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# Soil processes drive seasonal variation in retention of $^{15}\text{N}$ tracers in a deciduous forest catchment

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**Abstract.** Seasonal patterns of stream nitrate concentration have long been interpreted as demonstrating the central role of plant uptake in regulating stream nitrogen loss from forested catchments. Soil processes are rarely considered as important drivers of these patterns. We examined seasonal variation in N retention in a deciduous forest using three whole-ecosystem  $^{15}\text{N}$  tracer additions: in late April (post-snowmelt, pre-leaf-out), late July (mid-growing-season), and late October (end of leaf-fall). We expected that plant  $^{15}\text{N}$  uptake would peak in late spring and midsummer, that immobilization in surface litter and soil would peak the following autumn leaf-fall, and that leaching losses would vary inversely with  $^{15}\text{N}$  retention. Similar to most other  $^{15}\text{N}$  tracer studies, we found that litter and soils dominated ecosystem retention of added  $^{15}\text{N}$ . However,  $^{15}\text{N}$  recovery in detrital pools varied tremendously by season, with >90% retention in spring and autumn and sharply reduced  $^{15}\text{N}$  retention in late summer. During spring, over half of the  $^{15}\text{N}$  retained in soil occurred within one day in the heavy (mineral-associated) soil fraction. During summer, a large decrease in  $^{15}\text{N}$  retention one week after addition coincided with increased losses of  $^{15}\text{NO}_3^-$  to soil leachate and seasonal increases in soil and stream  $\text{NO}_3^-$  concentrations, although leaching accounted for only a small fraction of the lost  $^{15}\text{N}$  (<0.2%). Uptake of  $^{15}\text{N}$  into roots did not vary by season and accounted for <4% of each tracer addition. Denitrification or other processes that lead to N gas loss may have consumed the rest. These measurements of  $^{15}\text{N}$  movement provide strong evidence for the dominant role of soil processes in regulating seasonal N retention and losses in this catchment and perhaps others with similar soils.

**Key words:** Arnot Forest, New York, USA; deciduous forest;  $^{15}\text{N}$  tracer; N retention; seasonality; soil fractions; soil nitrogen; stream nitrate.

## INTRODUCTION

Processes of forest nitrogen (N) retention have long been inferred from seasonal patterns of stream nitrate ( $\text{NO}_3^-$ ) concentration, but these inferences have rarely been directly tested. That is, in most seasonally snow-covered catchments, stream  $\text{NO}_3^-$  concentrations rise during the dormant season, peak at snowmelt, and then drop to low levels during the growing season (e.g., Stoddard 1994, Wright et al. 2001). This pattern has been broadly attributed to the seasonal pattern and strength of terrestrial plant demand during the growing season (Vitousek and Reiners 1975, Stoddard 1994, Likens and Bormann 1995, Church 1997). However, in some temperate forest watersheds, stream  $\text{NO}_3^-$  concentrations peak in summer rather than winter, sparking questions on what drives seasonal N retention and loss in these and other catchments (e.g., Mulholland and Hill

1997, Band et al. 2001, Goodale et al. 2009, Brookshire et al. 2011, Ohte 2012). Multiple hypotheses have been proposed to explain high summer  $\text{NO}_3^-$  concentrations in streamwater, including: contributions from deep groundwater flow paths (Burns et al. 1998, Ohte 2012); summer decreases in catchment wetness, denitrification rates, and hydrologic connectivity (Band et al. 2001); and expected summer increases in rates of net nitrification under warmer summer temperatures (Goodale et al. 2009, Brookshire et al. 2011). Seasonal variation of in-stream processes can also affect stream  $\text{NO}_3^-$ , with light-stimulated autotrophic N uptake prior to leaf-out in spring and heterotrophic N uptake induced by organic matter inputs generated by autumn leaf-fall (e.g., Mulholland 2004, Roberts and Mulholland 2007, Goodale et al. 2009, Sebestyen et al. 2014). Improved understanding of the drivers of seasonal patterns of ecosystem N retention should yield new insights relevant for basic understanding of N dynamics and for management of pollutant N losses in streamwater.

Catchment input–output studies can capture the integrated net response of ecosystem N balance;

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however, they provide little information about where N is retained or lost (Likens and Bormann 1995, Church 1997). Tracer studies using  $^{15}\text{N}$  provide a powerful alternative approach for identifying key ecosystem N retention and loss processes (e.g., Nadelhoffer et al. 1999a, Holub and Lajtha 2004, Perakis et al. 2005). The tracer approach enables quantification of the fate of  $^{15}\text{N}$  added to forest ecosystems as measured over various timescales, typically a year or longer for tracer studies that encompass both tree and soil responses (e.g., Tietema et al. 1998, Nadelhoffer et al. 1999b, Templer et al. 2012) or sometimes at finer timescales in small-plot studies focused on soil responses (e.g., Zogg et al. 2000, Perakis and Hedin 2001, Providoli et al. 2006). Other than one small-plot study that contrasted the response of  $^{15}\text{N}$  added to mixed pine stands in May and October (Seely and Lajtha 1997), this tracer approach has not yet been used to examine seasonal variation in forest  $^{15}\text{N}$  retention and loss pathways, even though temperate forests experience very large seasonal differences in environmental conditions and biological activity that should affect N dynamics and fate.

We examined how  $^{15}\text{N}$  retention varied seasonally in a deciduous forest in the headwaters of the Upper Susquehanna Basin in central New York, USA. The region typically develops a winter snowpack, yet streams display  $\text{NO}_3^-$  peaks during summer rather than during the dormant season (Goodale et al. 2009). Previous measurements of soil and streamwater chemistry led us to hypothesize that soil processes might dominate control of summer  $\text{NO}_3^-$  peaks in these catchments, with N immobilization in soils and streams stimulated by autumn leaf-fall (Goodale et al. 2009). In this study, we compared  $^{15}\text{N}$  retention in spring, summer, and autumn using three sequential whole-ecosystem  $^{15}\text{N}$  tracer additions. Frozen conditions precluded a winter comparison. We hypothesized that root  $^{15}\text{N}$  uptake would be greatest in late spring and summer corresponding with the periods of plant growth and that soil  $^{15}\text{N}$  retention would be greatest in autumn, driven by heterotrophic N uptake fueled by the input of carbon in leaf-fall. We also examined the fate of  $^{15}\text{N}$  within two soil fractions (light and heavy) following the first  $^{15}\text{N}$  addition in spring, expecting microbial activity to produce greater  $^{15}\text{N}$  recovery in the particulate (light) fraction compared to the mineral-associated (heavy) fraction. We expected that gaseous  $^{15}\text{N}$  losses to denitrification would be greatest either during the warm summer period (Bohlen et al. 2001) or in the wet soils of spring (Groffman and Tiedje 1989) and that leaching losses would vary inversely with  $^{15}\text{N}$  retention in the combined plant and soil pools.

## METHODS

### Site description

The study was conducted within Cornell University's Arnot Forest, 25 km south of Ithaca, New York, USA.

Measurements centered on a 0.25-ha triangular plot that surrounded a perennial spring at the origin of Pine Creek (42°17' N, 76°8' W; 503 m elevation; Fig. 1). The plot spanned 50 m across its top, with a center axis that ran 100 m downhill along the creek. The plot was gridded at 10-m intervals to define subplots for tracer application and soil sampling.

Annual (1970–2000) mean temperature at the site averages 7.8°C, with monthly means ranging from –5.2°C in January to 20.4°C in July (NRCC 2009). Annual precipitation averages 930 mm/yr, with more in summer (93 mm/month) than winter (53 mm/month). Atmospheric N deposition to Connecticut Hill, a long-term monitoring site 14 km north of Arnot Forest, averaged  $\sim 10 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  during 2000–2009, including  $6 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  as wet deposition (52% as  $\text{NO}_3^-$ , 48% as  $\text{NH}_4^+$ ),  $2 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  as  $\text{HNO}_3$  vapor, and  $2 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  as  $\text{NH}_3$  (Butler et al. 2015).

Vegetation consists of second-growth mixed hardwoods established naturally after harvest in 1873–1887 and fires in 1900–1911 (Fain et al. 1994, Fahey et al. 2013). Soils are somewhat poorly drained Dystrudepts developed in channery silt loam and silty clay loam till over Devonian shale (Neeley 1965). Coarse fragments (>2 mm) average 22% by volume. Soil pH (1:2 soil:water) is  $4.4 \pm 0.1$  (mean  $\pm$  SE,  $n = 60$ ). Redoximorphic features were observed during lysimeter installation in summer 2006 (C. Goodale, *personal observation*). Earthworms have invaded Arnot Forest, especially in wetter soils, reducing forest floors from  $\sim 20 \text{ t C/ha}$  in worm-free sites to  $\sim 2 \text{ t C/ha}$ , composed mostly of stems and twigs (Bohlen et al. 2004, Fahey et al. 2011).

### Seasonal weather and phenology

The study year of 2007 averaged 0.4°C warmer and 4% wetter than the long-term mean, but provided broadly typical seasonal conditions (Fig. 2). A late 35-cm snowfall occurred in mid-April, two weeks prior to the first tracer addition. The snow melted completely and combined with 3 cm of rain to saturate soils at the time of the first tracer addition. Leaf-out occurred in mid-May. Soils dried throughout May and June, which were 0.8°C warmer than normal with half of normal rainfall. A rainy July followed, so that soils were moist at the time of the second tracer addition; three rainfall events (1.0, 2.5, and 2.1 cm) occurred between day 7 and 30 after this addition (Fig. 2). Late summer conditions were typical, followed by an unusually warm October (+4.5°C) and wet November and December (+40%). Leaf-fall began in late September, occurred primarily in mid-October, and was 90% complete at the time of the third tracer application.

### Tracer additions

On 30 April, 31 July, and 30 October 2007, we applied  $70 \text{ g }^{15}\text{N/ha}$  as 99 atom%  $^{15}\text{N-KNO}_3$ , along with 1.5 mm of water and an equimolar (0.31 mmol/L) amount of

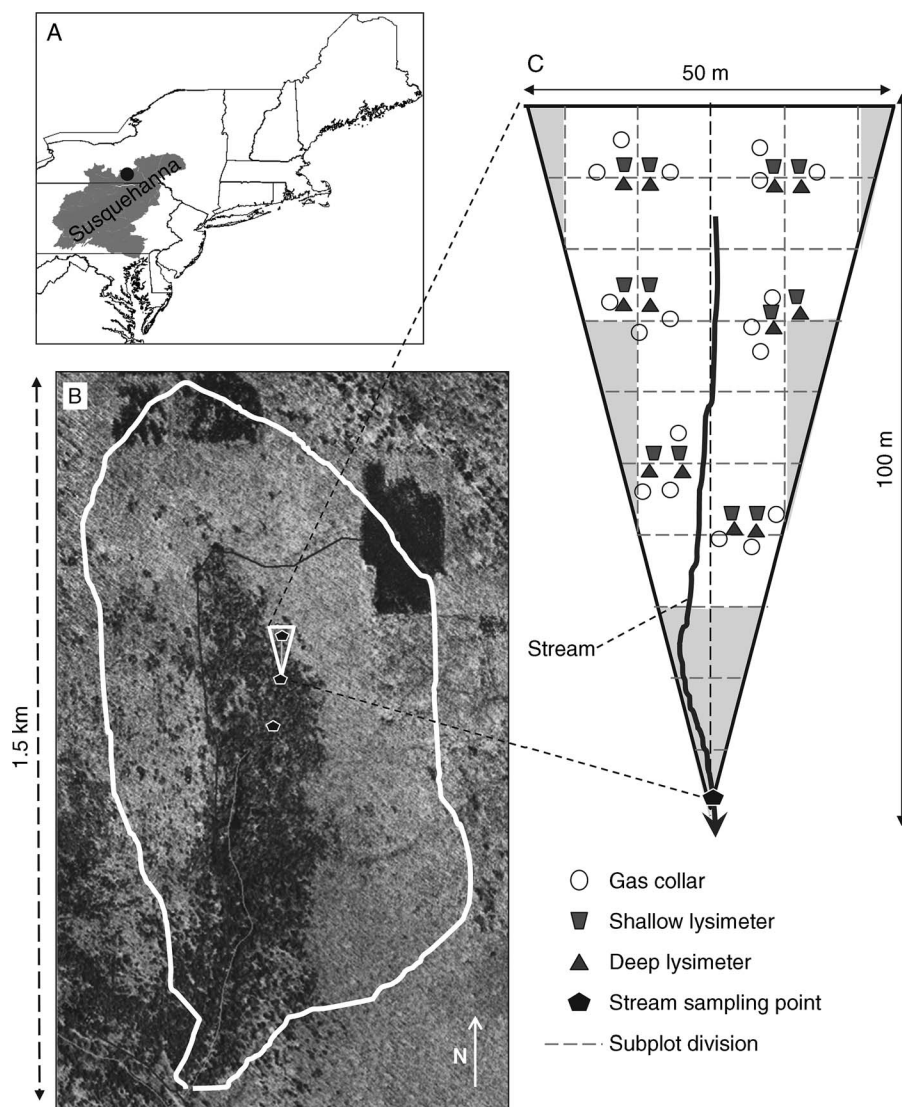


FIG. 1. (A) Site location (black dot) within the New York, USA portion of the Susquehanna basin; (B) the Pine Creek catchment and the tracer-addition plot (white outlines) displayed on a 2002 orthophoto (conifers, dark; hardwoods, gray); and (C) layout of instrumentation and soil sampling (white blocks) within the tracer-addition plot.

KBr added as an inert tracer, for cumulative additions of 4.5 mm of water and 0.21 kg N/ha. The total  $^{15}\text{N}$  addition amounted to 2% of the mean annual N deposition rate and <0.01% of the total forest N stock to 10 cm. The three dates were chosen to represent conditions of post-snowmelt and pre-leaf-out in spring, mid-growing season in summer, and closely following leaf-fall in autumn. Tracers were applied with a pair of handheld hose sprayers equipped with flow gauges and supplied by a 3785-L (1000 gallon) tank located near the top of the catchment (see Plate 1). Each application was divided into three to five passes per subplot to enhance evenness of distribution, and tracers were applied to the stream surface within the plot at the same areal rate as for the adjacent terrestrial upland.

#### Field sampling

To examine seasonal variations in the fate of tracer  $^{15}\text{N}$ , litter ( $\text{O}_i$  material), surface soil, roots, soil solutions, and stream  $\text{NO}_3^-$  were all collected before and in an exponential time series after each  $^{15}\text{N}$  application. For the spring and summer additions, sample collections occurred the day before (−1) and 1, 2, 7, 30, and 90 days after the  $^{15}\text{N}$  addition; for the autumn addition, sampling occurred on days −5, +1, +7, and +21 post-addition. The 90th day after the spring and summer additions also served as the pre-addition sampling date for summer and fall, respectively, for a total of 14 sampling dates in 2007. Snowfall in late November prevented further sampling following the autumn addition.



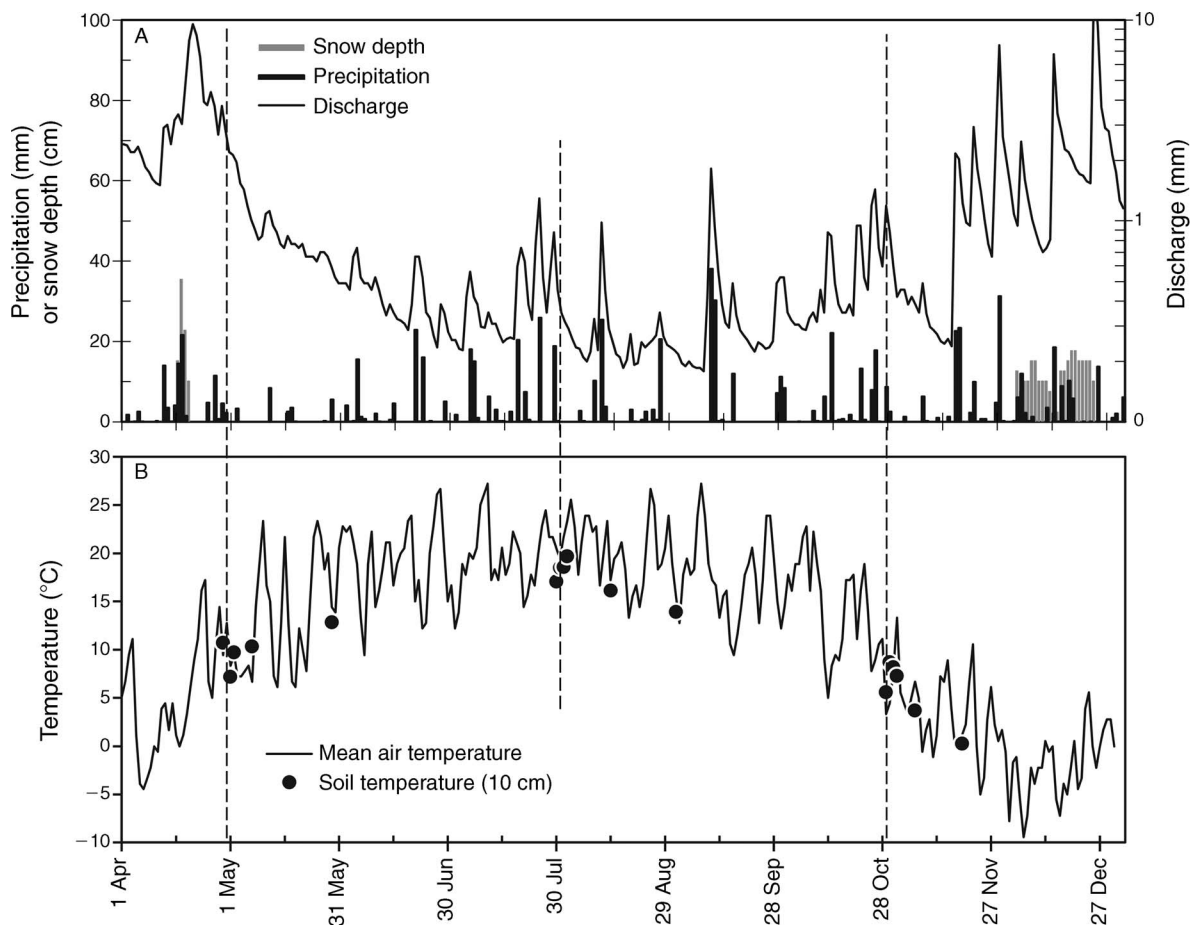


FIG. 2. Hydrology and temperature during study year (2007), including (A) precipitation, snow depth (Ithaca, New York, USA), and discharge (log scale), and (B) mean air (Ithaca, New York) and soil (Arnot Forest, New York, USA) temperature. Dashed vertical lines indicate dates of tracer additions.

Soil and root samples were collected from 20 central subplots, 87.5–100 m<sup>2</sup> (Fig. 1). The forest floor was typically absent except for a thin layer of surface litter (the O<sub>i</sub> layer). Soils to 10 cm depth below the O<sub>i</sub> layer were collected using two 7 cm diameter cores per subplot ( $n = 40$  cores) and included O<sub>e</sub>–O<sub>a</sub> material in those few cases where it occurred. O<sub>i</sub> material was collected from the 7-cm corers for the spring and summer studies and from inside a 20 cm diameter steel pipe in fall. To limit analytical costs, the O<sub>i</sub> layer, soil, and associated root samples were composited by subplot from 40 to 20 samples of each type per date. Root samples were further composited by subplot pairs ( $n = 10$ ) and then sorted into two root classes: fine ( $\leq 1$  mm diameter) and coarse ( $> 1$  mm). Movement of tracer into aboveground litter was quantified by collecting litterfall at  $\sim 3$ -week intervals (4 October, 29 October, and 19 November 2007) from 25 mesh-lined litter baskets, 57.2  $\times$  41.3 cm, distributed across the plot. Baskets were briefly removed during the fall tracer addition to prevent contamination.

To provide some information on N gas losses and belowground biological activity, soil fluxes of N<sub>2</sub>O and

CO<sub>2</sub> were measured using a closed chamber method in which collars were sealed with an opaque, gas-tight lid fitted with a septum. Eighteen PVC soil collars, each enclosing a 0.065 m<sup>2</sup> area, were installed to  $\sim 3$  cm depth in early April 2007, as six sets of three collars, with each set of collars located within 3 m of a set of lysimeters. Soil gases were collected one day before ( $-1$ ) and 1, 2, 7, 29, and 91 days following the spring addition, days  $-1$ , 0, +1, 2, 14, 32, and 90 following the summer addition, and days  $-1$ , 0, +1, 2, 7, 20 following the autumn addition, for 17 measurement dates. On each date, four gas samples were collected from each chamber over a 2-h period into pre-evacuated 22-mL vials.

Tracer movement to soil water was quantified using 24 lysimeters grouped into six sets of four, which included a pair of shallow (10 cm) zero-tension lysimeters and a pair of deep (50 cm) tension lysimeters, installed in July 2006. Three sets were located on each side of the stream (Fig. 1). The zero-tension lysimeters consisted of a 30.5 cm long by 25.4 cm diameter PVC pipe split in half and capped at one end, draining to a 2-L polyethylene collection bottle placed in a soil pit.

These lysimeters were installed via lateral excavation beneath the top 10 cm of undisturbed soil on the upslope side of the pits, which were then backfilled. The 50-cm lysimeters consisted of SoilMoisture 1900 Series ceramic cup lysimeters (SoilMoisture Equipment, Goleta, California, USA) sampled after sitting for roughly 24 h under 50 kPa of tension applied by a vacuum hand pump. Lysimeter water samples were composited by pair (to six pairs per depth) for chemistry and  $^{15}\text{N}\text{-NO}_3^-$  analyses.

Tracer exports in streamwater were quantified using ISCO automated samplers to collect streamwater at three locations downstream from a perennial spring: at 10 m, nearest the spring; at 86 m, where the stream exited the plot; and at 165 m, below the plot. Inferences of in-stream uptake of  $^{15}\text{NO}_3^-$  were limited to observations of tracer  $^{15}\text{NO}_3^-:\text{Br}^-$  ratios, constrained further by limited sampling resolution, low  $\text{Br}^-$  levels near or below detection limits, and an instrument malfunction that destroyed samples immediately following the spring tracer addition. For the first 24 h following each addition, stream samples were collected every 20 min and composited into 3-h intervals. For the next 30 d, samples were collected every hour and composited by day. Stream samples for  $^{15}\text{N}\text{-NO}_3^-$  analysis ( $n = 65$ ) were selected to span the stream response, covering 24 time points from the three sites. All soil solutions and streamwater samples were filtered in the field through ashed, prerinsed 0.7- $\mu\text{m}$  glass fiber filters. Soil cores and water samples were frozen on the day of collection to minimize microbial activity between time of collection and later processing.

#### *Laboratory processing and analyses*

Soil cores were thawed then sieved to 2 mm, and 10-g subsamples were dried at 110°C for 1 d for moisture determination. A second subsample was ground to a fine powder using a ball mill (Retsch mixer mill MM200; Verder Scientific, Newtown, Pennsylvania, USA) then dried and weighed for isotope analysis. Roots were collected during sieving, sorted to fine ( $\leq 1$  mm) and coarse ( $> 1$  mm) root categories, dried for one week at 50°C, and weighed. Roots were then frozen with liquid nitrogen and ground with a mortar and pestle to homogenize for a representative subsample, which was ground in a freezer mill to a fine powder for isotope analysis (Spex CertiPrep 6750; Spex CertiPrep, Metuchen, New Jersey, USA).  $\text{O}_i$  material was passed through a 6-mm sieve then dried at 60°C for 5–7 d and weighed. Litterfall was sorted to leaf, needle, twig, and other components, dried to a constant mass at 60°C and weighed.  $\text{O}_i$  material and leaf litter were homogenized with a coffee grinder, then a subsample was ground in the freezer mill to a fine powder for isotope analysis.

For the spring addition only, the 20 sieved soil samples ( $< 2$  mm) from each of five dates (day –1, +1, 2, 7, 30) were split using density fractionation (Sollins et al. 1999), which separates soil material into a fraction

dominated by particulate organic matter (light fraction) and a fraction dominated by mineral-associated material (heavy fraction). Cost prevented similar analyses for summer and fall. Twelve grams of dried soil were added to 36 mL of low-N sodium polytungstate (SPT) solution prepared to a density of 1.65 g/cm<sup>3</sup>. The soil-SPT slurry was agitated on a shaker table for 2 h, centrifuged for 12 min, and allowed to stand for 12–24 h, during which time particles fully separated into floating (light) and settled (heavy) fractions. Light-fraction material was aspirated from the top, then both fractions were separately filtered on ashed 7-cm Whatman GF/F filters (GE Healthcare Life Sciences, Pittsburgh, Pennsylvania, USA), rinsed with at least 250 mL of deionized water, dried at 55°C for 4 d, weighed, and ground to a fine powder for isotope analysis.

Concentrations of trace gases ( $\text{N}_2\text{O}$  and  $\text{CO}_2$ ) were measured at Cornell University (Ithaca, New York, USA) using a gas chromatograph fitted with an electron capture detector and thermal conductivity detector (Shimadzu GC-2014; Shimadzu, Kyoto, Japan). Chamber volume was calculated using the average of four depth measurements made in the field. Gas fluxes were calculated from the rate of increase in concentration over time. In cases where the concentration increase was not linear over the entire 2-h incubation, fluxes were calculated using the slope of the initial linear period. We did not measure gas  $^{15}\text{N}$  composition because of the very low  $\text{N}_2\text{O}$  concentrations observed and because the large size of the background  $\text{N}_2$  pool typically precludes use of the  $^{15}\text{N}$  tracer technique to detect  $^{15}\text{N}_2$  production (Yang et al. 2014). Thus, the potential seasonal role of denitrification as a driver of  $^{15}\text{N}$  losses was inferred primarily from patterns of “unrecovered”  $^{15}\text{N}$ , recognizing that this term includes not only  $^{15}\text{N}$  gas losses but also unmeasured hydrologic or plant uptake  $^{15}\text{N}$  fluxes and errors in all measured terms.

For all solutions,  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , and  $\text{Br}^-$  concentrations were measured using ion chromatography (Dionex ICS-2000; Dionex, Sunnyvale, California, USA). Dissolved organic carbon (DOC) and total dissolved N (TDN) were measured using oxidative combustion (Shimadzu TOC-V<sub>CPN</sub> with a TNM-1 chemiluminescent detector; Shimadzu, Kyoto, Japan) following acidification and sparging. Dissolved organic N (DON) was computed by difference ( $\text{DON} = \text{TDN} - \text{NO}_3^- - \text{NH}_4^+$ ). The  $^{15}\text{N}$  composition of  $\text{NO}_3^-$  was measured using a modification of the ammonia diffusion method described by Sigman et al. (1997). Briefly, samples were concentrated by boiling, while  $\text{NH}_4^+$  was removed from solution by conversion to  $\text{NH}_3$  with addition of  $\text{MgO}$  to increase sample pH. Next,  $\text{NO}_3^-$  was reduced to  $\text{NH}_4^+$  using Devardas alloy, then more  $\text{MgO}$  was added to repeat the conversion to  $\text{NH}_3$ ; this  $\text{NH}_3$  was diffused onto glass fiber filters acidified with 2.5 M  $\text{K}_2\text{SO}_4$  and suspended within Teflon packets (DuPont, Wilmington, Delaware, USA) for 48 h while heating to 60°C, followed by 7 d on an orbital shaker. Samples with potential for

TABLE 1. Dry mass, C, and N content for leaf litterfall, the litter layer ( $O_i$ ), soil, and roots, and late-fall (20 November) recovery of the cumulative  $^{15}\text{N}$  additions, sampled in Arnot Forest near Ithaca, New York, USA.

Ecosystem pool	Dry mass (Mg/ha)	C/N ratio	C stock (Mg/ha)	N stock (kg/ha)	Recovery of cumulative $^{15}\text{N}$ (%)
Leaf litterfall	$1.8 \pm 0.1$	$67.2 \pm 1.6$	$0.8 \pm 0.04$	$13 \pm 0.7$	0.3
Fine roots (<1 mm)	$2.5 \pm 0.4$	$38.7 \pm 3.2$	$1.1 \pm 0.2$	$29 \pm 3$	2.4
Coarse roots (>1 mm)	$6.9 \pm 1.8$	$87.2 \pm 7.7$	$3.4 \pm 0.9$	$39 \pm 10$	2.2
$O_i$ (litter layer)	$8.7 \pm 0.8$	$39.4 \pm 0.9$	$2.9 \pm 0.4$	$73 \pm 9$	24.7
Soil (<2 mm fraction)	$618 \pm 50$	$15.0 \pm 0.7$	$32.5 \pm 3.9$	$2052 \pm 123$	48.1
Total					77.7

Notes: Soil and roots were sampled from the top 10 cm. Values are mean  $\pm$  SE.

$^{15}\text{N}$  enrichment exceeding instrument range (indicated by high concentrations of the  $\text{Br}^-$  tracer) were spiked with unlabeled  $\text{NO}_3^-$  ( $\delta^{15}\text{N} = 4.4 \pm 0.02\%$ ), with sample  $\delta^{15}\text{N}$  values computed after correcting for these spikes.

All stable isotope analyses were conducted at the Cornell University Stable Isotope facility (Cornell University, Ithaca, New York, USA) using an isotopic ratio mass spectrometer (Finnigan MAT Delta Plus, Thermo Finnigan, San Jose, California, USA) following combustion with an elemental analyzer (Carlo Erba NC2500, Thermo Finnigan, San Jose, California, USA).

#### Quantitative analyses

$^{15}\text{N}$  composition is expressed using customary delta ( $\delta$ ) notation, as the difference between the ratio of  $^{15}\text{N}$  to  $^{14}\text{N}$  in a sample ( $R_{\text{samp}}$ ) and in the atmospheric  $\text{N}_2$  gas standard ( $R_{\text{std}}$ ), expressed relative to that standard in per mil units (‰)

$$\delta^{15}\text{N}(\text{‰}) = (R_{\text{samp}} - R_{\text{std}})/R_{\text{std}} \times 1000. \quad (1)$$

Repeated-measures analysis of variance was used to determine statistically significant differences in  $\delta^{15}\text{N}$  values among dates for each of the regularly sampled ecosystem N pools. Nitrogen stocks ( $M_{\text{poolN}}$ ; g N/ha) were computed using N concentration and mass measurements averaged over the study period (Table 1). Recovery of tracer  $^{15}\text{N}$  in each pool ( $^{15}\text{N}_{\text{Rec}}$ ) was computed as the difference in the atom%  $^{15}\text{N}$  of a pool after tracer addition ( $^{15}\text{N}_{\text{post}}$ ) compared to pre-addition ( $^{15}\text{N}_{\text{pre}}$ ) and multiplied by the pool's N stock; relative (%) recovery was computed by dividing by the mass of tracer added ( $M_{^{15}\text{Nadded}}$ , g  $^{15}\text{N}$ /ha) and multiplying by 100.

$$^{15}\text{N}_{\text{Rec}}(\%) = (\text{atom}\%^{15}\text{N}_{\text{post}} - \text{atom}\%^{15}\text{N}_{\text{pre}}) \times M_{\text{poolN}}/M_{^{15}\text{Nadded}} \times 100. \quad (2)$$

We estimated both overall tracer recovery and percentage recovery of each tracer application relative to measurements immediately preceding each addition.

#### RESULTS

The  $O_i$  layer and surface soil together dominated the overall recovery and seasonal patterns of  $^{15}\text{N}$  retention, with strong and persistent retention in these pools during spring and autumn (>90%), but transient

retention followed by large losses during summer (Figs. 3 and 4). Fine and coarse roots together retained only a small fraction ( $\leq 4\%$ ) of each of the three  $^{15}\text{N}$  additions (Figs. 3 and 4), with lagged temporal responses relative to those observed in the detrital pools. Unrecovered  $^{15}\text{N}$ , possibly lost to N gases, peaked in summer, with small losses in spring. Losses of tracer  $^{15}\text{N}$  as  $\text{NO}_3^-$  leaching from the surface soils also peaked following the summer addition, but this overall flux was very small: cumulative  $^{15}\text{N}$ - $\text{NO}_3^-$  leaching losses both in shallow soil water and in streamwater each amounted to <0.1% of the added tracer.

#### $^{15}\text{N}$ recovery in surface litter and soil

Following the spring addition, the small N pool in the  $O_i$  layer (73 kg N/ha; Table 1) showed immediate enrichment in  $\delta^{15}\text{N}$  by  $\sim 90\%$  for the following three months (Fig. 3A). The summer addition further enriched the  $O_i$  layer to nearly 210‰, but only for one week: by day 30,  $O_i$  layer  $\delta^{15}\text{N}$  values did not differ significantly from before the summer addition. The autumn addition increased  $O_i$  layer  $\delta^{15}\text{N}$  values by a similar amount as the prior two additions. Using these  $\delta^{15}\text{N}$  enrichments to compute tracer recovery (Eq. 2), the  $O_i$  layer retained 35% of the spring tracer addition from day 1 through at least day 90 afterwards (Fig. 4A, B). During the first week following the summer tracer application, the  $O_i$  layer retained additional  $^{15}\text{N}$  amounting to 44% of that application (Fig. 4A, B). However, the  $O_i$  layer then lost  $^{15}\text{N}$ , mostly between day 7 and 30 after the summer addition; by day 90, the  $O_i$  layer had lost as much  $^{15}\text{N}$  as it initially retained from this application (Fig. 4A, C). In autumn, the  $O_i$  layer retained additional  $^{15}\text{N}$  averaging 46% of that application (Fig. 4A, D).

In the large pool of N in the top 10 cm of soil (2052 kg N/ha), all three  $^{15}\text{N}$  additions immediately increased soil  $\delta^{15}\text{N}$  by approximately 5‰ (Fig. 3B). This soil enrichment persisted throughout the monitoring periods following the spring and autumn additions, but decreased by about half by day 30 after the summer addition. These enrichments corresponded to recovery of over half (55%) of the  $^{15}\text{N}$  added in spring in this soil pool, with a small decrease in  $^{15}\text{N}$  recovery after the first week. The heavy fraction (mineral-associated material) accounted for 26% of the spring tracer  $^{15}\text{N}$  addition



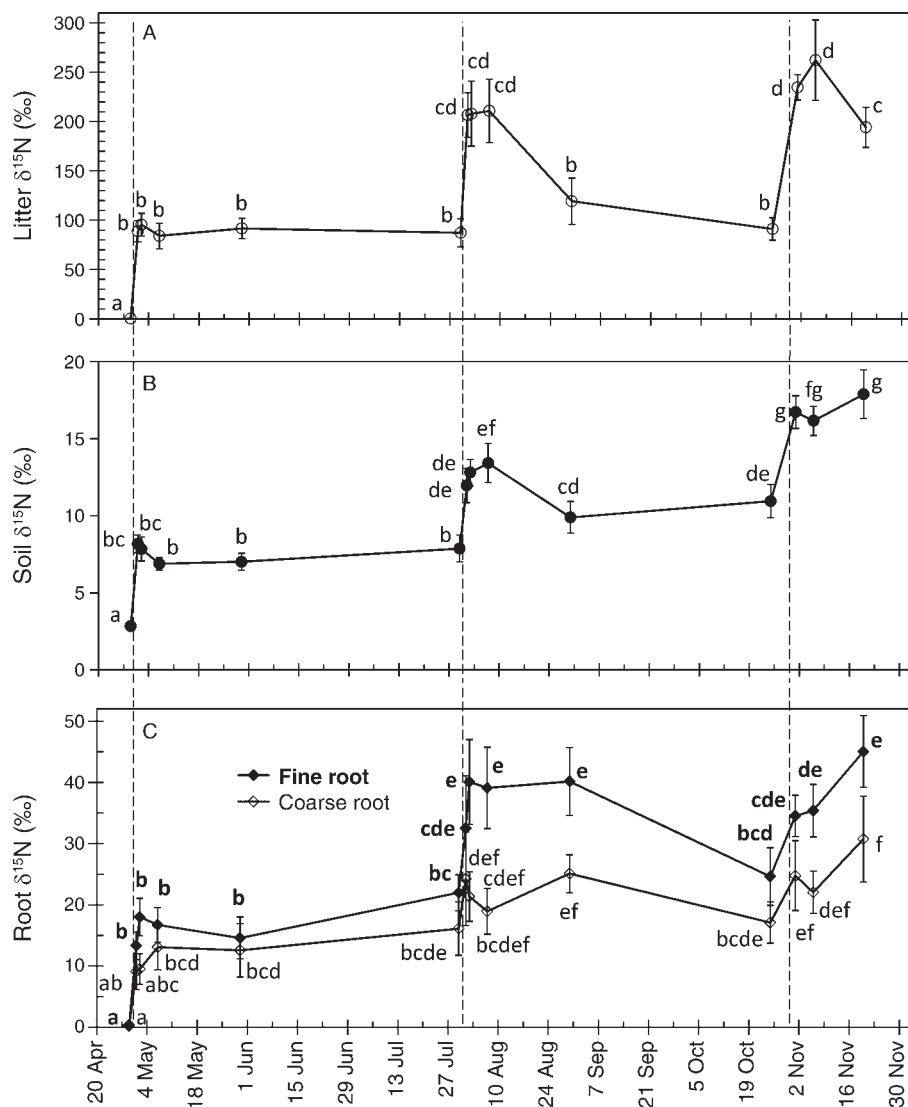


FIG. 3. Patterns of  $\delta^{15}\text{N}$  (mean  $\pm$  SE) enrichment of (A) litter in the  $\text{O}_i$  layer, (B) surface soil (0–10 cm), and (C) fine and coarse roots at 0–10 cm depth. Different letters indicate significantly different  $\delta^{15}\text{N}$  values ( $P < 0.05$ ) on different sampling dates; bold letters correspond to fine roots and lightface letters correspond to coarse roots. Dashed vertical lines indicate dates of tracer additions.

(Fig. 5) or over half (57%) of the  $^{15}\text{N}$  recovered in the soil pool (Table 2). Recovery of  $^{15}\text{N}$  in the heavy fraction did not vary significantly by date, with similar recovery on days 1–30 (Fig. 5). After the summer tracer application, additional  $^{15}\text{N}$  was recovered in soil during the first week, amounting to 52% of this tracer addition. Soil then lost  $^{15}\text{N}$  between day 7 and 30 afterward, reducing recovery in this pool to  $\sim 30\%$  of the summer addition. In autumn, soil retained additional  $^{15}\text{N}$  amounting to 59% of this tracer application. Overall, the combined detrital pools ( $\text{O}_i$  and soil) formed the largest fate for added  $^{15}\text{N}$  after all three tracer additions. Retention was brief in summer and greatest in autumn, consistent with patterns expected if the supply of particulate and dissolved organic C from leaf-fall

spurred microbial demand for N, although substantial recovery also occurred in the mineral soil and its heavy fraction, which were not expected to support high rates of microbial immobilization.

#### *$^{15}\text{N}$ recovery in plant pools*

The small pool of N in fine roots (29 kg N/ha) demonstrated similar temporal trends in  $^{15}\text{N}$  enrichment as the  $\text{O}_i$  and soil pools, increasing by the same amount ( $\sim 16\%$ ) after all three additions (Fig. 3C). Fine-root  $^{15}\text{N}$  enrichment persisted throughout the 90 days after the spring addition, but declined between day 30 and 90 after the summer addition. Coarse root N (39 kg N/ha) had a slower increase in  $^{15}\text{N}$  enrichment relative to the other pools: significant increases in  $\delta^{15}\text{N}$  occurred by

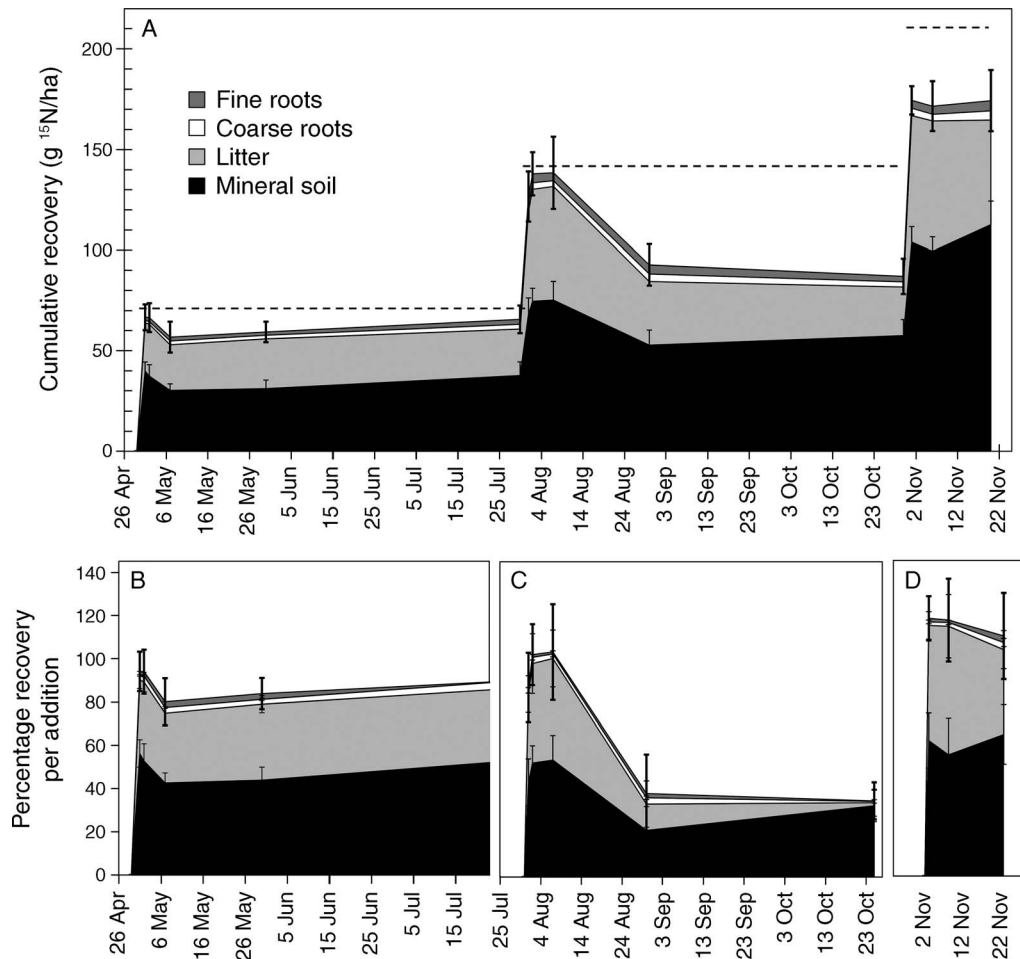


FIG. 4. (A) Cumulative (mean  $\pm$  SE) recovery of added  $^{15}\text{N}$  in the  $\text{O}_i$  litter layer and in soil and fine and coarse roots at 0–10 cm depth. Dashed horizontal lines indicate the cumulative amount of  $^{15}\text{N}$  added in increments of 70 g/ha on 30 April, 31 July, and 30 October 2007. Estimated recovery of new label in each pool is shown following the (B) spring, (C) summer, and (D) autumn tracer additions.

day 7 after the spring and day 20 after the summer  $^{15}\text{N}$  additions (Fig. 3C). Fine and coarse roots each accounted for a minor amount ( $\leq 2\%$  each) of tracer  $^{15}\text{N}$  that did not vary by season.

Litterfall consisted primarily of deciduous leaves (84%), with small contributions from hemlock needles (5%), twigs (8%), and other litter (3%; mostly seeds). The small flux of N in leaf litterfall (12.8 kg  $\text{N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ ) showed modest  $\delta^{15}\text{N}$  enrichment (13.6‰) and amounted to just 0.3% of the total  $^{15}\text{N}$  addition. The unvarying amount of  $^{15}\text{N}$  recovered in roots and the small amount of  $^{15}\text{N}$  found in roots or litterfall overall countered the expectation that plant uptake of  $^{15}\text{N}$  has a substantial role in its seasonal retention patterns.

#### Gas fluxes

Soil respiration showed typical seasonal patterns of slow rates in spring, a midsummer peak, and a decrease in autumn (Fig. 6A), with rates similar to those observed

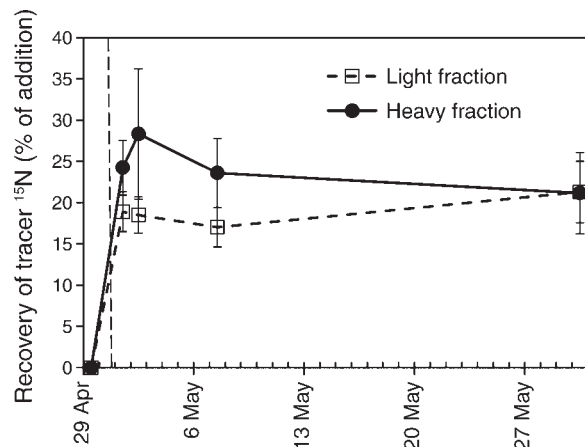


FIG. 5. Recovery of added  $^{15}\text{N}$  (mean  $\pm$  SE) in soil (to 10 cm depth,  $<2$  mm material) light and heavy fractions following the spring tracer addition. The dashed vertical line indicates the date of tracer additions.

TABLE 2. Characteristics and distribution of mass, C, N, and recovered  $^{15}\text{N}$  tracer (mean  $\pm$  SE) between light and heavy soil fractions (0–10 cm, <2 mm material) for the spring tracer addition.

Characteristics and distribution	Light fraction	Heavy fraction
C concentration (mg/g)	232 $\pm$ 8	36 $\pm$ 2
N concentration (mg/g)	8.5 $\pm$ 0.3	3.3 $\pm$ 0.2
C:N ratio (g/g)	27.5 $\pm$ 0.8	11.1 $\pm$ 0.3
$\delta^{15}\text{N}_{\text{pre}}$ (‰)	1.5 $\pm$ 0.3	1.5 $\pm$ 0.2
$\delta^{15}\text{N}_{\text{post}}$ (‰)	7.4 $\pm$ 0.8	4.6 $\pm$ 0.6
Dry mass (% total)	12.7 $\pm$ 2.5	87.3 $\pm$ 2.5
C stock (% total)	51 $\pm$ 6	49 $\pm$ 2
N stock (% total)	29 $\pm$ 7	71 $\pm$ 3
$^{15}\text{N}$ tracer (% total)	43 $\pm$ 6	57 $\pm$ 12

Note:  $n=20$  samples per date, for each of five dates (29 April, 1 May, 2 May, 7 May, 30 May).

previously at nearby sites within Arnot Forest (Fisk et al. 2004). Seasonal patterns of soil respiration broadly corresponded with temperature, although rates in spring, when soils were very wet, were lower than might have been expected based on soil temperature alone (Fig. 6B).

Fluxes of  $\text{N}_2\text{O}$  regularly fell below detection limits. Detectable fluxes of  $0.1\text{--}28\ \mu\text{g N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$  did occur at 1–6 of the 18 collars on all but one sampling date, and all but one collar had measureable fluxes on at least one date. Averaged across all collars, mean  $\text{N}_2\text{O}$  fluxes during spring ( $0.1\text{--}0.7\ \mu\text{g N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ) were lower than during summer ( $1.2\text{--}5.7\ \mu\text{g N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ) or autumn ( $0.7\text{--}3.2\ \mu\text{g N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ ; Fig. 6A). If the overall mean flux rate of  $1.2\ \mu\text{g N}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$  applied for 200 days of the April–November measurement period,  $\text{N}_2\text{O}$  loss amounted to roughly  $0.06\ \text{kg N/ha}$ , although the variability of  $\text{N}_2\text{O}$  fluxes made this estimate extremely crude.

We did not directly measure losses of tracer  $^{15}\text{N}$  as N gas ( $\text{NO}$ ,  $\text{N}_2\text{O}$ , or  $\text{N}_2$ ), but made some inferences about gaseous  $^{15}\text{N}$  losses based on the unrecovered or “missing”  $^{15}\text{N}$  tracer. High rates of total  $^{15}\text{N}$  tracer recovery during spring ( $83\text{--}94\% \pm 7\text{--}11\%$ ; mean  $\pm$  SE) indicated that N gas losses could not have exceeded 6–17% of the tracer addition (Fig. 4B). Much larger losses occurred during summer of up to two-thirds (66%) of this tracer addition. During days 1–7 after the summer addition, we recovered  $^{15}\text{N}$  amounting to all ( $101 \pm 19\%$ ) of this addition, but recovery fell to  $45 \pm 18\%$  by

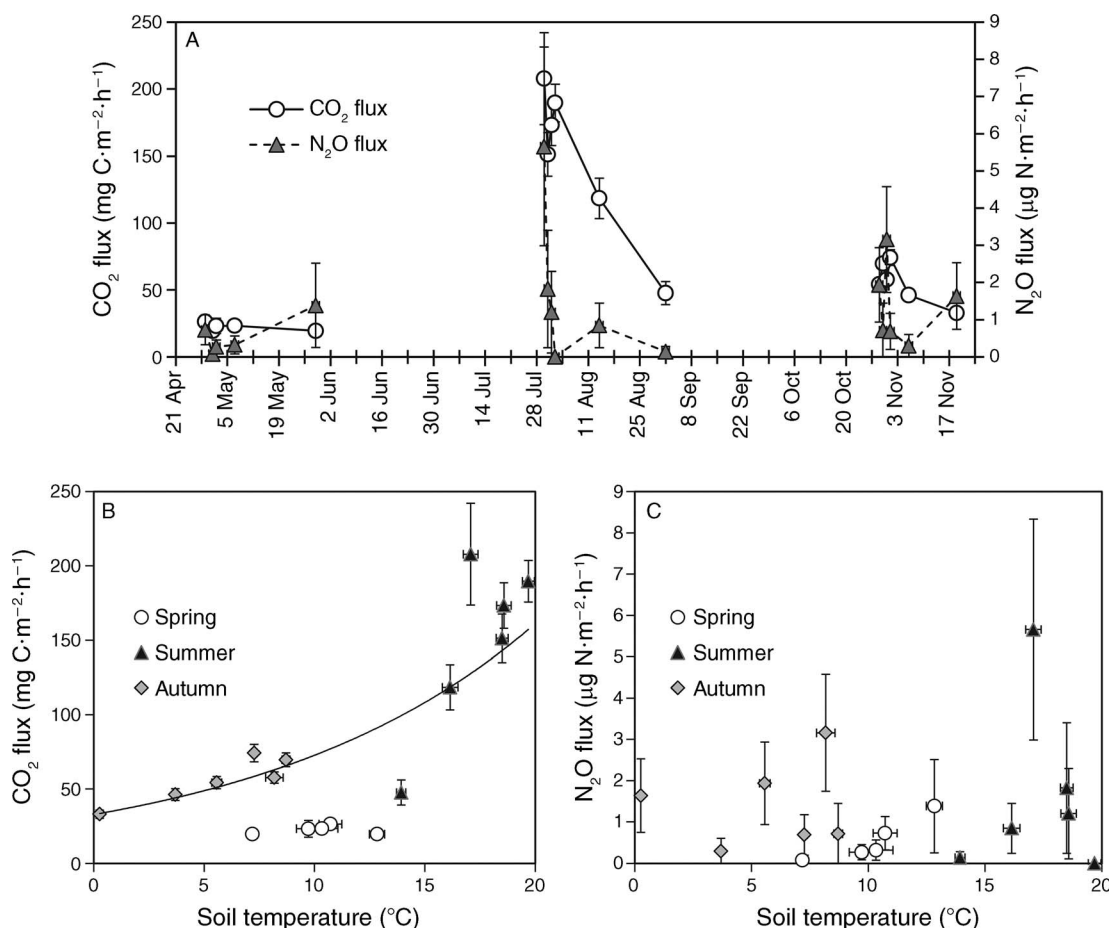


FIG. 6.  $\text{CO}_2$  and  $\text{N}_2\text{O}$  fluxes (mean  $\pm$  SE) (A) over the growing season and (B, C) in response to variations in soil temperature at 10 cm depth.

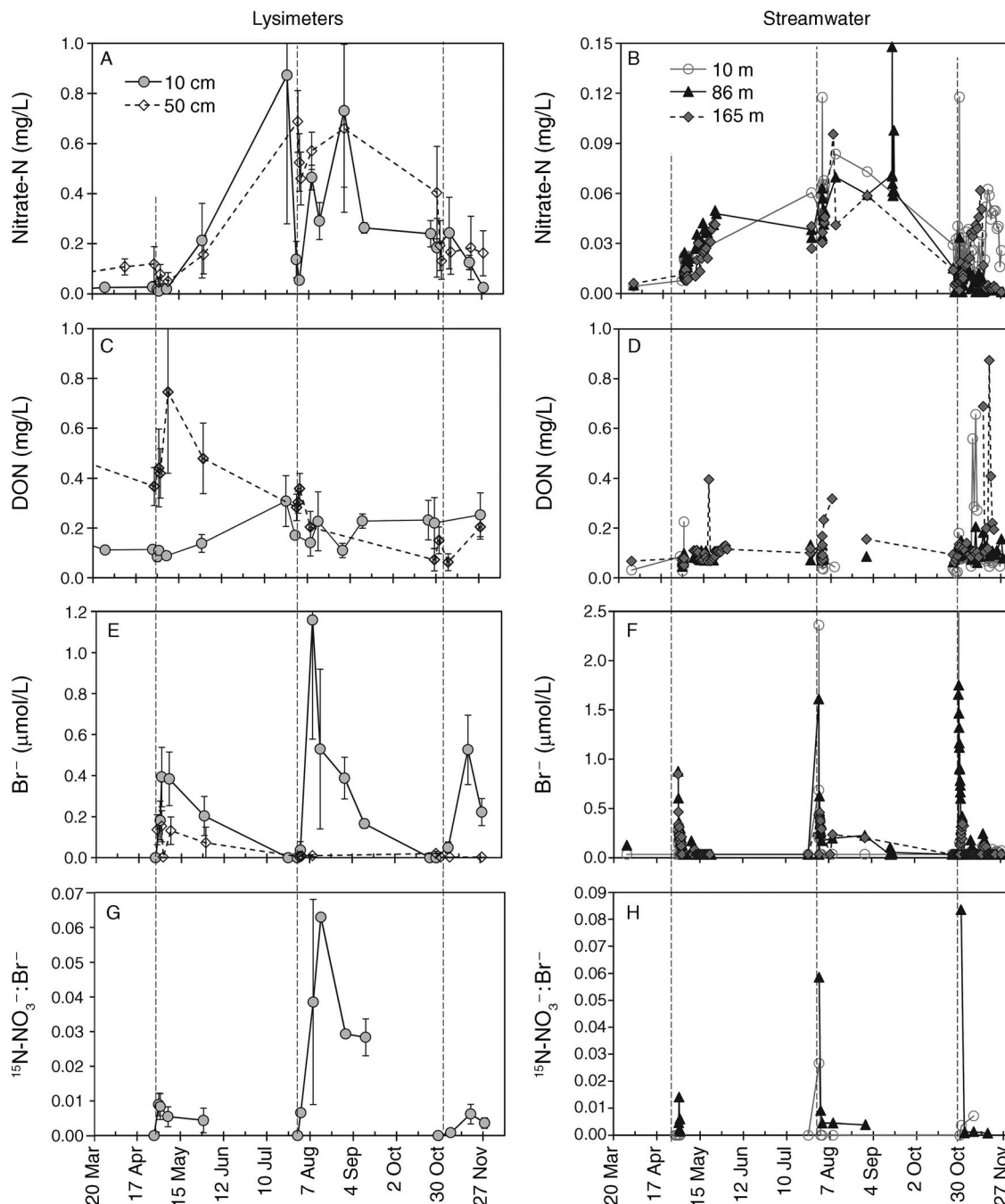


FIG. 7. Concentrations of soil water (left column) and streamwater (right column) for (A, B) nitrate, (C, D) dissolved organic nitrogen, (E, F) bromide, and (G, H) tracer  $^{15}\text{N-NO}_3^-:\text{Br}^-$  molar ratio. Values are mean  $\pm$  SE for 12 lysimeters composited to up to six analyses per date or for streams collected 10 m (mid-plot), 86 m (bottom of plot), and 165 m downstream from the spring source of Pine Creek. Dashed vertical lines indicate dates of tracer additions.

day 30 and  $34 \pm 9\%$  by day 90 (Fig. 4C). In autumn, recovery of additional  $^{15}\text{N}$  amounting to  $108 \pm 15\%$  of this application showed complete retention in the measured pools. Together, the  $\text{N}_2\text{O}$  measurements and patterns of unrecovered  $^{15}\text{N}$  indicate that larger gaseous losses of  $^{15}\text{N}$  likely occurred during summer than during the spring or fall.

#### Soil water

In the shallow lysimeters (10 cm depth),  $\text{NO}_3^-$  concentrations were very low throughout early 2007. They rose in late May, peaked in June and July at  $>0.75$  mg N/L, then dropped to  $\sim 0.20$  mg N/L from mid-September onward (Fig. 7A). Dissolved organic N

(DON) concentrations remained stable throughout the year at  $\sim 0.10$  to  $0.20$  mg/L (Fig. 7C). Tracer  $\text{Br}^-$  and  $^{15}\text{NO}_3^-$  were both detected in the shallow lysimeters after all three additions (Fig. 7E, G). Lysimeter  $\delta^{15}\text{N-NO}_3^-$  values reached 33–1712‰ (data not shown). Tracer  $^{15}\text{N}$  recovery amounted to  $<0.07$   $\mu\text{g } ^{15}\text{N-NO}_3^-/\text{L}$  following the spring and autumn tracer additions and up to  $0.44$   $\mu\text{g } ^{15}\text{N-NO}_3^-/\text{L}$  in early August. Bromide and  $^{15}\text{N-NO}_3^-$  were applied at equimolar concentrations:  $<1\%$  of the added  $^{15}\text{N-NO}_3^-$  was recovered relative to  $\text{Br}^-$  following the spring and autumn additions, with 3–6% recovered following the summer addition (Fig. 7G). This pattern supported the hypothesis that leachate  $^{15}\text{N-NO}_3^-$  loss should vary inversely with its retention in plant and detrital pools, driven at this site by variation in  $^{15}\text{N}$  retention in  $\text{O}_i$  material and soil. To roughly estimate the flux of tracer  $^{15}\text{NO}_3^-$  that reached 10 cm, measured concentrations of  $\text{NO}_3^-$  and tracer  $^{15}\text{N-NO}_3^-$  were multiplied by daily streamflow as an approximation of water flow (Fig. 2A), with concentrations on unmeasured dates interpolated using the mean of concentrations from adjacent measurement dates. For the cumulative period of 30 April to 31 December, these estimated fluxes amounted to  $\sim 0.3$  kg  $\text{NO}_3\text{-N}/\text{ha}$  with  $0.1$  g/ha of tracer  $^{15}\text{N}$  or a mere 0.05% of the cumulative  $^{15}\text{N}$  addition.

In the deep (50-cm) lysimeters,  $\text{NO}_3^-$  concentrations displayed similar seasonal patterns as in the shallow lysimeters, with smaller seasonal amplitude (Fig. 7A). The deep lysimeters rarely had detectable  $\text{Br}^-$  concentrations (Fig. 7E) but showed enriched  $^{15}\text{N-NO}_3^-$  following the tracer addition. Nonetheless, tracer  $^{15}\text{N-NO}_3^-$  concentrations at 50 cm were very low, averaging  $<0.05$   $\mu\text{g}/\text{L}$  of tracer  $^{15}\text{N-NO}_3^-$  and amounting to a negligible flux of tracer  $^{15}\text{N}$ .

*Streamwater.*—Consistent with past measurements (Goodale et al. 2009), stream  $\text{NO}_3^-$  concentrations were low during the winter and early spring, rose throughout May, peaked during summer, and decreased in autumn (Fig. 7B). Streamwater had similar  $\text{NO}_3^-$  seasonality as the lysimeters but roughly 1/10 their peak  $\text{NO}_3^-$  concentration (Fig. 7A, B).  $\text{NO}_3^-$  concentrations differed little among the three main stream sampling sites at 10, 86, and 165 m downstream from the creek's spring source, except during late autumn, when the 10-m site had higher  $\text{NO}_3^-$  concentrations than the sites downstream. Dissolved organic carbon (DOC; data not shown) and DON (Fig. 7D) concentrations were generally low. The  $\text{Br}^-$  tracer peaked quickly and then dissipated at the three main stream sampling locations following all three additions (Fig. 7F). It fell below detection within a day at the 10-m site following all three additions and persisted just above detection limits for up to 30 d following the summer addition at the two downstream sites. The  $^{15}\text{N}$  tracer showed similar patterns, although detectable enrichment of  $^{15}\text{NO}_3^-$  persisted from the first  $^{15}\text{N}$  addition throughout the course of the study. Tracer  $^{15}\text{NO}_3^-$  recovery relative to  $\text{Br}^-$  dropped rapidly over

time after all three tracer additions (Fig. 7H). On the evenings of the dates of the summer and autumn additions, stream export of tracer  $^{15}\text{NO}_3^-$  where the stream exited the study plot (86 m) peaked at 6% and 9%, respectively, of the paired  $\text{Br}^-$  tracer; corresponding samples from the spring addition were destroyed during analysis. Tracer  $^{15}\text{NO}_3^-$  recovery fell to  $<1\%$  of  $\text{Br}^-$  in the one to two days following all three  $^{15}\text{N}$  additions. The cumulative amount of tracers lost from the plot in streamflow amounted to roughly 17% of the  $\text{Br}^-$  and 0.09% of the  $^{15}\text{N-NO}_3^-$  applications.

#### *Cumulative $^{15}\text{N}$ tracer fate*

In late November, three-quarters (77%) of the cumulative  $^{15}\text{N}$  applied (210 g/ha) was recovered in the  $\text{O}_i$  layer (25%), surface soil (48%), and in fine (2%) and coarse (2%) roots (Table 1). The remaining 23% either moved to other plant pools or deep soil, or was lost from the ecosystem. Less than 1% moved into leaf litterfall or was lost as lysimeter or streamwater  $\text{NO}_3^-$ . A quarter of the loss or movement of  $^{15}\text{N}$  to unmeasured pools occurred about 1 week after the spring addition, while the rest occurred between 1 week and 1 month following the summer application.

#### DISCUSSION

The retention and loss of tracer  $^{15}\text{N}$  varied considerably across our three seasonal applications, dominated by strong retention in litter and soil in spring and autumn and large  $^{15}\text{N}$  losses in summer, which coincided with the timing of  $^{15}\text{N-NO}_3^-$  leaching losses and stream  $\text{NO}_3^-$  peaks. Here, we discuss the likely processes governing these individual and interconnected  $^{15}\text{N}$  fates, illustrating how soil processes are central to seasonal patterns in  $^{15}\text{N}$  retention at this and possibly other sites.

#### *Plant uptake*

We hypothesized that root  $^{15}\text{N}$  uptake would peak in late spring and summer, corresponding with the phenology of plant growth and leaf production in this deciduous forest. However, our measurements showed little  $^{15}\text{N}$  recovery in roots overall (3–4%), with no seasonal variation. These  $^{15}\text{N}$  measurements did not cover all plant pools, but the root measurements likely captured the most important plant components. The very low recovery of  $^{15}\text{N}$  in litterfall ( $<0.05\%$ ; Table 1) suggests that little tracer moved to other plant parts during 2007, and subsequent measurements in 2008 found minimal additional  $^{15}\text{N}$  in all aboveground plant pools (2.6%) or in deeper roots (2.5%; Goodale et al., unpublished data). These results and those from similar studies (Nadelhoffer et al. 1999b, Templer et al. 2012) show that trees acquire only a small portion of  $^{15}\text{N}$  added as a tracer of simulated atmospheric deposition and that roots form the largest pool of plant  $^{15}\text{N}$  recovery during the first 1–3 years after tracer addition.

Plant N uptake is difficult to measure directly, and its seasonality is rarely quantified. Mass balance constraints



indicate that trees must take up a large flux of N each year to produce new leaves, wood, and other tissues, and that they acquire the great majority of this N from mineralization and mycorrhizal-mediated uptake of N from the soil rather than from deposition (e.g., Johnson 1992, Likens and Bormann 1995, Cleveland et al. 2013). At Hubbard Brook, New Hampshire, USA, a site with low stream  $\text{NO}_3^-$  in summer (Likens and Bormann 1995), direct measurements of N uptake by sugar maple and red spruce (*Picea rubens*) roots show slightly lower uptake rates in May than in July and September (Socci and Templer 2011), with relatively stable temporal patterns of uptake across these months. The  $^{15}\text{N}$  approach used here traces plant uptake of N from deposition, but does not capture uptake from soil, thus missing the magnitude and any seasonality of this large flux of N. However, the tracers did show that plant uptake of added  $^{15}\text{N}$  did not vary seasonally, and that the large decline in  $^{15}\text{N}$  recovery in summer was not driven by plant uptake but instead was dominated by variation in soil retention processes.

#### Soil retention

Our tracer results showed strong retention of added  $^{15}\text{N}$  in detrital pools following the spring and autumn additions, but only transient retention following the summer addition. We can only speculate as to why detrital  $^{15}\text{N}$  retention varied so greatly across the three seasons and what processes drove these responses. Our measurements of  $^{15}\text{N}$  recovery in bulk soil pools represent the combined responses of  $^{15}\text{N}$  in several different soil components, including soluble extractable N, microbial biomass, soil organic matter (SOM), and clay particles. Past studies in temperate forests show that additions of  $^{15}\text{NH}_4$  or  $^{15}\text{NO}_3$  are usually consumed within a day and that microbial biomass often dominates the initial sink for added  $^{15}\text{N}$  on scales of hours to about a week after which recovery decreases sharply; retention by SOM can occur rapidly and persistently, dominating the remaining  $^{15}\text{N}$  recovery for months to years (Seely and Lajtha 1997, Zogg et al. 2000, Perakis and Hedin 2001, Holub and Lajtha 2004, Providoli et al. 2006). Incorporation of tracer  $^{15}\text{N}$  into SOM can occur through a range of processes, including: accumulation of N from microbial necromass; condensation of N in microbial enzymes with soil phenolic compounds; sorption of DON onto SOM; reaction of  $\text{NH}_4^+$  or  $\text{NO}_2^-$  with SOM; and incorporation of  $\text{NH}_4^+$  into clay lattices. The exact mechanisms of  $^{15}\text{N}$  incorporation remain uncertain, especially for  $^{15}\text{NO}_3^-$ , which must be reduced to a more reactive form of N to react with SOM (e.g., Johnson et al. 2000, Davidson et al. 2003, Colman et al. 2007, Morier et al. 2008, Lewis and Kaye 2012). Under reducing conditions,  $\text{NO}_3^-$  can be reduced abiotically to  $\text{NO}_2^-$ , especially at low pH (McBride 1994; Kizewski et al., *in press*). Lab  $^{15}\text{N}$  experiments with forest soils show more SOM retention of  $\text{NO}_2^-$  than  $\text{NO}_3^-$  or  $\text{NH}_4^+$  and greater retention by sterile soils

than live (Fitzhugh et al. 2003a, b, Lewis and Kaye 2012).

At our site, during the spring tracer addition, soils were mostly saturated following recent snowmelt and rain, resulting in low rates of soil respiration (Fig. 6B). If low these low soil respiration rates signify low rates of microbial growth and  $^{15}\text{N}$  immobilization, abiotic processes may have dominated  $^{15}\text{N}$  retention in these wet, iron-containing soils, perhaps via reduction by  $\text{Fe}^{2+}$  (Davidson et al. 2003). Much of this soil  $^{15}\text{N}$  retention occurred rapidly, and much of it went into the soil heavy fraction. Microbial immobilization and turnover do not easily explain this  $^{15}\text{N}$  sink. The movement of  $^{15}\text{N}$  into the heavy fraction within one day of tracer addition occurred more swiftly than expected for the timescale turnover of  $^{15}\text{N}$  immobilized in microbial biomass, typically reported as days to weeks (e.g., Seely and Lajtha 1997, Zogg et al. 2000, Perakis and Hedin 2001). Further, the heavy soil fraction typically consists of very old SOM considered relatively protected from microbial consumption (Sollins et al. 1999, Gaudinski et al. 2000). A tracer study in Switzerland also found that the majority of soil  $^{15}\text{N}$  retention occurred in their most stable soil pool, the clay fraction (Hagedorn et al. 2005). Thus, the cool and wet conditions in spring could have favored abiotic  $\text{NO}_3^-$  reduction and reaction with SOM, driving rapid  $^{15}\text{N}$  retention in detrital pools, especially in stable heavy-fraction material.

During summer, transient microbial  $^{15}\text{N}$  retention may have had a greater role in  $^{15}\text{N}$  retention than in spring. Soil respiration peaked in summer, when temperatures were highest (Fig. 6A), indicating faster rates of root and heterotrophic microbial respiration and a correspondingly greater capacity for microbial  $^{15}\text{N}$  immobilization. We did not partition  $^{15}\text{N}$  into microbial biomass, but past  $^{15}\text{N}$  studies have shown rapid but short-term immobilization of added  $^{15}\text{N}$  in microbial biomass, with sharp declines after 1–2 weeks (Seely and Lajtha 1997, Zogg et al. 2000, Perakis and Hedin 2001, Providoli et al. 2006). In our study, the abrupt decrease in  $^{15}\text{N}$  recovery in  $\text{O}_i$  material and soil between days 7 and 30 after the summer addition (Fig. 4) had a similar temporal pattern as that observed for turnover of soil microbial biomass in other studies. The capacity for microbial N immobilization may have been particularly C-limited at our site compared to many others. Invasion by nonnative earthworms has left only a small pool of litter and soil C in the top 10 cm (Table 1; Bohlen et al. 2004, Fahey et al. 2011), and the soil C/N ratio is low (15), below the typical threshold for microbial N immobilization (20–25).

The autumn tracer addition occurred when conditions were reasonably well-suited to microbial N immobilization: soils were moist but not saturated, and leaf-fall supplied fresh inputs of labile C. Retention of  $^{15}\text{N}$  in detrital pools persisted throughout the three weeks of autumn sampling, longer than for the summer tracer addition. Lower rates of respiration in cooler conditions



PLATE 1. Autumn tracer addition to Pine Creek catchment, Arnot Forest, New York, USA. Photo credit: C. L. Goodale.

and increased availability of C provided by leaffall could have perhaps sustained longer microbial immobilization in autumn than during summer.

#### *Nitrogen gas losses*

Gaseous losses of N can occur as  $\text{NO}$ ,  $\text{N}_2\text{O}$ , and  $\text{N}_2$  produced by both nitrification and denitrification. We expected little effect of nitrification on the fate of our  $^{15}\text{NO}_3^-$  tracer and focused on denitrification, in which microbes use  $\text{NO}_3^-$  as a terminal electron acceptor when  $\text{O}_2$  is limiting. Measured  $\text{N}_2\text{O}$  fluxes were very low, but denitrification losses of  $\text{N}_2$  can be large even when  $\text{N}_2\text{O}$  losses are not. At Hubbard Brook, New Hampshire, USA, measured  $\text{N}_2:\text{N}_2\text{O}$  loss ratios from surface soils average 73–210 (Kulkarni et al. 2014) or 5–15 (Morse et al. 2015). Applying these ratios to our roughly estimated  $\text{N}_2\text{O}$  flux of  $0.06 \text{ kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$  yields a range of possible  $\text{N}_2$  flux of 0.3–0.9 up to 4–12  $\text{kg N}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ .

Increased  $^{15}\text{N}$  loss to denitrification during the lower moisture conditions of summer may seem counterintuitive, but denitrification increases steeply with temperature and can occur rapidly under moist conditions with moderate  $\text{O}_2$  levels (Morse et al. 2015). The presence of soil redoximorphic features at this site indicated the presence of anaerobic microsites, and large rainfall events between day 7 and 30 after the summer  $^{15}\text{N}$  addition could have further stimulated denitrification. Our measurements of  $\text{N}_2\text{O}$  loss varied greatly but peaked in summer (Fig. 6A). Prior natural abundance measurements at Pine Creek and other nearby streams showed enrichment of  $\delta^{15}\text{N}_{\text{NO}_3}$  during summer along with increased stream  $\text{NO}_3^-$  concentration, speculated as possibly due to a summer increase in both nitrifica-

tion and denitrification (Goodale et al. 2009). At Hubbard Brook, New Hampshire,  $\text{N}_2\text{O}$  emissions, denitrification enzyme activity, and potential N mineralization and nitrification rates all increase during summer relative to spring or autumn (Bohlen et al. 2001, Werner et al. 2011), and dual isotopic natural abundance measurements of  $\text{NO}_3^-$  ( $\delta^{15}\text{N}_{\text{NO}_3}$ ,  $\delta^{18}\text{O}_{\text{NO}_3}$ ) in shallow groundwater provide strong evidence for midsummer denitrification (Wexler et al. 2014).

#### *Hydrologic N losses*

Leaching of tracer  $^{15}\text{N}\text{-NO}_3^-$  peaked in summer, when shallow lysimeters captured a small flush of  $^{15}\text{NO}_3^-$  in the first large rainstorm. Yet, losses of tracer  $^{15}\text{N}\text{-NO}_3^-$  to lysimeter water were very small, amounting to <1% of the paired  $\text{Br}^-$  tracer in spring and autumn and only 3–6% in summer, with a cumulative leaching flux <0.05% of added  $^{15}\text{N}\text{-NO}_3^-$ . At Plynlimon, Wales, leaching to 10–100 cm depth amounted to 19% of tracer  $^{15}\text{N}\text{-NO}_3^-$  relative to  $\text{Br}^-$  within four hours of application along with a 1-cm flush of water (Evans et al. 2008). In both studies, comparison to a conservative  $\text{Br}^-$  tracer confirms that most of the added  $^{15}\text{N}\text{-NO}_3^-$  was rapidly retained, transformed, or lost above or before collection of soil water and was not simply missed due to imprecision in lysimeter sampling or delayed solute transport.

Tracer  $^{15}\text{N}\text{-NO}_3^-$  also disappeared quickly from the stream, such that at most 9% of the  $^{15}\text{N}\text{-NO}_3^-$  tracer was recovered relative to  $\text{Br}^-$  as the stream exited the plot during the hours immediately following the tracer additions to the plot and to the stream surface. Overall, the stream exported <0.1% of applied  $^{15}\text{N}\text{-NO}_3^-$ ,

although additional losses might have occurred during unmeasured periods. At other sites, streamwater  $^{15}\text{N-NO}_3^-$  losses accounted for 5–10% of added  $^{15}\text{NH}_4^{15}\text{NO}_3$ , occurring primarily during the period of tracer application and up to three to six months thereafter (Nadelhoffer et al. 1999a, Schleppi et al. 1999, Kjonaas and Wright 2007), although these other studies all also included 25–35 kg N·ha<sup>-1</sup>·yr<sup>-1</sup> of experimental fertilizer additions, treatments which reduce ecosystem  $^{15}\text{N}$  retention and increase hydrologic losses (e.g., Tietema et al. 1998, Nadelhoffer et al. 1999b, Templer et al. 2012).

Rapid transformation of tracer  $^{15}\text{N-NO}_3^-$  to DON is a possible alternative form of tracer leaching  $^{15}\text{N}$  loss (e.g., Seely and Lajtha 1997, Dail et al. 2001, Perakis and Hedin 2001; Kizewski et al., *in press*). However, DON concentrations in our shallow lysimeters usually fell below those for  $\text{NO}_3^-$ , and DON was unlikely to have been as enriched as  $\text{NO}_3^-$  in  $\delta^{15}\text{N}$ . Both factors would produce a smaller flux of tracer loss as  $^{15}\text{N-DON}$  than the negligible  $^{15}\text{N-NO}_3^-$  losses we observed. At Gårdsjön, Sweden, catchment additions of  $^{15}\text{NH}_4^{15}\text{NO}_3$  yielded much smaller (28%) stream losses of tracer  $^{15}\text{N}$  as DON than as  $^{15}\text{N-NO}_3^-$  (Kjonaas and Wright 2007). Our sampling did not encompass all forms of hydrologic  $^{15}\text{N}$  loss, but overall, hydrologic losses of tracer  $^{15}\text{N-NO}_3^-$  were very small relative to changes in  $^{15}\text{N}$  retention in soil and surface litter.

#### *Seasonality of ecosystem N retention*

To date, seasonal variation in ecosystem N retention processes have largely been inferred from patterns of stream  $\text{NO}_3^-$  (e.g., Stoddard 1994, Brookshire et al. 2011, Ohte 2012). The typical pattern of low  $\text{NO}_3^-$  concentrations during the growing season and higher losses during the dormant season is often interpreted as a reflection of plant N demand or degree of N saturation of the plant–soil system (Stoddard 1994, Goodale et al. 2000, Lovett et al. 2000, Wright et al. 2001). Yet, some catchments demonstrate nearly the opposite pattern marked by a rise of  $\text{NO}_3^-$  in summer, spurring reexamination of the drivers of seasonal N retention processes (Mulholland 2004, Goodale et al. 2009, Brookshire et al. 2011, Ohte 2012). Catchments with relatively high stream  $\text{NO}_3^-$  in summer could simply reflect delayed export of  $\text{NO}_3^-$  by deep flow paths that decouple terrestrial processes from streams (Burns et al. 1998, Ohte 2012). At our site, however, streamwater and soil water both show similar seasonal patterns of summer  $\text{NO}_3^-$  peaks, with tenfold higher concentrations in soil leachate than in streamwater (Fig. 7). We previously proposed that these observations could reflect increases in net nitrification in soil along with partial consumption of  $\text{NO}_3^-$  by denitrification (Goodale et al. 2009).

In this tracer study, measurements of the fate of added  $^{15}\text{N}$  support the primary role of soil processes governing  $^{15}\text{N}$  retention and loss. Like most other  $^{15}\text{N}$  tracer

studies in forests (e.g., Seely and Lajtha 1997, Tietema et al. 1998, Nadelhoffer et al. 1999b, Templer et al. 2012), the great majority of  $^{15}\text{N}$  recovery occurred in the  $\text{O}_i$  layer and surface soils. Unlike these other studies, our tracer additions showed strong seasonal variation in these detritus-associated  $^{15}\text{N}$  sinks, ranging from near-complete and persistent  $^{15}\text{N}$  retention following the spring and fall additions, including a large, rapid  $^{15}\text{N}$  sink into mineral-associated (heavy) soil material, to transient retention and subsequent loss following the summer addition. Plants took up little if any of the  $^{15}\text{N}$  lost from soil during summer, which instead may have been lost partly as N gases. The large loss of  $^{15}\text{N}$  from soil allowed both denitrification and  $^{15}\text{N-NO}_3^-$  leaching to peak in summer. The concurrent decrease in detrital  $^{15}\text{N}$  retention, increase in  $^{15}\text{N-NO}_3^-$  losses to soil water, and peaks in soil- and streamwater  $\text{NO}_3^-$  concentrations all appear to together provide strong evidence for the primary role of soil processes in regulating seasonal patterns of  $^{15}\text{N}$  retention and loss at this site and perhaps for other catchments with similar seasonal stream  $\text{NO}_3^-$  patterns.

In other temperate deciduous forests, net mineralization and nitrification rates typically peak in summer relative to spring and fall (e.g., Nadelhoffer et al. 1983, Bohlen et al. 2001), associated with summer increases in microbial biomass (Bohlen et al. 2001) and the strong effect of temperature on nitrification (Stark 1996, Brookshire et al. 2011). Large increases in soil N-cycling rates in summer should increase the N supply to multiple fates that could include plant uptake, denitrification, and leaching. Seasonal leaching losses of  $\text{NO}_3^-$  should dip in summer if N consumption processes increase more quickly than the supply rate or rise if nitrification rates exceed increases in loss processes. We observe that streams with the typical summer  $\text{NO}_3^-$  dips often occur in regions where soils form thick surface organic layers, such as in the northern Appalachian Mountains (Goodale et al. 2000, Lovett et al. 2000), Scandinavia, and peaty regions of the UK (Wright et al. 2001). By contrast, many of the catchments with summer  $\text{NO}_3^-$  peaks occur in warmer regions in the southeastern U.S. and Japan and contain soils that often lack this thick organic layer or have lower carbon contents (Band et al. 2001, Mulholland 2004, Brookshire et al. 2011, Ohte 2012). The location of our site in central New York occurs geographically closer to the northern sites, yet stream  $\text{NO}_3^-$  seasonality follows the southern pattern, perhaps due partly to the site's thin forest floor forming a limit on its capacity to retain N as cycling rates increase in summer. Forest soils with thick organic horizons and high C:N ratios have a greater capacity to retain new  $^{15}\text{N}$  (Lewis and Kaye 2012) and to retain N overall (e.g., Gundersen et al. 1998) relative to lower-carbon sites; soil carbon status may also form an important factor governing seasonality of N retention. Combining new seasonal  $^{15}\text{N}$  studies with mass balance analyses at a range of sites is needed to test the role of



soil processes in producing varying seasonal N retention and loss patterns.

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