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Long-term movement of ^{15}N tracers into fine woody debris under chronically elevated N inputs

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Abstract

Two key questions in the study of large-scale C (carbon) and N (nitrogen) cycling in temperate forests are how N cycling in soil detritus controls ecosystem-level retention of elevated N deposition, and whether elevated N deposition is likely to cause increases in C pools. The large C:N ratios in woody detritus make it a potentially important contributor to N retention, if N immobilization increases, and a potentially important contributor to C sequestration, if pool sizes increase. We studied N concentrations, C:N ratios, and pool sizes of N and biomass in fine woody debris (FWD $<$ 5 cm diam.) 12 years into a long-term N-amendment study in two contrasting forests, a naturally-regenerated forest dominated by *Quercus* spp., and a 63-yr old plantation of *Pinus resinosa*. We also quantitatively recovered ^{15}N tracers (originally applied as $^{15}NH_4$ and $^{15}NO_3$) in FWD, eight years following their application in the same study, in both ambient and N-amended plots. We used these data to test predictions of tracer redistributions made by a biogeochemical process model that included ^{15}N. Results from the N pool-size analysis and the ^{15}N tracer-recovery analysis indicated that under elevated N inputs of 5 g N m^{-2} yr^{-1} (as NH_4NO_3) over the decadal time period, only 0.15%–0.76% of the elevated N inputs were recovered in FWD of N-amended plots relative to ambient. Any increase in N immobilization in wood appeared to be minimal, in agreement with model predictions. Under N amendments, pool sizes of C in FWD were not significantly different from ambient, whereas pool sizes of N were marginally higher. Patterns of $^{15}NH_4$ vs. $^{15}NO_3$ recovery, treatment differences, and forest-type differences suggested that plant uptake, rather than detrital immobilization, was the dominant mechanism of ^{15}N tracer movement into FWD. This result indicates that plant-soil cycling operating over a decadal time scale or longer controls C:N ratios and N pool sizes in woody debris.

Introduction

To facilitate the development of a predictive understanding of ecosystem responses to elevated nitrogen inputs, several large-scale manipulations of nitrogen inputs to forests are under way in temperate forests in North America and Europe. The forest floor (the surface organic layer, or O horizon of temperate-forest soil) has emerged a key ecosystem component in these studies of C and N cycling. Forest floors have shown high rates of retention of ^{15}N labels (Nadelhoffer et al., 1999a; Tietema et al., 1998), and C:N ratios in the forest floor have been found to correlate closely with rates of net nitrification and with whole-system aspects of N cycling and retention such as nitrate leaching (Currie 1999; Goodale and Aber 2001; Gundersen et al., 1998, 1999; McNulty et al., 1991).

The extent to which fine woody debris (FWD) contributes to N retention in the forest floor under elevated N inputs is largely unknown. FWD above a certain size class (0.5 cm, for example) is often excluded by seiving from studies of forest N stocks and dynamics. Two very different mechanisms are plausible for increased N retention in woody debris under increased N inputs.

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First, N immobilization in FWD would provide a direct mechanism for increased N retention in the forest floor. Woody debris has higher C:N ratios than other litter, but whether woody debris immobilizes N as it decays is a matter of current debate (Alban and Pastor, 1993; Chueng and Brown, 1995; Krankina et al., 1999). Second, any increase in N uptake and the flux of N in woody litter could provide an ecosystem-level mechanism of N retention.

The extent to which changes in stocks of FWD could potentially have an impact on changes in forest C budgets under elevated N deposition is also largely unknown. To some extent, elevated N deposition in temperate forests of North America and Europe may result in increased sequestration of atmospheric C (Nadelhoffer et al. 1999b; Townsend et al. 1996). To address this, it is critical to know which ecosystem components are responsible for retention of N deposition, what the C:N stoichiometries of those components are, and in cases where C:N ratios become narrower under N deposition, the extent to which this is due to increases in C pool sizes. Woody tissue (living and dead) is key because C:N ratios are much higher than in other ecosystem components such as foliage, fine roots, or soil organic matter.

The Chronic N Study at the Harvard Forest in central MA, designed to test hypotheses concerning ecosystem responses to chronically-elevated N inputs (Aber et al., 1993, Magill et al., 2000), provided a framework for us to study responses in woody-debris C and N pools. Experimentally-elevated inputs of N have been made in two forests at this site since 1988. Here we report N concentrations, C:N ratios, and pool sizes of N and biomass in FWD on the forest floor 12 years into the study. We also report the recovery and analysis of ^{15}N tracers in FWD eight years following their application to the soil surface. Recovery of the tracers eight years after their application allowed us to examine the net effects of a number of processes: Initial plant-soil partitioning, plant uptake, woody litter production, and N dynamics in FWD on the forest floor for several years.

To our knowledge, the present analysis is the first study of woody debris under large-scale N amendments over a decadal time scale. We had three main objectives: (1) We sought to determine whether the 12 years of N amendments have altered N concentrations or C and N pool sizes in the finer size classes of downed woody debris. (2) We sought to quantify the recovery of eight-year old ^{15}N tracers (originally applied as $^{15}NH_4$ and $^{15}NO_3$), under both ambient and N-amended conditions, in the finer size classes of woody debris. (3) We used the field results to make a direct test of model predictions. Previously, we used a biogeochemical process model, TRACE (Tracer Redistributions Among Compartments in Ecosystems), to predict ^{15}N recoveries in woody debris that would result from the multiple mechanisms controlling N retention and partitioning among living and dead biomass at this site (Currie and Nadelhoffer, 1999).

Methods

Site description

The Harvard Forest is a site in the Long-Term Ecological Research (LTER) network sponsored by the US National Science Foundation. Elevations of experimental plots in the Prospect Hill tract studied here are 370–390 m; monthly mean temperatures are −7°C in January and 19°C in July. Precipitation averages 110 cm/yr, distributed fairly evenly throughout the year (Van Cleve and Martin, 1991). We worked in the two forest stands that are part of the ongoing Chronic N study (Aber et al., 1993, Magill et al., 1997). The first is an even-aged red pine (*Pinus resinosa* Ait.) stand planted in 1926 on land that was previously pastured and cultivated (Motzkin et al., 1999). The second is a predominantly oak (*Quercus velutina* Lam., *Q rubra* L., *Betula lenta* L., *Acer rubrum* L.) stand. The mixed oak forest is on land that was pastured in the 19th century, reverted to forest by 1860, has regenerated naturally from a mix of disturbances since, and is regionally representative (Aber et al., 1993; Foster 1992; Foster et al., 1992; Motzkin et al., 1999). Soils in both forest stands are coarse-loamy, mixed, frigid Typic Dystrochrepts. Soils are well drained and contain well-defined O horizons (mor type).

Experiment design and background

In each forest there are four, 30 × 30 m experimental plots: a reference plot, a low-N amendment plot, a high-N amendment plot, and a N + S (nitrogen plus sulfur) amendment plot. The reference plots (which we refer to here as the ambient plots) receive ambient wet deposition of NH_4^+, NO_3^-, and DON (dissolved organic N) totaling 0.9 g N m^{-2} yr^{-1} (Currie et al., 1996). The low-N and high-N plots receive ambient deposition plus 5 or 15 g N $m^{-2}yr^{-1}$ (respectively) as NH_4NO_3. The N + S plots receive ambient deposition plus 5 g N $m^{-2}yr^{-1}$ as NH_4NO_3 together with 7.4 g S $m^{-2}yr^{-1}$ as $NaSO_4$. Amendments have been sprayed onto the forest floors in six equal applications per year, April through September, each year since 1988. Each 30 × 30 m treatment plot is divided into 36, 5 × 5 m subplots.

Here we consider only ambient and low-N plots, because only these plots were labelled with ^{15}N tracers. Enriched ^{15}N tracers were applied in 1991 and 1992 in both forest types; the midpoint of tracer applications was in late 1991. The isotopic composition of N fertilizers added to treatment plots in years other than 1991–1992 was similar to that of atmospheric N_2 (0.3663 atom% ^{15}N, or, $\delta^{15}N = 0$ ‰). During 1991 and 1992, either $^{15}NH_4^+$ or $^{15}NO_3^-$ was added to separate 15 × 30 m halves of ambient and low-N plots on the same days fertilizers were applied to treated plots. Ambient plot halves received either 17.9 mg ^{15}N m^{-2} yr^{-1} as dissolved $^{15}NH_4Cl$ (99.1 atom% ^{15}N) or 16.2 mg ^{15}N m^{-2} yr^{-1} as dissolved $K^{15}NO_3$ (98.6 atom% ^{15}N). The same amounts of either $^{15}NH_4$ or $^{15}NO_3$ were added to low-N plots by dissolving either $^{15}NH_4Cl$ or $K^{15}NO_3$ into the NH_4NO_3 solutions used for fertilizing low-N plot halves. This increased the $\delta^{15}N$ value of the NH_4^+ion on one half of each low-N plot from 0 to 965‰ (or from 0.3663 to 0.7173 atom% ^{15}N) and the $\delta^{15}N$ value of the NO_3^- ion added to the other half from 0 to 761‰ (or 0.6433 atom% ^{15}N).

The four contiguous, internal, 5 × 5 m subplots within each labeled plot half are considered 'isotope-internal subplots' (Nadelhoffer et al. 1999a). Samples for woody detritus ^{15}N analysis were collected from these isotope-internal subplots.

Quantification of woody debris

We used quadrats to quantify FWD biomass. In each forest type we marked out three 2.5 × 2.5 m quadrats at random within the ambient plots and three within the low-N plots (12 total). We separated FWD into three size classes based on diameter: 0.5 to < 1.0 cm, 1.0 to < 2.5 cm, and 2.5 to < 5.0 cm. Within the entire area of each quadrat we quantified woody debris in size classes from 1.0 cm to < 5.0 cm. We used two nested, 1 × 1 m sub-quadrats (established at random within each quadrat, 24 total) to quantify debris in the finest size class, 0.5 cm to < 1.0 cm. Some authors, ourselves included, define size classes up to 10 cm diameter as FWD (Currie and Nadelhoffer in review; Harmon and Sexton, 1996). However, given the limited size of our N-amended and ^{15}N-labelled plots, together with the fact that spatial heterogeneities in distributions of woody detritus increase with size, we omitted size classes > 5 cm diameter from the present study.

We classified debris into decay classes, with the number of decay classes varying by size class (Polit and Brown, 1996). The largest size class was separated into sound, intermediate, and rotten categories; finer size classes had fewer decay categories. We determined decay class by the presence of bark and branches, the degree of sapwood degradation, soundness of the heartwood, and the presence of moss and fungi (Polit and Brown 1996; Sollins 1982).

We included woody debris resting on the forest floor and in the upper litter layer (Oi horizon). To avoid the disturbance of tearing roots or disrupting the soil structure on these permanent plots, we did not dig into the deeper horizons of the forest floor. We also excluded live wood as well as dead wood attached or suspended above the ground. All pieces of downed, dead wood were cut where they crossed quadrat or sub-quadrat edges; these were sorted and weighed in the field (to 1 g) in each combination of size and decay class. Pieces of sloughed bark were included where they appeared to be from wood < 5 cm diam. After weighing, detritus was returned to the experimental plots.

Collection of samples

Representative samples of FWD used for field-moist to dry-mass conversion factors and for chemical analyses were collected from the 5 × 20 m isotope internal area in each ^{15}N-labelled area. Each sample consisted of a complete cylindrical piece of wood, including bark when present. From ambient and low-N plots in each forest, samples were taken from each combination of size and decay class. One set of three replicate samples from each size-decay combination was taken from plot halves (15 × 30 m) that had received $^{15}NO_3$ enrichment, while the other set of three replicates was taken from plot halves that had received $^{15}NH_4$ enrichment. In three cases, it was not possible to find three replicates in the 'sound' category in the 2.5–5.0 cm size class, so two replicates were used. Overall, 141 samples were collected from ^{15}N-enriched plots.

Additional samples were collected for determination of natural-abundance levels of ^{15}N in this material. For ambient conditions, we analyzed samples of

FWD collected in a concurrent study that employed transects well outside of the treatment plots. From each forest, 16 samples of FWD were analyzed for δ^{15}N, in all size-decay combinations. For N-amended conditions, we collected samples of FWD from N + S plots (which had received N amendments but no isotopic labeling). We analyzed 13 samples from the pine forest and 16 from the oak forest.

Sample preparation and chemical analysis

Samples were taken to Appalachian Laboratory in Frostburg, MD for analysis of C and N content. Samples were dried to constant mass at 70°C (subsamples of 2–15 g, depending on size class, were used to determine corrections from field-moist masses to 70°C mass, and applied to masses determined in the field). Samples were ground to #20 mesh in a Wiley Mill, stirred well, subsampled (200 mg) for further grinding to a fine powder in a jar mill, and tested to pass through a #100 mesh sieve. A 100-mesh grind (150 μm) is finer than sometimes recommended for soil fractions (cf. Boone et al., 1999). In wood, however, where N concentrations are as low as 0.1% and where analytical samples are as small as 10 mg, we found the 100-mesh grind to produce more reliable results, in agreement with Nelson and Sommers (1996). After grinding, samples were again dried to 70°C and cooled in a desiccator. We determined C and N concentrations by dry combustion on a Carlo-Erba NC 2100. In triplicate analyses of samples chosen at random, differences between the highest and lowest values, expressed as a percentage of mean values, ranged from 0.4 to 0.8% for C, and from 0.5 to 6.6% for N. Isotopic ratios were determined on two mass spectrometers at the Marine Biological Laboratory, Woods Hole, MA. We used a Europa 20/20 mass spectrometer for ^{15}N-enriched samples; duplicate values of δ^{15}N (run on 12% of samples) averaged ± 0.49‰. We used a Finnegan delta-S mass spectrometer for natural-abundance ^{15}N samples. Typical reproducibility among duplicates on this instrument is 0.2–0.3‰ δ^{15}N. Dry weight corrections (105° C) were performed on all samples individually; we report all results here for biomass, C, N, and ^{15}N on a dry weight, ash-included basis.

Data analyses

Statistics

Values of detrital biomass summed over size-decay classes by quadrat were tested and found to be normally distributed. For values of C concentration, N concentration, and mass ratios of C:N, log transformations were used to obtain normal distributions. Analyses of Variance (ANOVA) were used to test effects of forest type and N treatment on detrital biomass pools, C concentrations, N concentrations, and C:N ratios.

Recoveries of ^{15}N labels

We use the standard delta notation to express the atom% ^{15}N in field samples as the per mil deviation from the atmospheric standard:

$$\delta^{15}\mathrm{N} = \left[\frac{\mathrm{atom\%}^{15}\mathrm{N}_{\mathrm{sample}}}{0.3663} - 1\right] \times 1000 \qquad (1)$$

To compare ^{15}N recoveries among treatments in which ^{15}N enrichments differed, and to facilitate comparisons with recoveries in other ecosystem pools and with the results of other studies, we calculated percent recoveries of ^{15}N tracers. We refer to this quantity as PR^{15}N, calculated as follows (Currie and Nadelhoffer, 1999):

$$PR^{15}\mathrm{N}(C_i, t) = \frac{N_{Ci}(t)(\mathrm{atom\%}^{15}\mathrm{N}_{\mathrm{Ci}}(\mathrm{t}) - \mathrm{atom\%}^{15}\mathrm{N}_{\mathrm{b}})}{A(t-t_0)(\mathrm{atom\%}^{15}\mathrm{N}_{\mathrm{a}} - \mathrm{atom\%}^{15}\mathrm{N}_{\mathrm{b}})}, \qquad (2)$$

where C_i is an ecosystem compartment, N_{Ci} (t) is the amount of N [g/m^2] in C_i at time t, $A(t\text{-}t_0)$ is the sum of N amendments [g/m^2] to time t, and the ‘a’ subscript denotes amendment, ‘b’ denotes background. Because PR^{15}N is a quantity derived through a complex conceptual model, we report statistical analyses and tests on the underlying data, but not on quantities of PR^{15}N themselves.

Results

Detrital biomass, C, and N

We observed no significant differences in overall FWD mass between forest types or N treatments (Figure 1; two-way ANOVA over all data with forest type and N treatment as categorical variables). Mean values of summed detrital masses were higher on N-amended plots, but variances were large and differences were not statistically significant. Further, the two variables of forest type and N treatment together explained only 20% of the variance in summed detrital masses, leaving 80% of the variance to be attributed to random

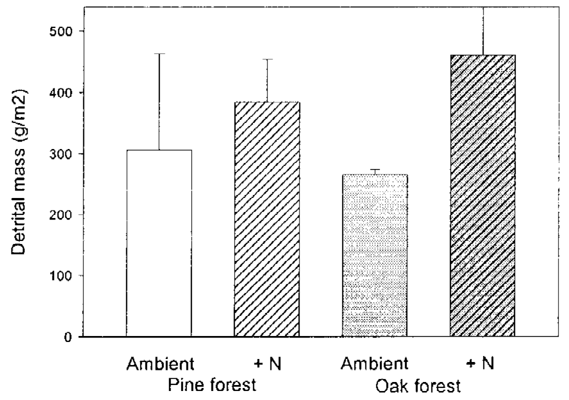

Figure 1. Detrital biomass of fine woody debris, summed across all size-decay classes, within each forest type and N treatment. Means and standard errors are shown for sets of three quadrats studied in each combination of forest and N treatment. Values are expressed on an oven-dry weight (105°C) basis. 'Ambient' and '+ N' are as in Figure 1. Analysis of variance indicated no significant differences between forests or N treatments.

variation. For use in subsequent calculations of N pools and $PR^{15}N$, within each forest we averaged detrital masses across N treatments. We took this step to avoid overestimating changes in N pool sizes as a result of the non-significant trends of higher detrital masses in N-amended plots.

Differences in N concentrations, C concentrations, and C:N ratios in FWD were all found to be significantly related to forest type (Figure 2). Concentrations of N were higher in the oak forest, while concentrations of C were slightly lower (as percent C, overall mean ± SE of 50.7 ± 0.2 *versus* 51.9 ± 0.3 in the pine forest) and C:N ratios were lower. Concentrations of N and C:N ratios were both found to be related to levels of N amendment at only marginal levels of significance ($p = 0.052$ and $p = 0.051$, respectively; two-way ANOVA over all data with forest type and N treatment as categorical variables). Samples from N-amended plots had marginally higher N concentrations and lower C:N ratios than samples from ambient plots (Figure 2). In each of these cases (N concentrations and C:N ratios), the statistical model using forest type and N treatment together explained < 15% of the variance in the data. Thus, although forest type and N treatment were significant, these effects were small compared to overall random variation among the pieces of woody debris.

We calculated differences in N pool sizes in FWD likely to have resulted from the 12 years of N amendments. In calculating pool sizes of N by size and decay class, we used detrital masses combined from the present study and from a separate, concurrent study. The concurrent study was designed to relate land use history to pool sizes of mass, C, and N in woody debris at larger scales, using 100 m transects and including coarse woody debris in much larger size classes (Currie and Nadelhoffer, in review). We incorporated data from the transect study here for size classes < 5 cm diameter. This produced more robust results by combining plot-sampling methods with line-intercept methods (Polit and Brown, 1996). The concurrent study also included woody detritus buried in the forest O horizon, which in the transect study was excavated to the upper mineral soil. To the means of unburied masses combined from the present and the transect studies, we added masses of buried material by size and decay class from the transect study. We then multiplied mean N concentrations for each forest type, N treatment, and size-decay class, by the appropriate detrital masses to determine sizes of N pools by forest, treatment, and size-decay class. We summed the resulting N pools across the six size-decay categories and calculated differences in these sums between ambient and low-N treatments (Table 1). We then calculated the changes in these pool sizes as percentages of the cumulative N amendments between the start of the study and our 1999 sampling (3.8 g N m^{-2} yr^{-1} in 1988 plus 10.5 subsequent years at 5 g N m^{-2} yr^{-1} = 56.3 g N m^{-2}). Because this calculation focussed on differences between ambient and N-amended plots, it did not include ambient N inputs, which we consider equal among treatment plots and forest types.

Isotope recoveries

Natural-abundance values of $\delta^{15}N$ in FWD, averaged over each forest type, ranged from −3.1 to −1.6‰ under ambient conditions and from −2.3 to −0.7‰ under N-amended conditions (Table 2). Most of the samples we collected on enriched plots had $\delta^{15}N$ values between 0 and +30 ‰, with some values above and below. Two $\delta^{15}N$ values, +96 and +200‰, stood out as being much higher than the others. Both of these were samples from ambient plots, one in the oak and one in the pine forest. These high $\delta^{15}N$ values propagated through our analyses of percent recoveries of ^{15}N tracers.

Even though there was substantial heterogeneity in the $\delta^{15}N$ values from piece to piece of woody detritus, three patterns emerged in the values of percent recovery of ^{15}N tracers. When expressed by individual size-decay classes, values of $PR^{15}N$ tended to be

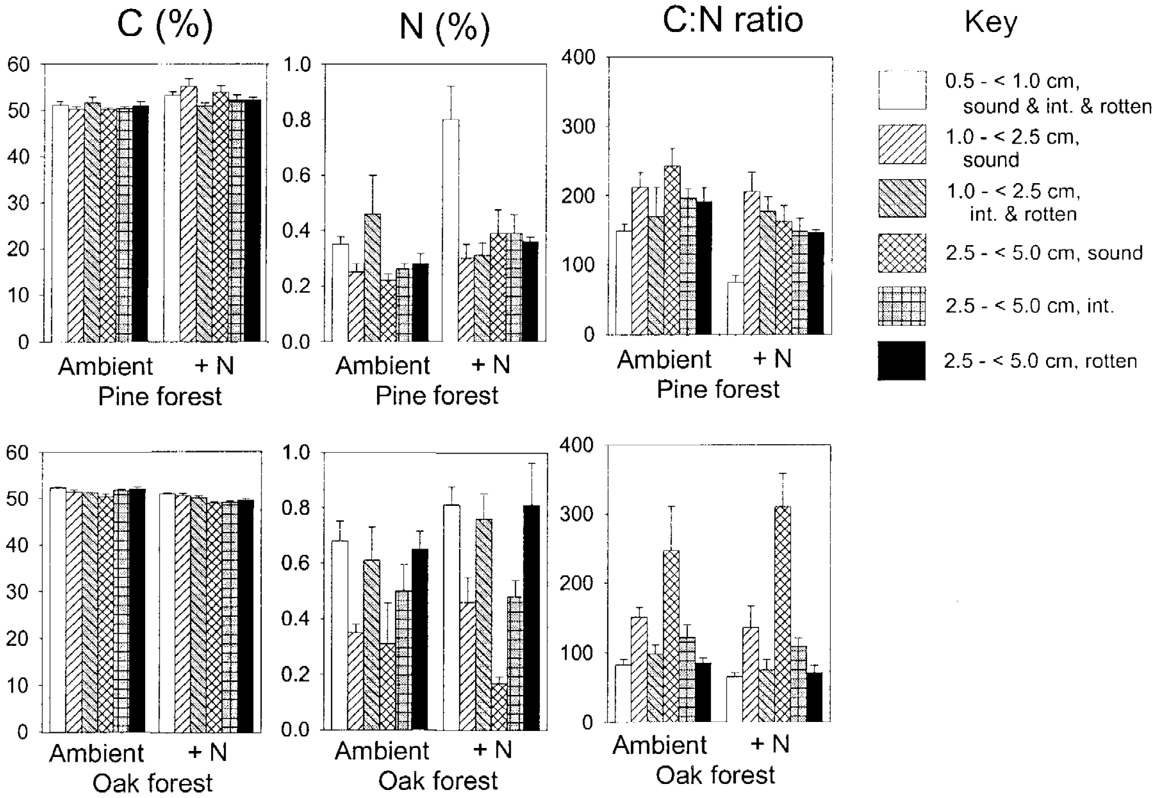

Figure 2. Chemistry of fine woody debris in size-decay classes. Means and standard errors are shown; values are expressed on an oven-dry weight (105 °C), ash-free basis. 'Ambient' refers to untreated plots, while '+ N' refers to plots that have received ambient N deposition plus 5 g N m^{-2} yr^{-1} since 1988. C:N ratio is calculated as a mass ratio. Sound, int., and rotten refer to decay classes, where 'int.' = intermediate stage of decay.

greater in N-amended plots relative to ambient. Tracer recoveries tended to be greater in the oak forest relative to the pine, particularly under N amendments, but also under ambient conditions (Figure 3). Summation over the size-decay classes, by form of original tracer, level of N amendment, and forest type (Figure 4) accentuated most of the patterns that were evident in the isotopic recoveries in separate size-decay classes (Figure 3). The exception was in the recovery of $^{15}NH_4$ under ambient conditions in the pine forest, in which the single highest value of $\delta^{15}N$ we obtained (200‰ in the pine ambient $^{15}NH_4$ case) propagated through the analysis. An additional pattern that emerged in the summed values of $PR^{15}N$ was, in the N-amended case, a greater recovery of $^{15}NO_3$ tracers relative to $^{15}NH_4$ tracers (Figure 4).

The percent recoveries of eight-year old ^{15}N tracers in FWD reinforced our finding, based on differences in N pool sizes (Table 1), of low percentage recoveries of experimental N amendments in FWD. Subtracting the $PR^{15}N$ values in ambient plots from those in N-amendment plots, the tracer-recovery analysis shows differences of 0.15% (pine forest) and 0.25% (oak forest) of the ^{15}N label in each case.

Discussion

Interpretation of N pool sizes and isotope recoveries

We are limited in our interpretations of ^{15}N tracer recoveries in two ways. First, our data do not address dynamics in ^{15}N storage in FWD over the eight-year period following tracer applications, because we had only one post-tracer sampling date, in 1999. We are able to compare tracer recoveries in FWD against natural-abundance $\delta^{15}N$ in areas where tracers were not used. For this reason, we focus on differences among plots and treatments: $^{15}NO_3$ vs. $^{15}NH_4$ labelled subplots, the oak forest vs. the pine forest, and ambient vs. N-amended treatments.

Table 1. Pool sizes of N in fine woody debris (g N m^{-2}) in ambient and N-amended plots. Treatment (+N) consisted of 5 g N m^{-2} yr^{-1} above ambient N inputs, applied each year beginning 1988. 'Treatment retention' is the percentage of cumulative, artificial N amendments since 1988 (56.3 g N m^{-2}) that could be accounted for in the increase in N storage in downed, fine woody debris < 5 cm diam.

Size class	Pine forest			Oak forest		
	Ambient	+ N	Treatment retention (%)	Ambient	+ N	Treatment retention (%)
0.5 to < 1.0 cm	0.265	0.605		0.429	0.511	
1.0 to < 2.5 cm	0.845	0.625		0.823	1.033	
2.5 to < 5.0 cm	0.515	0.723		1.314	1.455	
Sum	1.63	1.95	0.57%	2.57	3.00	0.76%

Table 2. ^{15}N natural abundances in fine woody debris, under ambient and N-amended conditions in pine and oak forests. Samples for ^{15}N natural abundance determinations were taken either from non-labeled transects in reference areas (ambient) or from non-labeled plots fertilized with 5 g N m^{-2} yr^{-1} each year from 1988 through 1999 (+ N). Means of $\delta^{15}N$ values (‰) ± 1 SE are shown for each forest type and N loading

Forest type	Ambient	+ N
Pine forest	−1.6 ± 0.2 (n = 16)	−0.7 ± 0.2 (n = 13)
Oak forest	−3.1 ± 0.2 (n = 16)	−2.3 ± 0.1 (n = 16)

The second limitation arises because we avoided any excavation of the deep forest floor (Oa horizon) for sample collection. Even so, some of the FWD we collected in 1999 was likely to have been present on the forest floor when the labeling took place in 1991 and 1992. We did sample throughout and beneath layers of fresh litter and litter accumulated over several years, through the upper Oe horizon. In addition, because FWD is irregularly shaped, curved, and branched, parts often protrude or are held slightly above the surface. Leaves and needles falling on such material, or on larger branches, often accumulates around rather than atop the FWD. We believe the occurrence of the two exceptionally high values of $\delta^{15}N$ we observed (+96 and +200‰) was most likely due to ^{15}N tracers that were incorporated into woody debris on the forest floor, possibly during applications in 1991–1992, because no living tree tissue here has been measured with such a high value of $\delta^{15}N$ (Nadelhoffer et al., 1999a). At the same time, our sampling design was likely to miss some proportion of the ^{15}N tracers on buried woody debris in smaller size classes. Thus, our calculations of percent recoveries of ^{15}N tracers may underestimate the importance of immobilization of ^{15}N in the years immediately following ^{15}N application.

Our interpretations of differences in detrital masses and pool sizes of C and N (independent of ^{15}N labels) are much less affected by the second limitation highlighted above. The N amendments (as NH_4NO_3) were made each year from 1988, up to and including 1999 and beyond. If a high amount of NH_4^+ or NO_3^- from N amendments were immobilized or otherwise incorporated rapidly in FWD, this would have been evident in our samples as differences in N concentrations or C:N ratios between ambient and N-amended plots. We tested these differences, finding them to be minor, of marginal significance, and explaining a very low percentage of variance (< 15%) in the data. Our calculations of differences in N recoveries in FWD among treatment plots, as percentages of N amendments over the 12-year period (Table 1), showed slightly higher recoveries of N amendments in FWD than did our calculations based on ^{15}N recoveries. Together, our two methods of assessment indicate that any elevated N immobilization in FWD in N-amended plots over the decadal time period was minor.

A concept frequently used in the interpretation of litter N dynamics is the critical C:N ratio (Aber et al., 1990; Pastor and Post, 1986). Above this ratio, detritus immobilizes N until the critical C:N ratio is reached through the combined processes of N immobilization and C mineralization. Partly decayed litter with a C:N ratio below the critical ratio exhibits net mineralization of N. Some studies have suggested that the critical C:N ratio is much higher for woody debris than for other detritus. Values of the critical C:N ratio may be

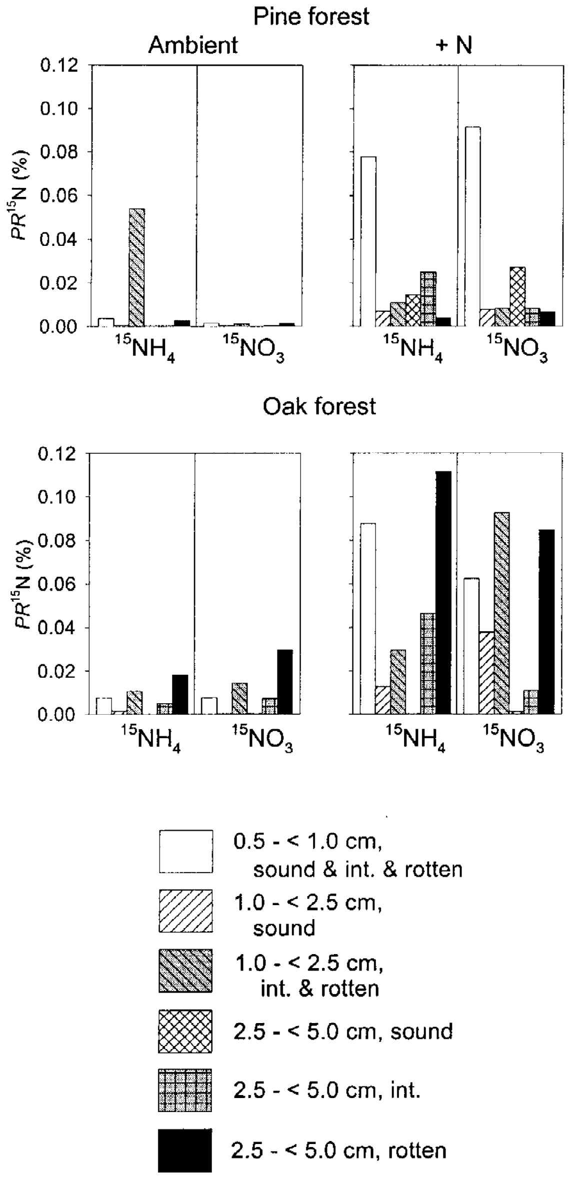

Figure 3. Percent recoveries of ^{15}N tracers ($PR^{15}N$) in size-decay classes of fine woody debris. The form of isotopic tracer listed ($^{15}NH_4$ or $^{15}NO_3$) is the form applied in 1991-1992 in 15 × 30 M isotopic tracer plots. Sound, int., and rotten refer to decay classes, where 'int.' = intermediate stage of decay.

over 100:1, or even over 200:1 for some tree species (Chueng and Brown, 1995; Edmonds, 1987). If so, this would be consistent with minimal rates of N immobilization at the C:N ratios we observed. We found C:N ratios in FWD of both forests to fall largely below 200:1, and in most detrital categories of the oak forest, below 100:1.

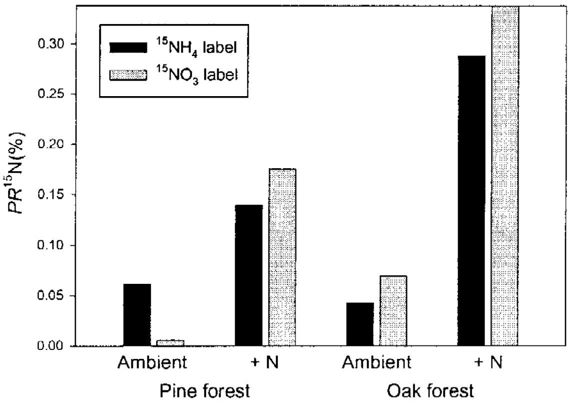

Figure 4. Summed percent recoveries of ^{15}N tracers ($PR^{15}N$) over all size-decay classes in the current study. N treatments, isotopic tracer treatments, and size / decay classes are as in Figure 3.

Interpretation of patterns in FWD mass

Based on our data, we cannot reject the null hypothesis that pools of FWD biomass showed no differences between ambient and N-amended plots after 12 yr of N additions. Both forests showed trends, though insignificant, toward increased FWD biomass under elevated N inputs. The apparent trend in the oak forest (Figure 1) owes much to the low mean value of FWD mass observed in the ambient treatment. This mean value was not verified when compared against the result (446 g m^{-2}) from our concurrent study, which used a line-intercept method along 100 m transects in the same forest stands (Currie and Nadelhoffer, in review). Because of the length and separation of the transects this could only be done under ambient conditions, disallowing a test of ambient vs. N-amendment differences, but allowing an independent assessment of woody biomass pools under ambient conditions.

Model-data comparisons

Some of our results allow tests, to varying degrees, of predictions made by the TRACE model. First, our finding that ambient and treatment-plot differences in net immobilization of N in FWD were minimal are consistent with our expectations embodied in the model structure. In the model, there was no net immobilization of N in woody debris; prior to the incorporation of well-decayed wood into humus, in the model, the pool of recognizable woody debris simply conserves N in woody detritus as C is mineralized. The prediction that net immobilization of N in recognizable FWD would be minimal appears to be in

Table 3. TRACE model predictions of percent recovery of ^{15}N labels in fine woody debris < 5 cm (Currie and Nadelhoffer 1999). Values are PR^{15}N expressed as percent (%).

Treatment	Pine forest		Oak forest	
	$^{15}NO_3$ label	$^{15}NH_4$ label	$^{15}NO_3$ label	$^{15}NH_4$ label
Ambient	0.16	0.70	0.71	2.20
Low-N	0.036	0.069	0.16	0.26

general agreement with our field data. Complete lack of net N immobilization in FWD, however, may be an underestimate, because the marginal differences in N concentrations that we observed may have resulted from minor increases in rates of N immobilization in N-amended treatments. Given our current data this is difficult to establish clearly, because the marginally higher N concentrations in FWD in N-amended plots could have arisen from marginally higher N concentrations in woody litterfall. At present, we have no information on N concentrations in woody litterfall.

We developed TRACE as a tool to interpret ^{15}N partitioning and redistribution (Currie et al., 1999). Prior to this field study, we published model predictions of PR^{15}N values in woody debris at the Harvard Forest (Currie and Nadelhoffer, 1999). Our calculated values of PR^{15}N in woody debris allow a test, though an imperfect test, of TRACE predictions. The model predicted PR^{15}N summed over all size classes of woody detritus, including coarse woody debris. To calculate the expected values of PR^{15}N in FWD $<$ 5 cm diameter, we multiplied the model predictions for 1999 PR^{15}N in overall woody debris by the fractions of the overall N pool in woody debris that is comprised by the N pool size in FWD $<$ 5 cm. These fractions are 0.119 for the pine and 0.154 for the oak forest; the bulk of the woody-debris N pool is in larger, well-decayed logs (Currie and Nadelhoffer, in review). Thus adjusted, model predictions for PR^{15}N in FWD $<$ 5 cm ranged from 0.036% to 2.20% for different combinations of forest, treatment, and form of ^{15}N label (Table 3). In general, predicted ^{15}N recoveries in FWD were somewhat higher than the observed values (Figure 4).

Apart from inaccuracies in modeled processes of N cycling, there are two additional factors that make these model-data comparisons of ^{15}N recoveries imperfect comparisons. Both would tend to cause our field-study results to be somewhat lower than TRACE predictions. The first was noted above: Some FWD on the forest floor in 1991–1992 may have become buried deep in the Oa horizons after immobilizing some ^{15}N tracers. The second factor is that TRACE models a homogeneous spatial area with no horizontal loss of ^{15}N. Since the sizes of our isotope-internal areas (5×20 m) were narrower than tree canopies, it is likely that some ^{15}N was carried horizontally out of our plots via woody litterfall. This, too, would cause our field-based calculations of PR^{15}N to be slight underestimates. Together, these factors limit the use of these model-data comparisons to make definite conclusions regarding the mechanisms of N partitioning and N dynamics. Still, we suggest that it is valuable to compare field results with model predictions if the limitations are recognized, and we suggest that learning to make improved model-data comparisons of this nature is an important direction for research.

TRACE embodied an expectation of gross N exchanges between woody detritus and pools of soil available N that appear too high when model predictions are compared against our field results. One of the powerful aspects of using ^{15}N in field studies lies in the sensitivity of δ^{15}N values to gross rates of N cycling; gross N exchanges can cause ^{15}N labels to move among N pools at different rates than net flows of N. In trace, while woody detritus had zero net immobilization of N, the N contained in woody debris still underwent nonzero gross exchanges with exogenous N pools. We had structured the model this way based on the findings of Downs et al. (1996), in which wood chips appeared to immobilize ^{15}N tracers in a particular year (the fourth year of the study) even when net N immobilization was zero. Thus, in model simulations, gross exchanges of N – simultaneous mineralization and immobilization of N, even while net N immobilization was zero – was the main process causing ^{15}N to appear in woody detritus. Since the model over-predicted ^{15}N recoveries in FWD, it appears that TRACE over-predicted gross N exchanges between FWD and pools of available N in soil.

A key set of model-data comparisons to make is to compare patterns of PR^{15}N values among treatments, isotope forms, and forest types. First, TRACE predicted greater PR^{15}N for $^{15}NH_4$ labels relative to $^{15}NO_3$ (Table 3) due to greater gross immobilization of NH_4^+ vs. NO_3^- in the model. Second, TRACE predicted higher ^{15}N recovery in FWD in ambient plots relative to fertilized (Table 3). The mechanism giving rise to both of these patterns in the model was gross exchange of N. The first pattern occurred because gross immobilization in the model had a preference for NH_4^+ (Currie et al., 1999); the second occurred because soil N inputs were lower in ambient plots, making the atom% ^{15}N values of the labels there greater, causing the model to predict proportionally greater ^{15}N immobilization per unit of gross N immobilization. These patterns are important to note because they both disagreed with patterns in the field recoveries of ^{15}N. As noted above, however, these conclusions and patterns in the field data must be interpreted with caution, because some FWD that retained ^{15}N tracers may have been buried in deep Oa horizons and excluded from sampling.

Plant N uptake

Differences in N uptake by trees in N-amended versus ambient plots could have produced the observed patterns of ^{15}N recoveries in FWD. Three major patterns in $PR^{15}N$ evident in FWD (Figure 4) are consistent with patterns in living vegetation observed by Nadelhoffer et al. (1999): (1) greater values of $PR^{15}N$ in N-amended plots versus ambient; (2) within N-amended plots in both forests, greater values of $PR^{15}N$ for $^{15}NO_3$ labels relative to $^{15}NH_4$ labels; and (3) greater $PR^{15}N$ in the oak forest vegetation relative to the pine forest. This suggests that the patterns of $PR^{15}N$ in FWD have been caused by differences in plant uptake, and the ^{15}N tracers we recovered in FWD eight years after labeling arose from differences in ^{15}N in woody litterfall.

At the Harvard Forest, uptake of N by vegetation increased in N-amended plots over ambient, as soil N sinks were overcome at higher rates of N inputs (Magill et al., 1997, Nadelhoffer et al., 1999b). This was evident in the patterns of ^{15}N recovery immediately following the two-year period of ^{15}N additions: Values of $PR^{15}N$ in vegetation were greater in the N-amended plots relative to the ambient plots (Nadelhoffer et al., 1999a). Tree uptake of N and recovery of ^{15}N in tree tissues were also greater in the oak forest vs. the pine, and recovery of $^{15}NO_3$ labels were greater than those of $^{15}NH_4$ labels in tree tissues in both forests (Nadelhoffer et al., 1999a). Together these patterns suggest that plant uptake, followed by woody litterfall, may have been the dominant mechanism of ^{15}N tracer movement into FWD over the eight-year period. It may also be the dominant mechanism that caused marginally greater pool sizes of N in FWD over the 12-year period of N amendments. This conclusion is consistent with our interpretations that FWD in both forest types exhibited minor fluxes of N immobilization on the forest floor.

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References

Aber J D, Melillo J M and McClaugherty C A 1990 Predicting long-term patterns of mass loss, nitrogen dynamics, and soil organic matter formation from initial fine litter chemistry in temperate forest ecosystems. Can. J. Bot. 68, 2201–2208.

Aber J D, Magill A, Boone R, Melillo J M, Steudler P and Bowden R 1993 Plant and soil responses to three years of chronic nitrogen additions at the Harvard Forest, Massachusetts. Ecol. Appl. 3, 156–166.

Alban D H and Pastor J 1993 Decomposition of aspen, spruce, and pine boles on two sites in Minnesota. Can. J. For. Res. 23, 1744–1749.

Boone R D, Grigal D F, Sollins P, Ahrens R J and Armstrong D E 1999 Soil sampling, preparation, archiving, and quality control. *In* Standard Soil Methods for Long-Term Ecological Research. Ed. G P Robertson, D C Coleman, C S Bledsoe and P Sollins. pp 3–28. Oxford University Press, New York.

Chueng N and Brown, S 1995 Decomposition of silver maple (*Acer saccharinum* L.) woody debris in a central Illinois bottomland forest. Wetlands 15, 232–289.

Currie W S 1999 The responsive C and N biogeochemistry of the temperate forest floor. TREE 14, 316–320.

Currie W S, Aber J D, McDowell W H, Boone R D and Magill A H 1996 Vertical transport of dissolved organic C and N under long-term N amendments in pine and hardwood forests. Biogeochemistry 35, 471–505.

Currie W S and Nadelhoffer K N 1999 Dynamic redistribution of isotopically labelled cohorts of nitrogen inputs in two temperate forests. Ecosystems 2, 4–18.

Currie W S and Nadelhoffer K N *In review* Carbon and nitrogen in woody detritus at the Harvard Forest: the imprint of land use history. Ecosystems.

Currie W S, Nadelhoffer, K J and Aber J D 1999 Soil detrital processes controlling the movement of ^{15}N tracers to forest vegetation. Ecol. Appl. 9, 87–102.

Downs M R, Nadelhoffer K J, Melillo J M and Aber J D 1996 Immobilization of a ^{15}N labelled nitrate addition by decomposing forest litter. Oecologia 105: 141–150.

Edmonds R L 1987 Decomposition rates and nutrient dynamics in small-diameter woody litter in four forest ecosystems in Washington, USA. Can. J. For. Res. 17, 499–509.

Foster D R 1992 Land-use history (1730-1990) and vegetation dynamics in central New England, USA. J. Ecol. 80, 753–772.

Foster D R, Zebryk T, Schoonmaker P and Lezberg A 1992 Post-settlement history of human land-use and vegetation dynamics of a Tsuga canadensis (hemlock) woodlot in central New England. J. Ecol. 80, 773–786.

Goodale C L and Aber J D 2001 The long-term effects of land-use history on nitrogen cycling in northern hardwood forests. Ecol. Appl. 11, 253–267.

Gundersen P, Emmett B A, Kjonaas O J, Koopmans C J and Tietema A 1998 Impacts of nitrogen deposition on nitrogen cycling: a synthesis. For. Ecol. Manage. 101, 37–55.

Gundersen P, Callesen I and De Vries W 1999 Nitrate leaching in forest ecosystems is related to forest floor C/N ratios. Environ. Pollut. 102, 403–407.

Harmon M E and Sexton J 1996 Guidelines for measurements of woody detritus in forest ecosystems. Publication No. 20. U.S. LTER Network Office: University of Washington, Seattle, WA, USA. 73 pp.

Krankina O N, Harmon M E and Griazkin A V 1999 Nutrient stores and dynamics of woody detritus in a boreal forest: Modeling potential implications at the stand level. Can. J. For. Res. 29, 20–32.

Magill A H, Aber J D, Hendricks J J, Bowden R J, Melillo J M and Steudler P A 1997 Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. Ecol. Appl. 7, 402–415.

Magill A H, Aber J D Berntson G M, McDowell W H, Nadelhoffer K J, Melillo J M and Steudler P 2000 Long-term nitrogen additions and nitrogen saturation in two temperate forests. Ecosystems, 3: 238–253.

McNulty S G, Aber J D and Boone R D 1991 Spatial changes in forest floor and foliar chemistry of spruce-fir forests across New England. Biogeochemistry 14, 13–29.

Motzkin G, Wilson P, Foster D R and Arthur A 1999 Vegetation patterns in heterogeneous landscapes: The importance of land use history and environment. J. Veg. Sci. 10, 903–920.

Nadelhoffer K J, Downs M and Fry B 1999a Sinks for ^{15}N-enriched additions to an oak forest and a red pine plantation. Ecol. Appl. 9, 72–86.

Nadelhoffer K J, Emmett B A, Gundersen P, Kjonaas O J, Koopmans C J, Schleppi P, Tietema A and Wright R F 1999b Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. Nature 398, 145–148.

Nelson D W and Sommers L E 1996 Total carbon, organic carbon, and organic matter. *In* Methods of Soil Analysis. Part 3. Chemical Methods. Ed. D L Sparks. pp 961–1010. Soil Science Society of America, Madison, WI.

Pastor J and Post W M 1986 Influence of climate, soil moisture, and succession on forest carbon and nitrogen cycles. Biogeochemistry 2, 3–27.

Polit J I and Brown S 1996 Mass and nutrient content of dead wood in a Central Illinois floodplain forest. Wetlands 16, 488–494.

Sollins P 1982 Input and decay of coarse woody debris in coniferous stands in western Oregon and Washington. Can. J. For. Res. 12, 18–28.

Tietema A, Emmett B A, Gundersen P, Kjønaas O J and Koopmans C J 1998 The fate of ^{15}N-labelled nitrogen deposition in coniferous forests. For. Ecol. Manage. 101, 19–27.

Townsend A R, Braswell B H, Holland E A and Penner J E 1996 Spatial and temporal patterns in terrestrial carbon storage due to deposition of fossil fuel nitrogen. Ecol. Appl. 6, 806–814.

Van Cleve K and Martin S 1991 Long-Term Ecological Research in the United States. 6th, revised edn. Long-Term Ecological Research Network Office, University of Washington, Seattle, WA, USA. 178 p.

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