Distribution of nitrogen-15 tracers applied to the canopy of a mature spruce-hemlock stand, Howland, Maine, USA

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Abstract In N-limited ecosystems, fertilization by N deposition may enhance plant growth and thus impact C sequestration. In many N deposition–C sequestration experiments, N is added directly to the soil, bypassing canopy processes and potentially favoring N immobilization by the soil. To understand the impact of enhanced N deposition on a low fertility unmanaged forest and better emulate natural N deposition processes, we added 18 kg N ha\(^{-1}\) year\(^{-1}\) as dissolved NH\(_4\)NO\(_3\) directly to the canopy of 21 ha of spruce-hemlock forest. In two 0.3-ha subplots, the added N was isotopically labeled as \(^{15}\)NH\(_4\)\(^+\) or \(^{15}\)NO\(_3\)^\(-\) (1% final enrichment). Among ecosystem pools, we recovered 38 and 67% of the \(^{15}\)N added as \(^{15}\)NH\(_4\)\(^+\) and \(^{15}\)NO\(_3\)^\(-\), respectively. Of \(^{15}\)N recoverable in plant biomass, only 3–6% was recovered in live foliage and bole wood. Tree twigs, branches, and bark constituted the most important plant sinks for both NO\(_3\)^\(-\) and NH\(_4\)\(^+\), together accounting for 25–50% of \(^{15}\)N recovery for these ions, respectively. Forest floor and soil \(^{15}\)N retention was small compared to previous studies; the litter layer and well-humified O horizon were important sinks for NH\(_4\)\(^+\) (9%) and NO\(_3\)^\(-\) (7%). Retention by canopy elements (surfaces of branches and boles) provided a substantial sink for N that may have been through physico-chemical processes rather than by N assimilation as indicated by poor recoveries in wood tissues. Canopy retention of precipitation-borne N added in this particular manner may thus not become plant-available N for several years. Despite a large canopy N retention potential in this forest, C sequestration into new wood growth as a result of the N addition was only \(\approx 16\) g C m\(^{-2}\) year\(^{-1}\) or about 10% above the current net annual C sequestration for this site.

Keywords Nitrogen-15 · Nitrate · Ammonium · Nitrogen deposition · Carbon sequestration · Forest elemental cycling

Introduction

Human activities have more than doubled the quantity of biologically available N in terrestrial ecosystems; however, these inputs are spread unevenly and are concentrated in areas of intensive agriculture, industry, and population centers (Galloway et al. 2004; Townsend et al. 1996). In ecosystems where N supply exceeds biological demand, there is potential for leaching, acidification, and loss of soil nutrients and eutrophication of fresh and marine waters (Boxman et al. 1995; Emmett et al. 1995; Kahl et al. 1993; McNulty and Aber 1993; Nixon et al. 1996; Fenn et al. 1998; UNEP and WHRC 2007). In N-limited systems, however, additional N inputs may increase plant productivity and thus C sequestration (Brix 1981; Tamm 1991; Vitousek and Howarth 1991; Townsend et al. 1996;
Holland et al. 1997; Magnani et al. 2007; Pregitzer et al. 2008). How much anthropogenic N contributes to long-term C storage in woody plant biomass as opposed to becoming immobilized in other ecosystem compartments with higher turnover rates is still debated (Aber et al. 1995; Nadelhoffer et al. 1999a; Schindler and Bayley 1993; Pepper et al. 2005; Currie et al. 2004). Many N manipulation studies suggest that soil, and not plant biomass, is the primary sink for increased N and thus infer that the resultant C storage attributed to increased N would not explain much of the “unexplained” C sink thought to exist in the terrestrial lands of the northern hemisphere (Aber et al. 1995; Nadelhoffer et al. 1999b; Currie et al. 2004). Others have argued, however, that the strong soil N sink could be a result of the experimental N being added directly to the forest floor—an addition method which accounts for only part of forest–atmosphere interactions and one thought to favor uptake by soil microorganisms rather than plants (Sievering 1999; Jenkinson et al. 1999).

From studies of throughfall, forest canopies are known to assimilate wet and dry N deposition (Johnson and Lindberg 1992; Arthur and Fahey 1993; Sievering et al. 2000) and this uptake mechanism may account for up to 30% of the annual growth requirement in some trees (Friedland et al. 1991; but see Bowden et al. 1989; Boyce et al. 1996). Furthermore, numerous studies of nursery stock and young forest trees have shown increased aboveground biomass in response to canopy N additions (Eilers et al. 1992; Hättenschwiler and Körner 1998). We sought to use $^{15}$N tracers of inorganic N to investigate the importance of foliar uptake to tree growth and thus C storage potential in a mature spruce-hemlock forest in central Maine. We hypothesized that: (1) canopy uptake of additional N ($\sim 6–9$ times ambient) in a closed-canopy, N-limited forest would be very high, $\sim 40\%$, consistent with previously reported N uptake of ambient inputs for this site (Lawrence and Fernandez 1991); and that (2) canopy N uptake would fuel additional C sequestration.

Materials and methods

Research site

The Howland Integrated Forest Study (HIFS) was established in 1987 in east-central Maine ($45^\circ12'N$, $68^\circ45'W$, 68 m a.s.l.). The site was a part of International Paper’s Northern Experimental Forest until it was sold to GMO Renewable Resources. 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 infestations. Red spruce ($Picea rubens$ Sarg.) and eastern hemlock ($Tsuga canadensis$ (L.) Carr.] are co-dominant species, with lesser quantities of white pine ($Pinus strobus$ L.), eastern white cedar ($Thuja occidentalis$ L.) and red maple ($Acer rubrum$ L.) (Hollinger et al. 1999).

The mean tree age for the site is $\sim 140$ years with some trees in excess of 300 years old. Mean live basal area, determined from 48 forest inventory and analysis plots [USDA Forest Service Forest Inventory and Analysis (FIA) Program] was 56.7 $\pm$ 16.5 m$^2$ ha$^{-1}$ at the initiation of this study (Hollinger et al. 2004). Leaf area index (silhouette method) was $\sim 6$ m$^2$ m$^{-2}$ and the mean tree height was 20 m (Scott et al. 2004). Epiphytic lichens are present at the site and represent about 3% of biomass present in litterfall collectors (unpublished data).

Soils are Aquic Haplorthods from the moderately well-drained Skerry series and Aeric Hapludults from the more poorly drained Westbury series, developed from a dense basal till that is characteristic of the region (McLaughlin et al. 1996). Annual precipitation averages $\sim 1,000$ mm with a mean temperature of $\sim 6^\circ$C, and a snow pack of up to 2 m from December up to and including March. Additional information on the HIFS can be found in Fernandez et al. (1990), Lawrence and Fernandez (1991), McLaughlin et al. (1996) and Hollinger et al. (1999), and at http://www.howlndforest.org/.

In 2000 and 2001 the HIFS received 0.65 and 0.8 kg N ha$^{-1}$ year$^{-1}$ as dry N deposition, respectively, and $>50\%$ of this as HNO$_3$. In addition to dry inputs, the site received 3 and 2 kg N ha$^{-1}$ year$^{-1}$ as wet N deposition in 2000 and 2001, respectively, mostly as NO$_3^-$ [CASTNET/NADP data for 2000–2001 (USEPA 2004)]. Analysis of foliage chemistry by Fernandez et al. (1990) characterized this forest as potentially deficient in both N and P.

Experimental design

Aerial application of dissolved N fertilizer to the canopy allowed us to add N to the forest ecosystem in a manner that more closely approximated wet N inputs compared to most previous studies. On five different dates during the growing season (May–August) a helicopter sprayed a fine mist of liquid NH$_4$NO$_3$ onto a 21-ha area consisting of a 120$^\circ$ circular sector between 250$^\circ$ and 10$^\circ$, radiating $\sim 440$ m out from an eddy-covariance tower. Each of the applications delivered 3.6 kg N ha$^{-1}$ as NH$_4$NO$_3$ for a total of 18 kg N ha$^{-1}$ year$^{-1}$ above ambient inputs, which is approximately 3–6 times the ambient wet input. The frequency and dose of N applications was 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 not have been practical. The total annual dose was toward the
lower end of what we calculated would be detectable and yet within the range of deposition rates experienced in forests of other parts of North America and Europe. Caution should be exercised in extrapolating the reported findings, both spatially and temporally, owing to the large dose relative to the ambient inputs.

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 have at least 24 h contact before subsequent rain events. Within the fertilized area we designated three 100 × 30-m (0.3-ha) intensively sampled sub-plots, two of which received additional helicopter fertilization in the form of labeled N either as 10% enriched (15NH4)2SO4 (hereafter, the 15NH4+ sub-plot) or Na15NO3 (the 15NO3− sub-plot) in a 0.3 kg N ha−1 dose with each flight, increasing the seasonal load of experimental N from 18 to 19.8 kg N ha−1 year−1 (a final enrichment of 1% 15N). A third intensively sampled sub-plot (the “fertilized” sub-plot) was established as a means of sampling the greater unlabeled fertilized area. A control plot receiving ambient N deposition was established >200 m away to avoid spray contamination. Spraying within sub-plot locations and avoidance of cross contamination was assured by low-level flights using an airborne AgNav global positioning system, marking the canopy along boarders of the sub-plot with bright cloth flagging, and by measuring the SO42− and Na contents of throughfall which might indicate overspray. In order to determine canopy removal of ambient precipitation N inputs to the control plot, precipitation was collected from open areas (former clear-cuts) within 1 km of the site. Canopy retention of ambient and experimental N inputs was measured using throughfall from all plot areas and reported elsewhere (Gaige et al. 2007).

Dimensional analysis and 15N recovery in plant biomass

Tree biomass

FIA-style plots were established in 2001 and measurements performed again in 2003. We established 12 transects every 30° centered on the CO2 flux tower. Subplots were created 50, 100, 200 and 400 m from the tower along each transect and this generated a total of 48 inventory plots; 16 were in the fertilized area and 32 in the control area. Every tree, live or dead, above 5 cm diameter at breast height was included in the inventory, although only live trees of the five dominant species (constituting >95% of the live overstory biomass) were used to estimate plant 15N recovery, based on the allometric equations of Young et al. (1980) and Jenkins et al. (2003).

Foliage sampling and analysis

Stem (tree bole), branches, green foliage, bark, and coarse and fine roots were randomly sampled from a total of 156 trees in both treated and untreated plots in 2003. Two branches (between 0.25 and 2 cm diameter) were removed from each tree via shotgun (#3 steel shot). Two of these branches, one each from a sunlit and shaded portion of the canopy were dried at 60°C until needles fell away from the branch and the sample achieved constant weight. These needle samples constituted an age-weighted method of determining 15N uptake by canopy foliage, and frozen prior to being lyophilized. Approximately 1–2 g of dry material was then ball milled (SPEX mill model 8000D) and subsamples removed for analysis by solid state isotope ratio mass spectrometry (IRMS) at the Stable Isotope Laboratory at the University of California, Davis. Samples with a high C:N ratio were run on a Europa 20–20 mass spectrometer with an ANCA/GSL combustion unit, whereas higher 15N enriched samples were run on an Europa Integra isotope ratio mass spectrometer; all with appropriate standards.

Branches and twigs

From the branches dried at 60°C, we removed current and past year twigs and a wood “cookie” (bark included) from the end of the branch most proximal to the main stem. The largest branches that were analyzed were about 0.5–2 cm in diameter. These dry samples were then milled and prepared for analysis in the same manner as dried green foliage. We used a wood volume-integrated method of estimating a single isotope enrichment of all wood for each tree sampled.

Bole wood analysis

We used a half-inch-diameter (1.27 cm) increment borer to remove a 4-cm length core at breast height from the same trees that were sampled for the foliage 15N recovery. Cores were removed in mid September 2003 and in most cases we removed enough material to include all of the visible sapwood, but rarely reached the pith of these mature trees. Sapwood increments were measured (translucent core wood) in the field and cores were then freeze dried and sanded by hand. Pressurized air was used to remove wood dust from the cores and then the cores were separated into three sub-samples; inner and outer bark; the 3 most recent years (2001–2003; years in which 15N was added to the forest canopy); and the 10 years prior to initiation of the canopy fertilization. The latter 10-year increment typically included some, but not all of the sapwood present in the wood cores; averaging 45% of the sapwood area. We assumed that the isotopic enrichment of the sapwood was
constant throughout and applied the enrichment of the 10-year increment to the volume of wood present in the sapwood area determined in the field. Bark and wood samples were milled and subsampled for IRMS.

Roots

Root samples were removed from 2003 soil cores to a depth of 21 cm. Roots were gently washed with water to remove soil, lyophilized, and then separated into coarse (>2 mm diameter) and fine roots. We collected 12 soil cores from each of the treated and control sub-plots for a total of 48 soil cores. No attempt was made to separate live and dead roots, and roots combined by size classes traversed forest floor and mineral soil horizons.

Forest floor and soil samples

For each of the 48 soil cores a 5.5-cm-diameter (21 cm length) tulip bulb corer was placed on top of the litter layer. A knife was used to cut the litter layer to allow the corer to penetrate the litter and underlying root mass. The corer was then hammered to 21-cm depth and the core retrieved intact. The core was transported on ice and then freeze dried. Freeze drying eased the separation of soil horizons intact. The core was transported on ice and then freeze dried. Freeze drying eased the separation of soil horizons intact. The core was transported on ice and then freeze dried.

Gaseous N losses

The application of NH₄NO₃ during the growing season may cause loss of inorganic N from the canopy by gaseous emission to the atmosphere of NH₃ or HNO₃ vapor. Deposition and/or emission of HNO₃ was measured using the modified Bowen ratio approach during the first 24-h period following application at control and treatment eddy flux towers (Sievering et al. 2001). Soil denitrification was not measured.

Estimating N enrichment, N recovery and C sequestration

We calculated the experimental ¹⁵N enrichment of ecosystem pools using the δ notation common to natural abundance and enrichment studies wherein slight enrichments are expected (Nadelhoffer and Fry 1994; Nadelhoffer et al. 1999c). This method accounts for differences in the initial ¹⁵N contents of all ecosystem pools and we indicate the divergence ¹⁵N enrichment from non-amended pools using the following equation:

\[
\text{Change in } \delta^{15}N(\%) = \delta^{15}N_{\text{experimental}} - \delta^{15}N_{\text{control}}
\]

Change in δ¹⁵N (‰) is the change in the enrichment of a pool due to fertilization and δ¹⁵Nexperimental and δ¹⁵Ncontrol are the ¹⁵N enrichments of a particular N pool in ¹⁵N exposed and control plots. To estimate the recovery of experimental ¹⁵N in ecosystem pools, we first estimated the mass and N content of the pool as described above, and then subtracted the natural abundance ¹⁵N content obtained from the experimental plot (atom%) to yield atom% excess (Nadelhoffer et al. 1999c). Atom% excess was then multiplied by the N content of the pool across the landscape to achieve an estimate of recovery of the isotope. We used Howland FIA data for 2003, tree species allometry for Maine tree species (Young et al. 1980), C, N content, and increment data to estimate the consequence of additional fertilizer for tree growth. Using the assumptions that ¹⁵N assimilated into wood species was indicative of the fate of the overall N addition, constancy of the C:N relationship in wood, and that ¹⁵N assimilated was in addition to, i.e., did not replace, native N uptake, we estimated the C sequestration caused by additional N inputs. The measurement precision of isotope analysis for foliage and soils (Europa Integra at the University of California Davis Stable Isotope lab) and all other above ground plant parts (Europa Hydra 20/20) was determined on duplicate standards after every ten experimental samples. The standard mean and 95% confidence interval for each instrument was δ¹⁵N = 110.0 ± 1.41 and δ¹⁵N = 1.43 ± 0.05, respectively.

Statistical analysis

We fertilized a contiguous 21-ha area in order to minimize edge effects and to permit study of effects of N addition on C fluxes by eddy covariance, which will be presented elsewhere. Replicating this large-scale aerial N fertilization experiment would have been too costly. Hence, we can report only on how this particular forest retained ¹⁵N and do not attempt to infer that similar forests would necessarily respond at similar retention rates. Three 0.3-ha study plots (30 x 100 m), each containing seven or more collectors for stemflow, throughfall, litter, and soil water, provide replication of some measurements within the full 21-ha area, thus affording estimates of within-treatment variance. Mean N retention by forest pools (e.g., canopy, wood, litter and soils) is presented with associated SD and
final uncertainties were propagated by a Monte Carlo method. Error associated with the summing of ecosystem pool $^{15}$N recoveries was calculated by propagating the SDs of each pool recovery; the square root of the sum of squares of the absolute value of each pool SD was used to assign error to total ecosystem $^{15}$N recovery.

**Results**

Spruce and hemlock constituted 42 and 37% of total stand biomass (Table 1) and therefore we performed the most intensive sampling for $^{15}$N analysis for these two species. Despite smaller contributions to total stand biomass, some species such as red maple and white pine made up an inordinate fraction of wood biomass in the treated area (Table 1). Red spruce was the species constituting the largest total biomass but larger woody tissue biomass and higher N content in hemlock wood resulted in this co-dominant species having a larger contribution of total tree N within the experimental area (Table 1). Constituting only 2% of stand biomass, white pine contained approximately 9% of stand N (Table 1). The C mass increment (the difference between the sum of the C mass of all trees in a plot in 2003 and 2001) was not significantly different for plots in the fertilized ($n = 16$) and control areas ($n = 32$), averaging $172 \pm 65$ and $197 \pm 42$ g C m$^{-2}$ year$^{-1}$ (mean and 95% confidence intervals), respectively. The mean annual C increment of all plots was $189 \pm 34$ g C m$^{-2}$ year$^{-1}$ (Fig. 1).

Forest floor and underlying soils to a depth of 21 cm contained about 1,200 g N m$^{-2}$. The forest floor on average was 4–9 cm deep and the underlying E horizon was typically 3–9 cm, thus the mineral B horizon sampled was often a small fraction of the 21-cm core depth and sometimes absent from the core. The litter layer (O$_1$) contained 32 g N m$^{-2}$ and the well-humified O$_2$ and B horizons (to 21 cm depth) contained 150 and 1,000 g N m$^{-2}$, respectively; however the N content calculated for the mineral soil did not include the complete depth of this horizon (Table 3). We did not detect any experimental $^{15}$N below the O$_2$ horizon in either the NH$_4^+$- or NO$_3^-$-amended plots and thus did not further characterize the deeper mineral horizons.

Foliage, branches, twigs, and bark were the most enriched ecosystem components from both NO$_3^-$ and NH$_4^+$ labeling, while recent bole wood (that growth which occurred during the 3-year $^{15}$N application) and the prior 10 years of sapwood were the least enriched pools (Table 2). Foliage of hemlock and spruce tended to be more enriched by the $^{15}$NO$_3^-$ addition; however, the only significant differences observed were for foliage of white pine, northern white cedar and red maple. There was significantly greater enrichment of pine and cedar in the $^{15}$NH$_4^+$ plot while red maple was more enriched in the $^{15}$NO$_3^-$ plot (Table 2). Little significant N localizations in

<p>| Table 1 Howland site forest inventory characteristics |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|</p>
<table>
<thead>
<tr>
<th>Species</th>
<th>Total biomass a (g m$^{-2}$)</th>
<th>Foliar biomass a (g m$^{-2}$)</th>
<th>Branch biomass a (g m$^{-2}$)</th>
<th>Stem biomass a (g m$^{-2}$)</th>
<th>Species’ contribution c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemlock</td>
<td>9,356</td>
<td>939</td>
<td>1,526</td>
<td>5,096</td>
<td>37 ± 20</td>
</tr>
<tr>
<td>Red spruce</td>
<td>10,785</td>
<td>1,085</td>
<td>1,019</td>
<td>6,393</td>
<td>42 ± 21</td>
</tr>
<tr>
<td>White pine</td>
<td>1,426</td>
<td>36</td>
<td>156</td>
<td>1,006</td>
<td>2 ± 10</td>
</tr>
<tr>
<td>White cedar</td>
<td>1,361</td>
<td>115</td>
<td>222</td>
<td>770</td>
<td>6 ± 07</td>
</tr>
<tr>
<td>Red maple</td>
<td>3,337</td>
<td>89</td>
<td>266</td>
<td>2,379</td>
<td>13 ± 11</td>
</tr>
</tbody>
</table>

<p>| Biomass N |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|</p>
<table>
<thead>
<tr>
<th>Species</th>
<th>Total biomass b (g N m$^{-2}$)</th>
<th>Foliar biomass b (g N m$^{-2}$)</th>
<th>Branch biomass b (g N m$^{-2}$)</th>
<th>Stem biomass b (g N m$^{-2}$)</th>
<th>Species’ contribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemlock</td>
<td>27.6</td>
<td>10.3</td>
<td>9.5</td>
<td>7.8</td>
<td>39.3</td>
</tr>
<tr>
<td>Red spruce</td>
<td>24.9</td>
<td>11.0</td>
<td>5.4</td>
<td>8.5</td>
<td>35.4</td>
</tr>
<tr>
<td>White pine</td>
<td>6.2</td>
<td>4.0</td>
<td>0.9</td>
<td>1.3</td>
<td>8.8</td>
</tr>
<tr>
<td>White cedar</td>
<td>3.6</td>
<td>2.1</td>
<td>0.2</td>
<td>1.3</td>
<td>5.1</td>
</tr>
<tr>
<td>Red maple</td>
<td>8.0</td>
<td>1.6</td>
<td>3.0</td>
<td>3.4</td>
<td>11.4</td>
</tr>
</tbody>
</table>

a Data from 36 forest inventory plots within a 63-ha area
b Above- and belowground biomass less root crowns calculated using allometry for Maine species (Young et al. 1980)
c Species’ contribution to total basal area; mean of 36 forest inventory and analysis plots ±1 SD
other plant parts were observed other than in hemlock bark, where we found very large enrichments in the plot exposed to \( {^{15}\text{NO}_3^-} \) (Table 2). Hemlock bark was by far the most enriched plant part found in the study and this was consistently observed in all trees sampled of this species. Recent wood was slightly more enriched than the remainder of the sapwood; however, no significant differences were observed between these two wood increments (Table 2). By multiplying the N contents of Howland tree species (Table 1) by the % \(^{15}\text{N} \) excess of various plant parts (converted from Table 2) it was determined that in addition to hemlock bark, wood and foliage of red maple were significantly more enriched by \( {^{15}\text{NO}_3^-} \) additions than these same tissues in other species (Tukey’s honest significant difference \( \alpha = 0.05 \)). Owing to large variations in plant tissue enrichment, and perhaps a lack of species differences, no other species differences were observed. High enrichment (by \( {^{15}\text{NO}_3^-} \)) of hemlock and red maple suggested that forests dominated by these species might be more N retentive than the other species in this study. Of the two species for which total plant recovery could be calculated, red spruce (41% of stand biomass) and eastern hemlock (36%), the hemlocks retained more than 75% of \( {^{15}\text{NO}_3^-} \), but an equivalent amount of \( {^{15}\text{NH}_4^+} \). Thus, hemlocks retained canopy-applied \( {^{15}\text{NO}_3^-} \) more efficiently than their biomass contribution to the stand would suggest, and this was purely a function of the affinity of hemlock bark for the \( {^{15}\text{NO}_3^-} \) ion.

Enrichments of the annual litter inputs for 2003 and for forest floor and belowground N pools are shown in Table 3. Smaller roots (<3 mm diameter) were more enriched than larger roots and both showed a slight, but not significantly greater enrichment in the plot exposed to the \( {^{15}\text{NO}_3^-} \) additions (Table 3). The litterfall collected in 2003 was roughly twice as enriched as the existing forest floor litter layer (O1), which was again about twice as enriched as the more decomposed O2 horizon. Roots, which comprised material obtained from all soil horizons, were more

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**Table 2** Aboveground plant part natural abundance and experimental enrichments by species

<table>
<thead>
<tr>
<th>Species/label</th>
<th>Foliage</th>
<th>Branch</th>
<th>Twigs</th>
<th>Stem (recent wood)</th>
<th>Stem (sapwood)</th>
<th>Bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern hemlock</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-2.2 (0.9)</td>
<td>-2.9 (1.0)</td>
<td>-3.9 (1.2)</td>
<td>-4.0 (0.9)</td>
<td>-0.7 (1.3)</td>
<td>-2.2 (1.4)</td>
</tr>
<tr>
<td>( ^{15}\text{NH}_4^+ )</td>
<td>24.1 (2.9)</td>
<td>58.7 (30.5)</td>
<td>40.9 (6.8)</td>
<td>9.5 (4.8)</td>
<td>2.9 (0.7)</td>
<td>1.8 (3.0)</td>
</tr>
<tr>
<td>( ^{15}\text{NO}_3^- )</td>
<td>41.2 (5.9)</td>
<td>49.3 (11.5)</td>
<td>53.8 (9.9)</td>
<td>15.8 (4.3)</td>
<td>5.4 (2.1)</td>
<td>339.3 (71.7)</td>
</tr>
<tr>
<td>Red spruce</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-3.5 (0.8)</td>
<td>-3.5 (0.9)</td>
<td>-6.2 (1.2)</td>
<td>-0.9 (1.8)</td>
<td>-1.9 (1.2)</td>
<td>-3.7 (0.9)</td>
</tr>
<tr>
<td>( ^{15}\text{NH}_4^+ )</td>
<td>17.7 (2.1)</td>
<td>82.0 (38.8)</td>
<td>41.6 (5.2)</td>
<td>5.0 (2.8)</td>
<td>2.0 (2.0)</td>
<td>0.0 (1.7)</td>
</tr>
<tr>
<td>( ^{15}\text{NO}_3^- )</td>
<td>33.5 (3.2)</td>
<td>71.4 (21.4)</td>
<td>49.9 (13.7)</td>
<td>16.3 (6.4)</td>
<td>7.2 (2.1)</td>
<td>43.0 (33.7)</td>
</tr>
<tr>
<td>White pine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.9 (0.8)</td>
<td>-1.2 (1.0)</td>
<td>-1.0 (0.4)</td>
<td>0.5 (1.2)</td>
<td>0.63 (n.a.)</td>
<td>-0.2 (0.1)</td>
</tr>
<tr>
<td>( ^{15}\text{NH}_4^+ )</td>
<td>51.1 (4.8)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>1.7 (n.a.)</td>
<td>4.0 (3.0)</td>
</tr>
<tr>
<td>( ^{15}\text{NO}_3^- )</td>
<td>23.1 (4.5)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>7.9 (5.2)</td>
<td>4.1 (2.1)</td>
<td>17.5 (5.2)</td>
</tr>
<tr>
<td>White cedar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.9 (1.5)</td>
<td>n.d.</td>
<td>-4.1 (0.1)</td>
<td>-2.1 (0.9)</td>
<td>-3.0 (n.a.)</td>
<td>-1.6 (0.9)</td>
</tr>
<tr>
<td>( ^{15}\text{NH}_4^+ )</td>
<td>52.2 (5.8)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>12.5 (n.a.)</td>
<td>3.4 (1.1)</td>
<td>6.5 (9.5)</td>
</tr>
<tr>
<td>( ^{15}\text{NO}_3^- )</td>
<td>31.0 (4.2)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>8.3 (n.a.)</td>
<td>5.5 (3.2)</td>
<td>19.9 (14.0)</td>
</tr>
<tr>
<td>Red maple</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-3.1 (0.6)</td>
<td>n.d.</td>
<td>-1.4 (2.4)</td>
<td>-2.1 (0.3)</td>
<td>0.1 (n.a.)</td>
<td>-2.9 (0.2)</td>
</tr>
<tr>
<td>( ^{15}\text{NH}_4^+ )</td>
<td>24.4 (2.7)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>19.2 (13.0)</td>
<td>7.0 (5.6)</td>
<td>11.5 (2.9)</td>
</tr>
<tr>
<td>( ^{15}\text{NO}_3^- )</td>
<td>115.2 (10.1)</td>
<td>n.d.</td>
<td>n.d.</td>
<td>30.3 (27.6)</td>
<td>23.8 (6.6)</td>
<td>17.0 (4.9)</td>
</tr>
</tbody>
</table>

* Control is the natural abundance of the plant tissue in untreated plots
* n.d. Not determined due to sample loss or insufficient sample
* n.a. not applicable due to too few replicates for error determination

---

![Fig. 1 Enrichment of litterfall, forest floor, and belowground N pools (\( \delta \% \)) receiving either \( ^{15}\text{NH}_4^+ \) or \( ^{15}\text{NO}_3^- \). Average enrichment above untreated plot values (n = 12). Error bars are ±1 SD of the mean. O1 Litter and duff >2 mm, O2 O horizon material <2 mm](image-url)
Table 3  Forest floor and belowground N pools and enrichment

<table>
<thead>
<tr>
<th>Pool</th>
<th>Total N mass a (g N m⁻²)</th>
<th>δ¹⁵N</th>
<th>δ¹⁵N amended b (%)</th>
<th>δ¹⁵N amended c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter layer</td>
<td>31.5</td>
<td>18.4 a</td>
<td>17.2 a</td>
<td></td>
</tr>
<tr>
<td>Litterfall</td>
<td>2.5</td>
<td>62.0 a</td>
<td>46.7 b</td>
<td></td>
</tr>
<tr>
<td>O horizon</td>
<td>153.4</td>
<td>4.3 a</td>
<td>7.6 a</td>
<td></td>
</tr>
<tr>
<td>Mineral horizon</td>
<td>1,010.5</td>
<td>~0</td>
<td>~0</td>
<td></td>
</tr>
<tr>
<td>Coarse roots</td>
<td>5.4</td>
<td>6.9 a</td>
<td>13.1 a</td>
<td></td>
</tr>
<tr>
<td>Fine roots</td>
<td>8.1</td>
<td>10.0 a</td>
<td>22.5 b</td>
<td></td>
</tr>
</tbody>
</table>

Different lowercase letters indicate significant differences in enrichment of the same pool under differing N amendments

Table 4  Flux of experimental ¹⁵N in 2003 litterfall a based on annual and cumulative (2001–2003) ¹⁵N inputs

<table>
<thead>
<tr>
<th>Plot</th>
<th>Recovery b of ¹⁵N based upon annual load (%)</th>
<th>Recovery b of ¹⁵N based upon cumulative load (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹⁵N added as NO₃⁻</td>
<td>4.41</td>
<td>1.47</td>
</tr>
<tr>
<td>¹⁵N added as NH₄⁺</td>
<td>3.43</td>
<td>1.14</td>
</tr>
<tr>
<td>Total litter N flux</td>
<td>7.84</td>
<td>2.61</td>
</tr>
</tbody>
</table>

a Litter included leaves, cones, lichens, branches and other debris

Recovery of ¹⁵N and ecosystem retention

We calculated that inputs of litter N to the forest floor in 2003 accounted for 3–5% of the annual ¹⁵N input (Table 4). However, the ¹⁵N present in litter beyond year 1 was a combination of recent and past-year ¹⁵N fertilization and thus we also present ¹⁵N recovery as a function of the cumulative load of ¹⁵N. When the litter N flux was considered in this way, roughly 1–1.5% of the total experimental ¹⁵N load was lost from the canopy and recovered in litter collected in 2003. The annual flux of ¹⁵N by way of litter was high considering that live foliage prior to autumn 2003 contained only 2.5% of the ¹⁵N added by that date and that most trees in this forest keep needles for several seasons (~3–6 years).

We did not detect any significant differences in 2003 sun or shade foliage, nor in using age classes independently to determine foliar retention of the label (data not shown). About 2.5% of ¹⁵N added to the canopy, the same for both ¹⁵N plots, was retained in the foliage using the age-weighted method (Table 5). After 3 years of ¹⁵N additions, differences we observed initially in the enrichment by age class of needles, emergent needles being more enriched than previous-year needles, was likely masked by uptake and translocation of the label between age classes.

Recovery of ¹⁵N in twigs and branches were considered together as both these woody samples were analyzed with the bark intact and the allometry we used did not allow independent determination of the biomass of these plant parts (Young et al. 1980). We estimated that 23 and 11% of ¹⁵N was retained in these tree parts for ¹⁵NH₄⁺ and ¹⁵NO₃⁻ plots, respectively (Table 5). Twigs and branches proved to be an important sink for N, but for data analyzed by this method ¹⁵N was detected in mineral soils and root enrichment was determined on roots bulked for all depths.
method, it was not possible to determine if the $^{15}$N was localized on or in the bark, in underlying wood, or in both tissues. Additionally, sunlit branches provide habitat for lichens, mosses, and cyanobacteria which may be responsible for some N retention (Lang et al. 1976; Reiners and Olson 1984). Very little (<2%) of the $^{15}$N was found in bark-free bolewood put on during the $^{15}$N additions and summing all bolewood recovery gave an estimate of between 1 and 3% of added N localized in wood of the main stem. When bark samples from the bole of the trees were included in the whole-tree $^{15}$N recovery estimate, $^{15}$NH$_4^+$ recovery changed only slightly but $^{15}$NO$_3^-$ recovery increased from 2 to 47%, and this was due solely to the enrichment of the bark of one tree species, hemlock (Tables 2, 5). Woody materials not directly exposed to the $^{15}$N addition (bole wood and roots) were the least enriched tree parts, and together accounted for less than 5% of ecosystem N recovery. However, exposed needles and leaves also contained very little experimental $^{15}$N.

Summing $^{15}$N retention in all plant parts led to an estimate of 29 and 61% recovery for $^{15}$NH$_4^+$ and $^{15}$NO$_3^-$, respectively (Table 5). Retention in other ecosystem pools (soils) accounted for an additional 9% of $^{15}$NH$_4^+$ and 7% of $^{15}$NO$_3^-$.

Thus, total ecosystem retention was estimated to be 38% of $^{15}$NH$_4^+$ and 67% of $^{15}$NO$_3^-$ added, counter to expectations given our assumption that NO$_3^-$ would be more easily lost from the system via leaching. We estimated gaseous N losses from the canopy (5–10%) on a gross N (but not $^{15}$N) basis, and leaching losses from the B horizon were 2–4% but did not include losses during snowmelt events or by denitrification. Summing $^{15}$N only, we were able to account for 42% of $^{15}$NH$_4^+$ and 69% for $^{15}$NO$_3^-$.  

**Discussion**

The observed low recoveries (Table 5) are typical for larger-scale $^{15}$N additions (Nadelhoffer et al. 1995, 1999a; Bubb et al. 1999; but see Buchmann et al. 1995). Incomplete and even poor recovery is a common feature of many ecosystem $^{15}$N tracer studies and this is often explained by volatilized, unmeasured or incompletely measured fluxes such as denitrification, leaching from the soil, inadequate or absent measurement of spring snowmelt losses (Platek et al. 2005) and photolysis or other light-generated N reactions which could cause loss of NO$_3^-$ (Dominé and Shepson 2002). There are also large uncertainties associated with small enrichments of ecosystems pools with high N content, such as the forest floor, soils and bark (Nadelhoffer et al. 2004). We found that bark and branches were a significant sink for both forms of $^{15}$N (Table 5), but we did not obtain samples from larger branches owing to a need to minimize destructive sampling and keep analytical costs reasonable.

The surprising result of hemlock bark retention of $^{15}$NO$_3^-$ may be due to either adsorption/absorption of incident label at the time of addition or later by way of stemflow or perhaps physiological assimilation and storage within bark (Wetzel and Greenwood 1989). Because we did not separate the outer and inner bark in our analysis, we cannot distinguish among these mechanisms. Few, if any, previous studies on forest floor-applied $^{15}$N have shown large enrichment of bark by $^{15}$N as compared to other plant pools, and rarely has bark been separately analyzed from wood (Bowden et al. 1989; Nadelhoffer et al. 1999b; Templer et al. 2005). We speculate that for hemlock, adsorption of NO$_3^-$ to bark surfaces may account for our observation. Sapwood enrichment indicated substantial movement of recently assimilated N into previously formed wood, and this has been observed by others (Schleppi et al. 1999; Nadelhoffer et al. 1999a).

Other interspecies differences were observed, including higher retention of $^{15}$NO$_3^-$ than $^{15}$NH$_4^+$ in foliage of all species except white pine and white cedar. However, no significant differences among other plant parts of softwood species existed for the two N forms added. Red maple foliage was at least twice as enriched by $^{15}$NO$_3^-$ as foliage of any other species. This observation, along with higher enrichment of red maple stem wood, suggested that hardwood species may be more retentive of deposition N, in either form, than the softwood species which dominate this site.

The low flux of $^{15}$N via the litterfall pathway was consistent with estimates of N recycling efficiency in softwoods, the varying age classes of the species in this forest (with some being deciduous), and the fact that not all litter fell during autumn and may have contained considerable N not translocated prior to litter drop (Cole 1981). Moreover, the estimation of $^{15}$N localized in the canopy was for green foliage and not other plant parts that contribute to litter and to our $^{15}$N recovery estimate.

Some uptake and translocation of the applied label was certain to occur during the 3-year period among N pools with faster turnover. For example, foliage from both deciduous and needle-leaf trees can make substantial inputs to the forest floor compartment on an annual basis. It is also well known that the timing of N additions can vary the N partitioning into different plant pools such as vegetative parts, fruits, or storage pools such as roots and bark (Meyer and Tukey 1965; Pregitzer et al. 1990; Proe and Millard 1995). Adding N several times during the growing season, we reasoned, would better approximate ambient inputs and help to overcome any phenology-based uptake patterns that might obscure longer-term fates using a single pulse type of N addition.
N uptake by an ecosystem can vary for a number of reasons; (1) tree growth rate; (2) the nutrient base in soils supporting growth, including changes in available N; (3) duration of needle retention in coniferous species; (4) the age or “maturity” of the ecosystem; and (5) the efficiency of recycling previously fixed N (N translocation) (Cole 1981). The N demand of coniferous ecosystems is thought to be relatively low owing to lengthy needle retention, efficient N recycling, and the ability of these species to produce large amounts of aboveground biomass per unit N assimilated. Boreal and temperate coniferous forests, for instance, have been shown to have an N demand of 5–50 kg N ha\(^{-1}\) year\(^{-1}\) and can produce 90–130 kg C ha\(^{-1}\) year\(^{-1}\) in aboveground biomass per kilogram of N assimilated (Cole and Rapp 1981). It would seem, therefore, that even low uptake of additional N could have a large C consequence in N-limited but slow-growing ecosystems.

Previous studies have largely applied \(^{15}\)N to juvenile trees or to the forest floor of natural and planted stands. These previous studies reported a moderate-to-high N assimilation potential of foliar-applied N in the case of 6- to 30-year-old conifers (Bowden et al. 1989; Eilers et al. 1992; Vose and Swank 1990), yet \(^{15}\)N applied to the forest floor mostly remained in the litter or in underlying soils (Buchmann et al. 1996; Nadelhoffer et al. 1995, 1999b; Templer et al. 2005; Wright and Tietema 1995). Assuming that mature trees can assimilate N in the same fashion as 10-year-old trees, Eilers et al. (1992) extrapolated that at the mature stand level, Norway spruce might be capable of assimilating 9 kg N ha\(^{-1}\) year\(^{-1}\). This uptake potential could meet 20–100% of annual N demand of conifer ecosystems and lead to a concomitant fixation of 800–1,200 kg C ha\(^{-1}\) year\(^{-1}\) (Cole and Rapp 1981). Data on the C:N ratio and biomass of the bole, branch, and foliar compartments at the Howland Forest (Johnson and Lindberg 1992; this study) provided a means by which to bound the present and potential C storage due to canopy N uptake. The aboveground C:N ratio for sapwood is \(\sim\) 800 at Howland. Taking 0.1–0.5 g N m\(^{-2}\) as the annual growing season canopy N uptake, and the result that 3% of the experimental \(^{15}\)N was associated with the wood fraction, we estimate that C storage of the fertilization was \(~16\pm10~g~C~m^{-2}~year^{-1}\). Because of large plot-to-plot variability in growth increment, we were not able to detect treatment effects of this magnitude over the short duration of this study. Given the observed variance and our sample size, the treatment effect would have to be almost 80 g C m\(^{-2}\) year\(^{-1}\) to be significant. Using eddy flux measurements, we have found (Hollinger et al. 2004) that annual C storage at Howland averaged about 184 ± 33 g C m\(^{-2}\) year\(^{-1}\) for the period from 2001 to 2003, in substantial agreement with the mensuration-based estimate. Thus, based on the \(^{15}\)N results, we predict that annual C storage at Howland would increase by <10% as a result of 18 kg ha\(^{-1}\) N fertilization. Although our study represents only the first few years after increased N deposition, it does not support a recent study inferring a strong increase in C sequestration with higher wet N deposition rates (Magnani et al. 2007).

Conclusion

We measured \(^{15}\)N retention of trees after 3 years of canopy applications to a mature spruce-hemlock stand (mean age \(~\sim\) 140 years). We were able to account for 29 and 61% of a 19.8 kg N ha\(^{-1}\) year\(^{-1}\) addition in above- and belowground plant parts exposed to \(^{15}\)NO\(_3\)\(^{-}\) and \(^{15}\)NH\(_4\)\(^{+}\), respectively. Between 10 and 25% of the \(^{15}\)N retained was in or on twig and branch materials, which constituted 16 and 9% of stand biomass for hemlock and spruce, respectively. It was not possible to confidently infer branch C uptake as a result because bark and wood enrichments were not independently measured for twigs and branches. In the main stem of the trees, however, we were able to separate bark from wood and recovered less than 5% of the \(^{15}\)N in bolewood but recovered 45% of \(^{15}\)NO\(_3\)\(^{-}\) in or on hemlock bark. This suggested that physico-chemical interactions with plant surfaces could be the predominant pathway for canopy N retention and that there might be little short-term C uptake as a result. Using only that \(^{15}\)N recovered in recent wood growth and stand allometry, we were able to put an upper limit on the potential for C sequestration due to fertilization; approximately 16 g C m\(^{-2}\) year\(^{-1}\), which was <10% of annual net C storage for this site. Other major \(^{15}\)N sinks included the forest floor and humus layer, which retained 3–8 and 1–4%, respectively. Regardless, plants and not soils were the most important short-term sink for canopy-added \(^{15}\)N, which was contrary to most studies wherein \(^{15}\)N was applied directly to the forest floor. However, this may not be a result of plant uptake and use of this additional N. Instead it appears that in the short term, most of the N was retained on plant surfaces; branches and main-stem bark, with little being assimilated into woody material and little effect upon C sequestration.

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References


