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Reports

Nitrogen turnover in the leaf litter and fine roots of sugar maple

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Abstract. In order to better understand the nitrogen (N) cycle, a pulse of $^{15}NO_3^-$ was applied in 1998 to a sugar maple (*Acer saccharum*) dominated northern hardwood forest receiving long-term (1994–2008) simulated atmospheric N deposition. Sugar maple leaf litter and live fine-root ^{15}N were quantified for four years prior to labeling and for 11 subsequent years. Continuous sampling of ^{15}N following addition of the tracer enabled calculation of leaf litter and fine-root N pool turnover utilizing an exponential decay function. Fine-root ^{15}N recovery peaked at 3.7% ± 1.7% the year the tracer was applied, while leaf litter ^{15}N recovery peaked in the two years following tracer application at ~8%. These results suggest shoots are primarily constructed from N taken up in previous years, while fine roots are constructed from new N. The residence time of N was 6.5 years in leaf litter and 3.1 years in fine roots. The longer residence time and higher recovery rate are evidence that leaves were a stronger sink for labeled N than fine roots, but the relatively short residence time of tracer N in both pools suggests that there is not tight intra-ecosystem cycling of N in this mature forest.

Key words: Acer saccharum; *experimental NO_3^- deposition; fine roots; leaf litter; long-term tracer experiments; nitrogen cycling; nitrogen sinks; northern hardwood; sugar maple.*

Introduction

The fate and consequences of atmospheric nitrogen (N) deposition are of great scientific and social interest (Schlesinger 2009). Because soil N availability often limits the productivity of temperate forests (LeBauer and Treseder 2008), N deposition could potentially increase carbon (C) storage in these ecosystems. Excess N in soil can also directly suppress litter decay (Zak et al. 2008), thereby providing an additional mechanism for greater ecosystem C storage. Nitrogen not internally stored or cycled in plant tissues or soil organic matter can leach to groundwater, with an oversupply of N eventually leading to N saturation and further consequences for downstream aquatic and marine ecosystems (Aber et al. 1989, Pregitzer et al. 2004). Understanding the mechanisms that control N cycling in forests is the most powerful approach to informed predictions and policy decisions aimed at forecasting and mitigating the consequences of anthropogenic N supplied through the production and use of fertilizer and combustion of fossil fuels.

In forests, N can be cycled within trees through remobilization from one plant module to another, such as in the re-translocation of N from senescing leaves to storage in woody tissues in autumn and the redeployment of this stored N to developing shoots in spring (Wetzel et al. 1989, Stepien et al. 1994). Nitrogen can also be internally cycled within the soil in many different ways, e.g., from litter decay to soil solution or from soil solution into the microbial community (Zak et al. 2004). Finally, N can be cycled from soil to plant and back to soil, e.g., taken up by roots/mycorrhizae from the soil, internally cycled in the tree, and then eventually deposited back to the soil via litter production. Nitrogen that is not stored or cycled within the plant–soil system must ultimately be transferred to some other ecosystem (herbivory, leaching, erosion, harvest, and similar fluxes) or lost back to the atmosphere (Chapin et al. 2002).

Trees and soil are the dominant pools of N in forests, and the incorporation of N into biomass is one potential sink for atmospheric N deposition (Zak et al. 2004). During the growing season, most of the N in trees is found either in the canopy (leaves and reproductive parts) or in fine roots (<1 mm in diameter) because these are the most metabolically active plant modules and they contain the highest concentrations of N-rich proteins and enzymes (Reich et al. 2008). Trees return most of their N to soil primarily through the death and decay of leaves, fine roots, and reproductive parts. Stem mortality and foliar leaching are normally very minor fluxes of N from plant to soil (Nave et al. 2009).

In order to examine the cycling of anthropogenic N, we applied tracer amounts of $^{15}NO_3^-$ in 1998 to plots in a mature forest receiving experimental NO_3^- additions (Zak et al. 2004). ^{15}N is often used to evaluate terrestrial N cycling, but the decadal fate of added ^{15}N has rarely been assessed (Nadelhoffer et al. 2004, Schlesinger 2009) and even long-term studies have sampled ^{15}N dynamics infrequently. Because we sampled leaves and fine roots four years prior to the pulse of $^{15}NO_3^-$ and for 11

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subsequent years, we were able to describe the rate at which the internal pool of N turns over within these mature trees. Our objective was to detail the movement of ^{15}N from trees to the soil by following its turnover in leaf litter and fine roots in order to determine how strongly the N supply to these pools is influenced by soil N.

Methods

Study site and experimental design

The study site is located in northern Lower Michigan (45°33′ N, 84°52′ W). The northern hardwood forest is dominated by sugar maple (*Acer saccharum* Marsh.), which accounts for 86% of the total overstory biomass (total aboveground overstory biomass = 301 Mg/ha; Zak et al. 2008). The dominant trees are ~32 m tall, total stand basal area is 33 m^2/ha, and the dominant trees are 91 years old, on average (as of 2004). Soils are sandy (86% sand) Typic Haplorthods of the Kalkaska series. The A horizon has a pH of 5.0 and the mineral soil exhibits a high degree of base saturation (>80%; MacDonald et al. 1993). Net soil N mineralization during the growing season is ~6.8 g $N \cdot m^{-2} \cdot yr^{-1}$ (calculated from Zogg et al. 1996). Mean annual temperature is 6.0°C and the mean annual precipitation is 871 mm, which is evenly distributed throughout the year. Annual wet + dry NO_3^--N atmospheric N deposition averages 0.58 $g \cdot m^{-2} \cdot yr^{-1}$ and wet + dry total atmospheric N deposition averages 0.91 $g \cdot m^{-2} \cdot yr^{-1}$ (Zak et al. 2008).

Three plots receiving ambient atmospheric N deposition were established in 1987, and three plots receiving experimental NO_3^- deposition were established in 1993. All plots are 900 m^2, with a 10-m treated buffer on all sides of the $+NO_3^-$ plots. The experimental NO_3^- deposition treatment was initiated in 1994 and is composed of 3 g NO_3^--$N \cdot m^{-2} \cdot yr^{-1}$ applied as solid $NaNO_3$ pellets, which are broadcast over the forest floor in six 0.5 g/m^2 increments over the growing season. The routine long-term measurement protocols are described in detail elsewhere (Burton et al. 2004, Pregitzer et al. 2004, 2008, Zak et al. 2004). In 1998, plots receiving the experimental NO_3^- deposition were each labeled with 24 g of ^{15}N (0.027 g $^{15}N/m^2$). The isotope was applied by mixing 99% atom excess $Na^{15}NO_3$ with the June, July, and August application of the routine experimental NO_3^--N treatment, introducing the label to the forest floor. Previous observations show the label was quickly assimilated into the N cycle at this site (Zak et al. 2004).

Sample collection and analysis

Litterfall was collected in four randomly located 0.5-m^2 litter traps per plot (see Plate 1). Collections occurred monthly from April through September and biweekly during periods of heavy leaf fall in October and November. Foliage from a subset of traps was sorted by species, dried in an oven at 65°C, and weighed to determine leaf litter biomass. In each plot, the sorted foliar litter samples from the dominant tree species, sugar maple, were composited for all dates within a year. Additional details on litter flux have been reported elsewhere (Pregitzer et al. 2008).

Table 1. Description of fine-root sampling methods in northern hardwood forest, lower Michigan, USA.

Year	Collection month	Size class	Sampling depth (cm)
1995	July	<1.0	10
1997	August	<1.0	10
1998	August	<1.0	5
1999	September	<0.5	10
2000	September	<0.5	25
2001	August	<1.0	5
2002	May	<1.0	5
2003	June	<1.0	5
2004	August	<0.5	10
2007	August	<1.0	5

Note: Sampling depth is for mineral soil plus organic layers (average depth ~3 cm).

Root collections sampled the organic layer (~3 cm) and mineral soil, but depth of sampling varied by year (Table 1). The samples were originally collected for use in determining either root biomass (2000) or root respiration (all other years). Samples were not sorted by species. All root samples were cleaned with deionized water, sorted by diameter class, and oven-dried at 65°C. We analyzed roots <1.0 mm in diameter in most years, but in 1999 and 2000 only roots <0.5 mm in diameter size were available for analysis. From previous root biomass measurements (Burton et al. 2004), we determined that 83% of the mass of roots <1.0 mm in diameter occurs in the <0.5 mm diameter class.

All tissue samples were finely ground in a ball mill and analyzed for ^{15}N and N concentration with an elemental analyzer (Costech 4010; Costech Analytical Technologies, Valencia, California, USA) coupled to a continuous-flow isotope ratio mass spectrometer (Delta Plus; Finnigan MAT, Bremen, Germany). For N concentration, atropine at varying masses was used to create the calibration curve (0.015–0.06 mg N; leaf and fine-root samples: 2 mg). For $\delta^{15}N$, samples were measured against a N_2 reference gas calibrated with IAEA reference materials (IAEA N1, 0.4‰; IAEA N2, 20.3‰; IAEA 310A, 47.2‰; International Atomic Energy Agency, Vienna, Austria). The standard deviation of measurements of a laboratory standard was 0.5‰ for $\delta^{15}N$. We calculated the percentage recovery of ^{15}N based on the following equation:

$$\text{Percentage recovery} = \left[(\%^{15}N_t \times N_t \times \text{biomass}_t) - (\%^{15}N_{\text{init}} \times N_{\text{init}} \times \text{biomass}_{\text{init}})\right] / {}^{15}N_{\text{addn}} \times 100$$

where $\%^{15}N_{\text{init}}$ and $\%^{15}N_t$ are the proportions of N in biomass that are ^{15}N prior to the ^{15}N addition (1997) and at time t subsequent to the ^{15}N addition; N_{init} and N_t are the N concentrations (mg/g) of the biomass pool

TABLE 2. Leaf litter and fine-root biomass, N concentration, N content, and ^{15}N recovery in a northern hardwood forest receiving NO_3^- deposition treatments since 1994.

Year	Biomass (g/m^2)		N conc. (mg/g)		N mass (g/m^2)		^{15}N mass (mg/m^2)		^{15}N recovery in $+NO_3^-$ plots (%)
	Control	$+NO_3^-$	Control	$+NO_3^-$	Control	$+NO_3^-$	Control	$+NO_3^-$	
Leaf litter									
1994	365.7 (20.6)	328.6 (27.1)	6.3 (0.4)	6.7 (0.5)	2.3 (0.2)	2.2 (0.1)	8.3 (0.7)	7.9 (0.5)	
1995	314.2 (10.2)	276.0 (28.8)	6.9 (0.4)	8.0 (0.1)	2.2 (0.1)	2.2 (0.2)	7.9 (0.2)	8.0 (0.9)	
1996	423.2 (15.1)	367.6 (24.5)	7.6 (0.2)	8.9 (0.1)	3.2 (0.2)	3.3 (0.2)	11.8 (0.8)	12.0 (0.8)	
1997	275.8 (8.4)	252.2 (12.9)	6.4 (0.2)	8.7 (0.4)	1.8 (0.1)	2.2 (0.2)	6.5 (0.3)	8.0 (0.8)	
1998	355.3 (9.2)	310.1 (27.4)	6.4 (0.3)	8.3 (0.5)	2.3 (0.2)	2.6 (0.3)	8.3 (0.6)	9.6 (1.2)	4.3 (1.1)
1999	334.0 (7.8)	363.7 (31.9)	6.6 (0.4)	8.2 (0.1)	2.2 (0.2)	3.0 (0.3)*	8.1 (0.6)	11.8 (1.1)	7.7 (2.4)
2000	353.8 (7.0)	356.3 (49.8)	6.9 (0.4)	9.5 (0.3)*	2.4 (0.2)	3.4 (0.5)*	8.9 (0.6)	13.0 (2.0)	8.4 (3.1)
2001	283.7 (8.7)	287.3 (4.4)	7.9 (0.2)	11.1 (0.2)*	2.3 (0.0)	3.2 (0.0)*	8.2 (0.1)	12.1 (0.1)	5.8 (1.2)
2002	226.5 (11.8)	212.2 (21.1)	10.5 (0.3)	14.3 (0.8)*	2.4 (0.1)	3.0 (0.1)*	8.7 (0.3)	11.4 (0.6)	4.1 (0.4)
2003	331.8 (8.7)	329.5 (25.7)	6.6 (0.3)	9.7 (0.2)*	2.2 (0.1)	3.2 (0.2)*	7.9 (0.4)	12.0 (0.9)	4.2 (0.2)
2004	288.6 (10.6)	322.7 (2.1)	8.3 (0.4)	10.4 (0.5)	2.4 (0.2)	3.3 (0.2)*	8.8 (0.8)	12.5 (0.6)	4.4 (1.0)
2005	310.0 (8.2)	327.6 (22.6)	7.9 (0.1)	9.4 (0.3)	2.5 (0.1)	3.1 (0.1)	9.0 (0.4)	11.5 (0.5)	3.0 (0.5)
2006	289.8 (7.5)	275.6 (44.5)	9.3 (0.4)	11.5 (0.9)	2.7 (0.0)	3.1 (0.3)	9.8 (0.2)	11.5 (1.2)	2.8 (0.4)
2007	311.7 (6.5)	297.9 (24.7)	10.8 (0.7)	10.6 (0.6)	3.4 (0.3)	3.2 (0.4)	12.3 (1.1)	11.8 (1.4)	2.8 (0.5)
2008	317.5 (5.5)	302.5 (18.7)	8.3 (0.4)	9.5 (0.3)	2.6 (0.1)	2.9 (0.3)	9.6 (0.3)	10.7 (1.0)	1.8 (0.2)
Fine roots									
1994	436.8 (7.4)	485.5 (22.9)							
1995			18.8 (2.4)	15.5 (0.8)	8.2 (0.7)	6.8 (0.3)	27.2 (0.0)	23.6 (0.0)	
1996	400.3 (57.8)	450.8 (40.0)							
1997			15.4 (1.3)	15.1 (0.2)	6.7 (0.3)	6.6 (0.1)	25.3 (1.5)	24.0 (0.2)	
1998			16.2 (0.6)	15.1 (1.1)	7.1 (0.4)	6.6 (0.3)	26.0 (1.0)	25.4 (0.3)	3.7 (1.4)
1999			11.3 (0.7)	11.7 (1.1)	5.0 (0.6)	5.0 (0.3)	18.5 (2.6)	24.7 (0.3)	1.4 (0.2)
2000	436.1 (60.6)	459.4 (69.0)	15.1 (0.2)	15.2 (0.7)	6.7 (0.6)	6.5 (0.2)	24.4 (2.1)	24.9 (0.2)	1.5 (0.3)
2001	490.2 (40.6)	354.7 (27.5)	15.7 (0.8)	16.3 (0.9)	6.9 (0.2)	7.0 (0.6)	25.2 (0.8)	24.6 (0.2)	0.8 (0.1)
2002			16.9 (2.2)	16.0 (0.9)	7.6 (0.2)	7.0 (0.2)	27.8 (0.8)	24.4 (0.3)	0.5 (0.1)
2003			17.4 (0.0)	14.6 (1.9)	7.7 (0.7)	6.3 (0.6)	28.1 (2.5)	24.5 (0.0)	0.3 (0.2)
2004			18.7 (0.6)	17.5 (0.8)	8.2 (0.5)	7.6 (0.2)	30.0 (1.1)	24.7 (0.4)	0.3 (0.1)
2007			15.6 (0.6)	16.0 (0.1)	6.8 (0.4)	7.0 (0.3)	25.0 (0.9)	24.7 (0.4)	0.2 (0.1)

Notes: In 1998, N additions were applied as $^{15}NO_3^-$. Treatment means ($n = 3$) are displayed with standard errors in parentheses. Years with significant pairwise differences ($P < 0.05$) between control and NO_3^- plots in leaf litter N concentration and leaf litter N mass are denoted with an asterisk next to these data for the NO_3^- amended plots. ^{15}N mass in fine roots was calculated using constant values through time for biomass (438.5 ± 17.6 and 431.3 ± 19.7 for control and $+NO_3^-$, respectively) and N concentration (15.8 ± 0.6 and 15.2 ± 0.6 for control and $+NO_3^-$, respectively). See *Methods* for details.

in 1997 and at subsequent time t; $biomass_{init}$ and $biomass_t$ are the biomass pools (g/m^2) in 1997 and at subsequent time t; and $^{15}N_{addn}$ is the amount of added ^{15}N. The value for $^{15}N_{addn}$ increased annually to take into account the amount of ^{15}N (9.9 g ^{15}N per plot) supplied through the annual additions of $NaNO_3$ ($\delta^{15}N = 0.7‰$) to each plot. Each percentage recovery value was based on an individual ^{15}N value for that pool and year. However, the procedure for determining the values for biomass and tissue N concentration varied between leaf litter and roots. For leaf litter, we used annual plot-level sugar maple biomass and N concentration data. For fine roots, sampling of the roots analyzed for ^{15}N varied over the course of the study in depth, date, and size class designations (Table 1). As a result of the sampling differences, we used biomass and N concentration data that were the average (at the plot-level) of these data over the 1994–2007 period. Root biomass measurements included all species and were made in 1994, 1996, 2000, and 2001 to 25 cm depth (Burton et al. 2004).

To determine the rate of N turnover in leaf litter and fine roots, we fit an exponential decay function (Olson 1963) to the percentage recovery data from the leaf litter and fine roots that included the peak in percentage recovery and all subsequent data points. Nitrogen turnover was then calculated as the inverse of the decay rate constant (k^{-1}).

Differences in fine-root and leaf litter mass, N concentration, and N mass were analyzed using a repeated-measures analysis of variance (PROC MIXED, restricted maximum likelihood; SAS version 9.1.3, SAS Institute, Cary, North Carolina, USA) and post hoc LSMEANS (Tukey's) adjusted for multiple comparisons. Fit of the exponential decay functions was assessed using regression (PROC REG, SAS).

RESULTS

N additions began in 1994, but significant increases in litter N concentrations did not occur until 2000 (Table 2). The increase in leaf litter N concentration ($P < 0.001$) resulted in a greater leaf litter N flux ($P < 0.001$), even though the NO_3^- deposition treatment never increased leaf litter mass (Table 2; Pregitzer et al. 2008). Both leaf litter mass and leaf litter N concentration varied from year to year (year: $P < 0.001$ for both mass and N; Table 2). Across years, the leaf litter biomass and N concentration was 374.8 ± 7.6 g/m^2 and

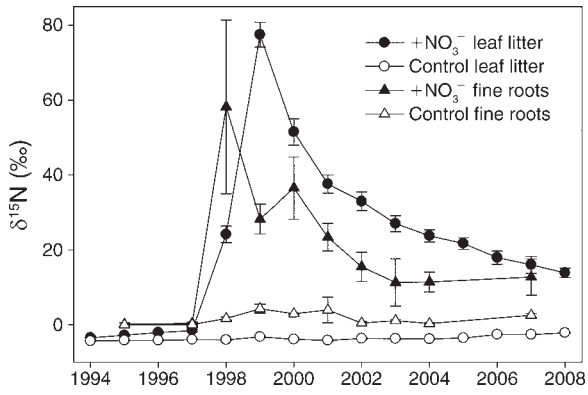

FIG. 1. Values of $\delta^{15}N$ for leaf litter and live fine roots from 1994 through 2008 in a northern hardwood forest, lower Michigan, USA. Pulse additions of ^{15}N-NO_3^- as a tracer were added to the NO_3^- deposition treatment in 1998.

7.8 ± 0.2 mg/g (mean ± SE) for the control and 380.5 ± 8.2 g/m^2 and 9.6 ± 0.3 mg/g for +NO_3^-.

In contrast with leaf litter mass, fine-root mass was relatively consistent during the four years it was sampled (year: $P = 0.795$). The experimental NO_3^- deposition treatment did not influence the concentration of N in fine roots ($P = 0.260$) or fine-root biomass ($P = 0.946$; Table 2). Across years, the mean fine-root biomass and N concentration was 438.5 ± 17.6 g/m^2 and 16.1 ± 0.5 mg/g for the control and 431.3 ± 19.7 g/m^2 and 15.3 ± 0.4 mg/g for +NO_3^-.

The $\delta^{15}N$ of leaf litter peaked in 1999, the year following the application of the tracer and gradually declined thereafter (Fig. 1). Peak recovery of ^{15}N was greater in leaf litter (8.4% ± 3.1%) than in fine roots (3.7% ± 1.4%; Table 2). For fine roots, recovery peaked in 1998. For leaf litter, recovery peaked in 2000 for leaf litter, but was only slightly greater than in 1999. The $\delta^{15}N$ of fine roots peaked the year the label was applied (1998) and also declined thereafter (Fig. 1). The exponential decay functions (both $P < 0.001$) predicted ^{15}N turnover rates of 3.1 years for fine roots and 6.5 years for leaf litter (Fig. 2).

DISCUSSION

Trees acquire N primarily from the soil through root/mycorrhizal uptake or directly through their foliage. Once acquired, the pool of N within the tree is used primarily to construct and maintain N-rich proteins and enzymes. Indeed, the respiratory activity of all land plants is highly correlated to tissue N concentration (Reich et al. 2008). Proteins and enzymes can turn over quickly and N often cycles within the plant on time steps that match the construction and senescence of N-demanding structures (Reich et al. 2008). In general, the internal pool of N in plant leaves and root tips, which contain the majority of plant total N, appears to cycle relatively rapidly within the whole tree. For example, once ^{15}N was introduced to the stem transpiration stream as an artificial sap solution, leaves and fine roots were uniformly labeled within just a few days (Horwath et al. 1992).

Trees can also produce and subsequently remobilize storage proteins (Millard 1996). In autumn, deciduous temperate and boreal trees retranslocate N from metabolically active leaves and synthesize storage proteins, which enable the efficient storage of N within the tree over the dormant season (Wetzel et al. 1989, Stepien et al. 1994). In spring, these storage proteins are remobilized and some of this N is preferentially used to construct new shoots. New shoot growth in highly determinate species like sugar maple begins in early spring when the soil is cold and active transpiration is very low. Internal N reserves are critical for new shoot growth in spring, because conditions for root N uptake are not optimal when buds break (Menino et al. 2007). Several studies utilizing ^{15}N have demonstrated that new shoot growth in spring preferentially utilizes stored N (Proe et al. 2000, Menino et al. 2007) and our results confirm these observations. At our site, the ^{15}N tracer peaked in leaf litter one year after application. The peak one year after the tracer addition is because the majority of N used in construction of shoots in 1998 was stored N (rather than tracer N). As the growing season progresses, shoots of deciduous trees typically rely more upon soil N than N in storage proteins (Proe et al. 2000, Menino et al. 2007). The fact that the ^{15}N recovery in fine roots peaked in the year the ^{15}N was applied suggests that the new roots of sugar maple are not built from stored N, but instead are built from mineral N. However, there are few studies quantifying the seasonality of ^{15}N in fine roots, and reliable information about the remobilization of N from fine roots still eludes the scientific community.

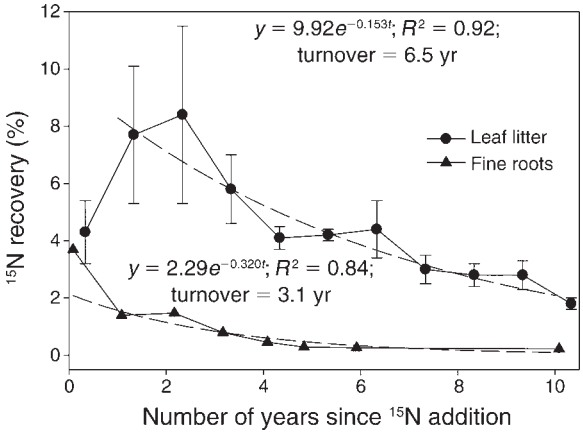

FIG. 2. Percentage recovery of ^{15}N from leaf litter and live fine roots in the years following the pulse addition of tracer ^{15}N-NO_3^- to the NO_3^- deposition treatment plots ($n = 3$ replicates). Dashed lines represent the fit (both $P < 0.001$) of an exponential decay function to the percentage recovery data through time.

PLATE 1. (Top) Northern hardwood forest canopy as seen from a tower allowing canopy access near Pellston, Michigan, USA. (Bottom) Leaf litter in early October in the Upper Peninsula of Michigan. Long-term litter collections (almost 25 years) have enabled greater insight into the mechanisms controlling nitrogen cycling in northern hardwood forests. Photo credits: top, Brian Parmenter; bottom, A. J. Burton.

Tracers recovered at any time after labeling are the net result of initial uptake, residence time (average pool turnover time), and subsequent inputs and losses to other ecosystem pools (Nadelhoffer et al. 2004). The $\delta^{15}N$ of leaf litter peaked in 1999, the year after labeling, and the pool of ^{15}N had an average turnover of 6.5 years (Fig. 2). ^{15}N could be lost in leaf litter and root mortality and subsequently taken back up from the soil, inflating our estimates of turnover. However, this influence should be small because these pools contained at most only a small fraction of the total label applied. Although reuptake prevents a completely accurate estimate of turnover, it is clear that continued uptake of non-labeled N from the soil dominated the N found in leaf litter as time progressed from the pulse of ^{15}N in 1998 (Fig. 2).

It has historically been assumed that because N is tightly retained within the intra-ecosystem cycle under N-limiting conditions, the large majority (80%) of the N required for growth over the life of a stand comes from internal remobilization and plant–soil–plant cycling rather than uptake from the soil (Miller 1986). The forests we studied are mature, have high biomass and high leaf area, and there is virtually no free growing space in the canopy (Pregitzer et al. 2008). The plots we labeled with ^{15}N also exhibit distinctive signs of N saturation (large soil leaching losses of N), in which N

availability exceeds biological demand (Pregitzer et al. 2004). In our case, the conventional wisdom suggesting that N cycles tightly within the tree and from tree-to-soil-to-tree is not the case. The pool of N in leaf litter turns over approximately every 6.5 years, which means the pool of N within the canopy was continually being replaced by non-labeled N. The rapid dilution of tracer N within leaves and the flux of N from the soil are evidence that both within the tree–soil–tree and ecosystem context, the N cycle is open. Other N fertilization studies have not sampled frequently enough to calculate turnover but have shown long-term ^{15}N recovery in the foliage that is similar to our recovery in leaf litter: 2.5–7.4% in *Pinus resinosa* Ait. and mixed *Quercus* forests after seven years (Nadelhoffer et al. 2004) and 1.6–4.5% in *Pinus contorta* Dougl. after eight years (Preston and Mead 1994).

An interesting question is whether or not our leaf litter results are the general case, or a consequence of our chronic N deposition treatment. Nadelhoffer et al. (2004) found that foliar ^{15}N recovery was greater at seven years than in the initial sampling in unfertilized forests. This appears contrary to declining ^{15}N recovery, but the initial sampling by Nadelhoffer et al. (2004) occurred in the same year ^{15}N additions concluded and our results indicate this early sampling may have missed peak uptake. In fact, several other pieces of evidence suggest the patterns we observed may widely reflect N use and internal cycling in trees. For example, young nonbearing orange trees are highly dependent on new inputs of N from the soil (Menino et al. 2007). Proe et al. (2000) report that current N supply has no significant impact on the amount of N remobilized to support new shoot growth, and as the growing season progresses, uptake of N from the soil becomes increasingly important for the formation of N reserves deployed the following spring. In mature almond trees, new N taken up from the soil represents ~50% of total canopy N (Weinbaum et al. 1987). Taken together, our results and those from other ^{15}N studies suggest that the pool of N within the tree is diluted relatively quickly by new N taken up from the soil. Given these observations, the closed view of the intra-ecosystem N cycle in mature forests requires revision. However, generalizations about tree N use should not be taken seriously until further ^{15}N turnover studies are conducted in mature natural forests, including those not exposed to chronic atmospheric N deposition. With a pool turnover of only 6.5 years, it is safe to conclude that the leaf litter N cycle in our study is relatively open. Because the canopy contains most of the N in these trees (Zak et al. 2004), trees in the NO_3^- deposition treatment have little capacity to retain N and appear to simply cycle it faster when exposed to N additions (Pregitzer et al. 2008).

The average residence time for ^{15}N in the pool of live fine-root tissue N was 3.1 years (Fig. 2). The difference in percentage recovery (Fig. 2) between leaf litter and fine roots demonstrates that leaves are a much stronger sink for N than fine roots in our forest. Other studies of ^{15}N have found similar long-term ^{15}N recovery rates in fine roots (1.6–5.6%) and have found that roots are often (but not always) smaller sinks for ^{15}N than foliage (Preston and Mead 1994, Nadelhoffer et al. 2004). In general, leaves are the strongest plant sink for N, representing 40–60% of the total amount of N acquired from the soil (Menino et al. 2007). In our study, direct comparisons of ^{15}N turnover between leaf litter and fine roots should be interpreted with caution. Our fine-root data come from live fine roots and the data do not represent senescent fine-root tissue. We still do not understand if N is retranslocated from fine roots before they die and the existing evidence is equivocal. Millard (1996), Proe et al. (2000), and Luyssaert et al. (2005) all discuss problems with estimates of nutrient resorption from foliage during senescence if the estimates are not derived from the use of tracer techniques. Equivalent data for fine roots that would enable such a discussion are not available, and our continuous time course for the pool dilution of ^{15}N in fine roots is problematic compared to that for leaf litter. Fine roots in our experiment were sampled in different ways through time (Table 1), and we now know there is significant variation in the concentration of fine-root tissue N and corresponding rates of fine-root respiration, depending on the position of an individual root on the branching root system (Pregitzer et al. 1998, 2002). Thus, a direct comparison of pool turnover between the leaf litter and fine-root pools cannot be done with certainty. However, 6.5 years and 3.1 years for leaf litter and live roots represent relatively short N pool residence times. Compared to leaves, construction of new fine roots appears to be less dependent on N transferred from internal storage pools and fine roots may cycle N faster than leaves, which are the dominant N sink.

The idea that trees conserve N by withdrawing amino acids and proteins from leaves in autumn, synthesizing specific proteins for storage over winter, and then remobilizing stored N in the spring to conserve N should be reconsidered. Our results and those from other ^{15}N studies (Proe et al. 2000, Menino et al. 2007) suggest the use of stored N to construct new shoots in the spring may be a physiological mechanism that has evolved in response to cold soils and a poorly developed vascular system in the early spring. Early in the spring when cold soils limit diffusion in the soil and the metabolic processes required to actively transport N into the root system, the determinate shoots partially differentiated in the buds the prior year expand rapidly in the absence of a fully functional transpiration system. At our study site in May, it is probably impossible for trees to transport enough N from soil to support rapidly expanding shoots, and therefore, over time trees may have evolved mechanisms to utilize internally stored N to fuel the expansion of a new cohort of N-rich leaves. As the tree's vascular system becomes fully functional, soil warms, and the growing season progresses, trees rely

mostly on N taken up from the soil, and so the pool of internally stored tree ^{15}N in our study declined exponentially. We must now understand if all temperate and boreal trees, regardless of soil N availability, follow a similar seasonal and interannual pattern of N cycling. Fine roots appear to cycle N faster than leaves and apparently rely less on internally stored N for their construction.

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