest ecosystems. Cambridge University Press. International Biological Programme 23:341–409.

Dail DB, Hollinger D, Davidson E, Fernandez I, Scott N, Goltz S, Sievering H, Lee JT. 2007. Fate of ^{15}N additions to a mature Spruce-Hemlock canopy. Oecologia (in review).

D'Elia CF, Steudler PA, Corwin N. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. Limnol Oceanogr 22:760–4.

Eilers JR, Brumme R, Matzner E. 1992. Above-ground N-uptake from wet deposition by Norway spruce (*Picea abies* Karst.). For Ecol Manag 51:239–249.

Fernandez IJ, Lawrence GB, Richards KJ. 1990. Characteristics of foliar chemistry in a commercial spruce-fir stand of northern New England USA. Plant Soil 125:288–292.

Friedland AJ, Miller EK, Battles JJ, Thorne JF. 1991. Nitrogen deposition, distribution and cycling in a sub-alpine spruce-fir forest in the Adirondacks, New York, U.S.A. Biogeochemistry 14:31–55.

Galloway JN, Cowling EB. 2002. Reactive nitrogen and the world: 200 years of change. Ambio 31:64–71.

Hansen K. 1996. In-canopy throughfall measurements of ion fluxes in Norway spruce. Atmos Environ 30:4065–76.

Hollinger Aber DY. J, Dail B, Davidson EA, Goltz SM, Hughes H, Leclerc MY, Lee JT, Richardson AD, Rodrigues C, Scott NA, Achuatavarier D, Walsh J. 2004. Spatial and temporal variability in forest–atmosphere CO_2 exchange. 2004. Global Change Biol 10:1–18.

Hollinger DY, Goltz SM, Davidson EA, Lee JT, Tu K, Valentine HT. 1999. Seasonal patterns and environmental control of carbon dioxide and water vapor exchange in an ecotonal boreal forest. Global Change Biol 5:891–902.

Kinkel LL. 1997. Microbial population dynamics on leaves. Ann Rev Phytopath 35:327–47.

Kristensen HL, Gundersen P, Callesen I, Reinds G. 2004. Atmospheric N deposition influences soil nitrate concentration differently in European Coniferous and Deciduous Forests. Ecosystems 7:180–92.

Lawrence GB, Fernandez IJ. 1991. Biogeochemical effects of acidic deposition on a low elevation spruce-fir stand in Howland, Maine. Can J For Res 21:867–75.

Lawrence GB, Fernandez IJ. 1993. A reassessment of areal variability of throughfall deposition measurements. Ecol Appl 3:473–80.

Lewis J. 2003. Stemflow estimation in a redwood forest using model-based stratified random sampling. Environmetrics 14:559–71.

Liechty HO, Mroz GD, Reed DD. 1993. Cation and anion fluxes in northern hardwood throughfall along an acidic deposition gradient. Can J For Res 23:457–67.

wLovett GM. 1992. Atmospheric deposition and forest nutrient cycling: a synthesis of the integrated forest study. Ecol. Stud. 91, In: Johnson D, Lindberg S, New York: Springer, pp 634–55.

Lovett GM, Lindberg SE. 1993. Atmospheric deposition and canopy interactions of nitrogen in forests. Can J For Res 23:1603–16.

Lovett GM, Nolan SS, Driscoll CT, Fahey TJ. 1996. Factors regulating thoughfall flux in a New Hampshire forested landscape. Can J For Res 26:2134–44.

Magill AH, Aber JD, Hendricks JJ, Bowden RD, Melillo JM, Steudler PA. 1997. Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. Ecol Appl 7:402–15.

McLaughlin JW, Fernandez IJ, Richards K. 1996. Atmospheric deposition to a low-elevation spruce-fir forest, Maine, USA. J Environ Qual 25:248–59.

McNulty SG, Aber JD, Newman SD. 1996. Nitrogen saturation in a high elevation New England spruce-fir stand. For Ecol Manag 84:109–21.

Nadelhoffer K, Emmett B, Gundersen P, Tietema A, Wright R. 1999. Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. Nature 398:145–47.

Nadelhoffer KJ, Johnson LC, Laundre JA, Giblin AE, Shaver GR. 2002. Fine root production and nutrient content in wet and moist arctic tundras as influenced by chronic fertilization. Plant Soil 242:107–13.

Papen H, Geßler A, Zumbusch E, Rennenberg H. 2002. Chemolithoautotrophic nitrifiers in the phyllosphere of a spruce ecosystem receiving high atmospheric nitrogen input. Curr Microbiol 44:56–60.

Parker GG. 1983. Advances in ecological research. New York: Academic, p 58–135.

Pizzicannella F, Nirel P, Landry J-C. 1995. Mise au point d'une méthode de détermination de l'azote organique dissous. Arch Sci Genève 49:59–68.

Potter CS, Ragsdale HL, Swank WT. 1991. Atmospheric deposition and foliar leaching in a regenerating southern Appalachian forest canopy. J Ecol 79:97–115.

Scott NA, Rodrigues CA, Hughes H, Lee JT, Davidson EA, Dail DB, Malerba P, Hollinger DY. 2004. Changes in carbon storage and net carbon exchange one year after an initial shelterwood harvest at Howland Forest. ME Env Manage 33(Suppl. 1):S9–22.

Sievering H, Fernandez I, Lee J, Hom J, Rustad L. 2000. Forest canopy uptake of atmospheric nitrogen deposition at eastern U.S. conifer sites: carbon storage implications?. Global Biogechem Cycles 14:1153–9.

Shearer G, Kohl DH. 1993. Natural abundance of ^{15}N: fractional contribution of two sources to a common sink and use of isotope discrimination. In: Knowles, R, Blackburn TH, Eds. Nitrogen isotope techniques. San Diego: Academic.

Solórzano L, Sharp JH. 1980. Determination of total dissolved nitrogen in natural waters. Limnol Oceanogr 25:751–4.

Sørensen P, Jensen ES. 1991. Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoroethylene trap for ^{15}N determination. Anal Chim Acta. 252:201–3.

Stadler B, Michalzik B. 1998. Aphid infested Norway spruce are ''hot spots'' in throughfall carbon chemistry in coniferous forests. Can J Fort Res 28:1717–22.

Stadler B, Michalzik B, Muller T. 1998. Linking aphid ecology with nutrient fluxes in a coniferous forest. Ecology 79:1514–25.

Stark JM, Hart SC. 1996. Diffusion technique for preparing salt solutions, kjeldahl digests and persulfate digests for nitrogen-15 analysis. Soil Sci Soc Am J 60:1846–55.

Swan HSD. 1971. Woodlands report WR/34. Pulp and Paper Resources Institute of Canada, p 27.

Tietema A, Emmet BA, Gundersen P, Kjønaas OJ, Koopmans CJ. 1998. The fate of 15N labeled nitrogen deposition in coniferous forest ecosystems. For Ecol Manage 101:19–27.

Tomaszewski T, Boyce RL, Sievering H. 2003. Canopy uptake of atmospheric nitrogen and new growth nitrogen requirement at a Colorado subalpine forest. Can J For Res/Rev Can Res 33(11):2221–7.

Townsend AR, Braswell BH, Holland EA, Penner JE. 1996. Spatial and temporal patterns in terrestrial carbon storage due to deposition of fossil fuel nitrogen. Ecol Appl 6:806–14.

U.S. Department of Agriculture, Forest Service. 2002. Forest inventory and analysis national core field guide; volume II: field data collection procedures for phase 3 plots, version 1.6, Sect. 14, pp 265–298.

USEPA. 2004. Howland, ME (HOW132). Clean Air Status and Trends Network. ''http://www.epa.gov/castnet/sites/how132.html'' (10 September 04).

Vitousek PM. 1982. Nutrient cycling and nutrient use efficiency. Am Nat 119:553–72.

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman DG. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7:737–50.

Wright RF, Rasmussen L. 1998. Introduction to the NITREX and EXMAN projects. For Ecol Manage 101:1–7.

Young HE, Ribe JH, Wainwright K. 1980. Weight tables for tree and shrub species in Maine. Univ of Maine Life Sci and Agric. Exp. Stn. Misc. Rep. 230. University of Maine, Orono, ME. 84 pp.