Ecology, 85(6), 2004, pp. 1725–1739

A STABLE ISOTOPE TRACER STUDY OF NITROGEN UPTAKE AND TRANSFORMATION IN AN OLD-GROWTH FOREST STREAM

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Abstract. The understanding of nitrogen dynamics in streams of temperate forest biomes historically has been constrained by a combination of anthropogenic disturbances and technical limitations. We report here on a study in an undisturbed stream in Oregon, USA, using a stable isotopic tracer to quantify uptake, transformation, and retention of nitrogen. We added $^{15}NH_4Cl$ for six weeks to Mack Creek, a third-order stream in a 500-year-old-growth coniferous forest and monitored ^{15}N in dissolved, aquatic, and terrestrial riparian food web components. Data collected before, during, and for four weeks after the tracer addition allowed us to derive uptake rates of inorganic N and to trace its fates. Short uptake lengths (35–55 m) and residence times (8–12 min) of ammonium indicated strong demand. Despite nitrate concentrations of 55–68 μg/L, nitrification rates were also high, with 40–50% of the $^{15}NH_4^+$ converted to nitrate over the 220-m study reach. Aquatic bryophytes and biofilm on large wood (''epixylon'') showed the highest biomass-adjusted uptake rates. All aquatic consumers sampled, both vertebrate and invertebrate, showed incorporation of tracer ^{15}N by the end of the experiment; small invertebrate grazers were more strongly labeled than their food sources. Increased ^{15}N label in 15 of the 17 riparian plant species sampled suggested transfer of aquatic N to the terrestrial ecosystem. At the end of the release, 81% of the added tracer was accounted for, with 49% exported (primarily as $^{15}NO_3^-$) and 32% retained within the stream and riparian biota (primarily by bryophytes, epixylon, and fine benthic organic material). Our results suggest that, in streams within undisturbed primary forests, uptake and retention of nitrogen may be highly efficient and that there may be strong connections between terrestrial and aquatic ecosystems.

Key words: food web; nitrification; nitrogen cycle; old-growth forest; retention; riparian; stable isotope; stream ecosystem; tracer addition.

INTRODUCTION

Nitrogen is a potentially limiting nutrient in aquatic and terrestrial ecosystems (Grimm and Fisher 1986), controlling both ecosystem productivity and dynamics. The impacts of anthropogenic changes to the global nitrogen budget have been increasingly noted in freshwater ecosystems (e.g., Vitousek et al. 1997). In temperate forested stream ecosystems, most nitrogen studies (e.g., Vitousek 1977, Meyer et al. 1981, Mulholland et al. 2000*a*) have been conducted in regions subjected to varying degrees of anthropogenic disturbance. Human influences range from land use, such as agriculture and forest harvest (e.g., Likens et al. 1970), to atmospheric nitrogen deposition (e.g., Goodale et al. 2000). Studies from less-impacted regions in North (Sollins et al. 1980, Triska et al. 1984) and South (Hedin et al. 1995) America demonstrate these anthropogenic influences have altered the form and processing of nitrogen (Perakis and Hedin 2002).

Numerous studies have clearly demonstrated that the transformation and assimilation of nitrogen within stream ecosystems are complex and highly efficient (e.g., Davis and Minshall 1999, Dent and Grimm 1999, Mulholland et al. 2000*a*, Peterson et al. 2001). Although streams have been viewed as conduits for nitrogen from terrestrial to marine ecosystems (e.g., Howarth et al. 1996), they can also modify inputs from terrestrial ecosystems, altering the form of nitrogen and timing of its processing as it is transported from drainage basins along river networks (Martí and Sabater 1996, Alexander et al. 2000, Peterson et al. 2001). Within the aquatic ecosystem, the rates and pathways of nitrogen uptake and transformation are varied. The dissolved nitrogen species, ammonium and nitrate, can be directly incorporated by aquatic primary producers, such as algae, or by components of the detrital pool, such as bacterial and fungal films on dead material

Manuscript received 21 January 2003; revised 10 September 2003; accepted 16 October 2003. Corresponding Editor: B. Downes.

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(Grimm and Fisher 1986). In addition, ammonium can be transformed into nitrate by nitrifying microorganisms (e.g., Richey et al. 1985). Alternatively, dissolved inorganic nitrogen could be taken up by streamside vegetation and potentially removed from the aquatic ecosystem (e.g., Pinay et al. 1998). Until recently, quantification of these various pathways and transfer of nitrogen through the food web has been limited by lack of a whole-system technique to detect the potential fates of dissolved nitrogen (Peterson et al. 1997).

The stable isotope of nitrogen (^{15}N) provides a tracer method for simultaneous study of the dynamics of inorganic forms of dissolved nitrogen and their uptake and transformation by stream biota, without the confounding effects of fertilization (Peterson et al. 1997, Mulholland et al. 2000*a*, Tank et al. 2000). We conducted a continuous six-week addition of ^{15}N-labeled ammonium to trace nitrogen fates in a third-order stream within a forested montane basin. This experiment was part of the Lotic Intersite Nitrogen eXperiment (LINX) project, a comparative study of stream nitrogen dynamics across different biomes in North America (Mulholland et al. 2000*a*, Peterson et al. 2001). Unlike other forested streams of the LINX cohort (Eastern United States: Michigan, North Carolina, New Hampshire, Puerto Rico, Tennessee), this experiment occurred in a pristine, old-growth, coniferous forest in western Oregon, a region with low atmospheric inputs of nitrogen (Vanderbilt et al. 2002). The contrast in the extent of human disturbance of these forested sites creates an opportunity to better understand the effects of forest age and riparian interactions on nitrogen cycling in stream ecosystems.

Based on previous studies in streams of North America and our experiences with nutrient addition experiments in local streams, we hypothesized that (1) nitrification would account for the largest fraction of ammonium uptake and transformation; (2) biotic uptake of ammonium would be highest for epilithic biofilms; (3) storage of added ^{15}N would be greatest in the fine benthic organic material pool due to its large standing stocks; (4) uptake of aquatic ^{15}N would be negligible in nearby terrestrial species such as riparian plants or terrestrial consumers of aquatic species.

Site Description

Mack Creek is a third-order stream located within the H. J. Andrews Experimental Forest in the western Cascade Mountains of Oregon, USA. The 640-ha basin is dominated by 400–500-year-old coniferous trees, predominantly Douglas-fir (*Psuedotsuga menziesii*), western hemlock (*Tsuga heterophylla*), and western red cedar (*Thuja plicata*). Understory streamside vegetation includes two species of maple and >50 species in the shrub and herb layers (Gregory et al. 1991). The site annually receives a mean of 2300 mm precipitation (Bierlmaier and McKee 1989). Summer months (late June through August) are typically dry, with the bulk of the precipitation falling as either rain or snow from November through March. Mean discharges in the study reach are 569 L/s at winter base flow and 59 L/s during summer. Mean annual air temperature is 8.5°C. The reach contains no summer-flowing tributaries and is a high gradient (>9%), single constrained channel dominated by bedrock (35%), cobble (23%), and gravel (34%) substrata, with few areas of fine sediment deposition. Aquatic habitats within the reach are 45% pools and 55% fast-water (riffles, rapids, and cascades). Mean wetted stream width during the study was 5.1 m, and mean depth was 15 cm, although some pools had depths up to 60 cm. The reach included two large (>2 m high) channel-spanning jams, consisting of hundreds of pieces of wood, which trapped substantial volumes of cobble and gravel.

Methods

The basic LINX protocol consisted of an intensive two-week characterization of existing physical, chemical, and biological conditions within the study reach. We then added ^{15}N-labeled NH_4Cl continuously for six weeks and tracked its fate through stream and riparian ecosystems during and for four weeks after the tracer addition. The ^{15}N addition was deliberately scheduled for July–August during the anticipated season of lower stream discharge and higher rates of biotic activity. We also performed limited sampling one year afterwards. Details of each portion of the study are provided below.

Ecosystem characterization

Continuous discharge was measured at a gaging station approximately 500 m downstream of the addition site. Site-specific discharge estimates were calculated from short-term (2 h) conservative tracer (chloride) releases conducted before and during the ^{15}N-tracer addition (Mulholland et al. 2000*a*). Benthic metabolism was estimated using the methods of Mulholland et al. (2001). Preliminary estimates of uptake lengths for inorganic nutrients (NH_4^+-N, NO_3^--N, PO_4^{-3}-P) were derived from short-term (2 h) enrichment methods (Stream Solute Workshop 1990) one week prior to the start of the ^{15}N-tracer addition and were used to establish the length of the study reach. Water samples for major dissolved nutrients were collected weekly at all sample sites, filtered (Whatman GF/F glass fiber), and frozen for later analysis. Nitrate (precision to 1 μg/L) was analyzed using automated cadmium reduction, ammonium (2 μg/L) with automated phenate methods, total dissolved nitrogen (6 μg/L) with Kjeldahl digestion, and phosphorus (1 μg/L) with persulfate digestion (at the Cooperative Chemical Analytical Laboratory, Oregon State University, Corvallis, Oregon, USA).

Standing stocks (in grams per square meter) of all food web components were measured one week prior to the start of the ^{15}N-tracer addition. Samples of each component, ranging from primary producers to vertebrate consumers, were collected from pools and riffles.

Standing stocks were calculated for the entire reach based on habitat-weighted means. All samples were dried at 60°C, weighed, and combusted at 500°C to calculate ash-free dry mass (AFDM). Subsamples were dried and analyzed for nitrogen and carbon content (as percentages) using a Carlo Erba Model 1500 Elemental Analyzer (Carlo-Erba, Milan, Italy; at the University of Georgia Soils Laboratory, Athens, Georgia, USA). Total standing stock of N per unit area for each component was calculated from percentage of N values and areal dry mass.

Primary uptake components consisted of submerged bryophytes, epilithon (biofilm, primarily diatoms, on rocks), biofilm on submerged wood (dubbed ''epixylon'' and consisting primarily of bacteria and fungi with some diatoms), suspended particulate organic material (SPOM, >1.2 μm), fine benthic organic material (FBOM, <1.0 mm), and coarse benthic organic material (CBOM, >1.0 mm). Epilithon, SPOM, and FBOM were sampled using procedures outlined in Mulholland et al. (2000*a*) and Tank et al. (2000). CBOM was separated into leaves and small (<5 cm) chunks of well-rotted wood. Accumulations of deciduous leaves in the stream were absent during summer, so conifer needles (primarily Douglas-fir and western hemlock) were collected. Bryophytes were scraped from substrata, separated into liverworts and mosses, rinsed, and examined under a microscope to remove any trapped invertebrates. Standing stocks of epixylon were determined from scrapings of known surface areas. These values were extrapolated to the entire reach by calculating surface areas of all wood submerged within the wetted channel at low flow. Wood surface areas were based on annual large wood surveys (S. V. Gregory, *unpublished data*) and 10 line transects (Wallace and Benke 1984) for small pieces of wood (<1 m long and <10 cm diameter).

Benthic invertebrate consumers were collected in 15 replicate Surber samples (0.1 m^2, 250-μm mesh). Taxa were divided into functional feeding groups (collector, scraper, shredder, gatherer, predator; Merritt and Cummins [1984]), and a dominant species was selected to represent each group for isotope sampling. Vertebrate consumer biomass was measured at the end of the tracer addition using two-pass electroshocking (Armour et al. 1983). Vertebrates included tailed-frog tadpoles (*Ascaphus truei*), Pacific giant salamanders (*Dicamptodon tenebrosus*), and cutthroat trout (*Onchorhyncus clarkii clarkii*). Trout were further categorized as young-of-the-year and adult.

^{15}N tracer addition

A battery-powered peristaltic pump dripped 2 mL/min stock solution of 0.03 g/L ^{15}N-NH_4Cl continuously for 42 days, from 21 July to 1 September 1998. Stock concentrations were calculated to increase the $\delta^{15}N$ of dissolved NH_4^+ by 500‰, given an initial discharge of 75 L/s and background NH_4^+-N concentrations of 4 μg N/L. We located the injection site in a turbulent cascade to ensure thorough mixing. Sampling stations were established 5 m above (for background isotopic values) and 25, 50, 75, 115, 150, 190, and 220 m below the dripper.

Food web components were sampled at all stations prior to the start of the addition and then weekly for six weeks during and four weeks after the injection (except day 7 sampling, postponed two days due to pump difficulties). Water was pumped through GF/F filters to collect SPOM. For all benthic food web components, we collected 3–6 samples per station and combined them to make one composite sample. Because numbers of vertebrate consumers were low, we collected only one trout fry and one tailed-frog tadpole per station per date. Salamanders, adult tailed frogs, and adult trout were collected only on the last day of isotope addition. On the final day of tracer release, we sampled riparian vegetation growing within 1 m of the edge of the active channel (mean high flow channel), which was as much as 2 m lateral distance from the wetted channel. Leaves of 17 plant species were collected 50 m above the addition site (for background isotopic signatures) and adjacent to the 75-m site. We also collected leaves, roots, and stems of four herbaceous species, *Athyrium filix-femina*, *Oxalis oregona*, *Stachys cooleyae*, and *Tolmiea menziesii* adjacent to each sampling station. Ouzels (dipper birds, *Cinculus mexicanus*) were caught in mist nets within the addition reach and in an adjacent basin, and blood samples were taken from the live birds. Invertebrate and small vertebrate consumers (tadpoles and trout fry) were held overnight to clear their guts prior to drying (Mulholland et al. 2000*b*). Larger aquatic vertebrates were killed humanely and their intestinal tracts discarded. All organic matter samples were dried at 50°C and ground to a fine powder. Analysis for ^{15}N content was performed with a Finnigan Delta S mass spectrometer (Finnigan, San Jose, California, USA; at the Ecosystems Center, Woods Hole, Massachusetts, USA).

Samples for dissolved inorganic nitrogen (DIN) were collected six hours after the addition began, 20 and 41 days later, and 12 hours after the addition ceased. Samples for $^{15}NH_4^+$ and $^{15}NO_3^-$ analysis were taken at all eight sample stations, plus two additional samples for $^{15}NO_3^-$ located 370 m and 420 m below the dripper. Collection and analysis of DI^{15}N fractions followed LINX protocols (Mulholland et al. 2000*a*, Hamilton et al. 2001). Estimates of $\delta^{15}N$ for dissolved organic nitrogen (DON) were derived by subtracting the contributions of $^{15}NH_4^+$ and $^{15}NO_3^-$ from total dissolved N.

Equations and calculations

All ^{15}N:^{14}N ratios are expressed as $\delta^{15}N$ (in per thousands) according to the equation

$$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R is the ^{15}N:^{14}N ratio and the standard is air. All

$\delta^{15}N$ values reported here have been corrected for background natural abundance and therefore include only tracer ^{15}N: these values are shown as $\Delta^{15}N$. If background $\delta^{15}N$ changed over the course of the study, mean component background values were used. Standing stocks were estimated once prior to the addition; for a given compartment, the total N biomass was assumed to be constant, while the level of ^{15}N-labeled biomass varied. Biomass of ^{15}N for each compartment was a function of its $\Delta^{15}N$ value and N standing stock. Except where noted, data points represent single composite samples for a given station at a given time.

Calculations of dissolved nitrogen parameters included fluxes, uptake lengths, and rates (for both NH_4^+ and NO_3^-), mass transfer coefficients (V_f, or uptake velocity, the velocity of a dissolved nutrient moving from water to substrata), nitrification rate, NH_4^+ regeneration rates, and N turnover rates and times. We used models and methodology described in Mulholland et al. (2000*a*) and Tank et al. (2000) (expanded in Hamilton et al. [2001] and Merriam et al. [2002]). Uptake lengths (the distance a dissolved nutrient travels before being immobilized) were calculated from both the 2-h enrichment prior to the isotope release and from the $\Delta^{15}N$ values of dissolved NH_4^+ and NO_3^- on days 0, 20, and 41; the latter were corrected for NH_4^+ regeneration from the biota (Mullholland et al. 2000*a*). Uptake rates for each primary food web component were derived from their $\Delta^{15}N$ on day 9, the first major sampling date after the addition began, relative to the mean level of dissolved $\Delta^{15}NH_4^+$ on day 0 and day 9 (Mulholland et al. 2000*a*). Retention and export of ^{15}N were calculated by mass balance of all ^{15}N added over the six-week period (4.062 g). Retention for each component within the 220-m reach was based on the downstream decline in component-specific ^{15}N biomass ($^{15}N_b$) on day 42. If the slope of the regression of $\ln(^{15}N_b)$ vs. distance was not significant at $P < 0.05$, we substituted the mean $^{15}N_b$ for the reach (Hamilton et al. 2001). We estimated rates of export of ^{15}N as $^{15}NH_4^+$, $^{15}NO_3^-$, DO^{15}N, and particulate SPO^{15}N during the 42-day addition. Fluxes for $^{15}NH_4^+$ and $^{15}NO_3^-$ at the downstream-most site were interpolated between day 0 and day 20, between day 20 and day 41, then summed over the duration of the release. We have DO^{15}N measurements only for day 42 and assumed a linear increase from day 0 to day 42 (Hamilton et al. 2001).

Results

Stream conditions

Discharge and water temperature in Mack Creek during the ^{15}N tracer addition were typical for mid- to late summer. Discharge declined over the 42 days of the isotope release (Fig. 1); stream temperature averaged 13.1 ± 1.2°C over the same period. Concentrations were low for NH_4^+ (mean 2 μg N/L) and moderate for NO_3^- (mean 54 μg N/L). Nitrate concentration increased over the duration of the experiment, presumably as a function of declining discharge, whereas NH_4^+ remained low (Fig. 1). Solar radiation (as PAR) and benthic metabolism were measured on days 9 and 10 during a period of partially overcast skies (Mulholland et al. 2001). Mean light intensity during this period was 3.8 $mol \cdot m^{-2} \cdot d^{-1}$. Gross primary productivity (GPP) was 1.9 g $O_2 \cdot m^{-2} \cdot d^{-1}$, but community respiration (CR) averaged 11.0 g $O_2 \cdot m^{-2} \cdot d^{-1}$, indicating the stream ecosystem was heterotrophic (P/R ratio = 0.17).

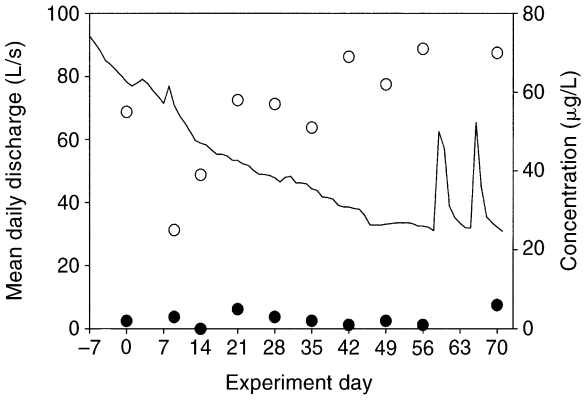

FIG. 1. Discharge (solid line) and ammonium (solid circles) and nitrate (open circles) concentrations at Mack Creek in the western Cascade Mountains of Oregon, USA. Addition began on day 0 (21 July 1998) and ended on day 42 (1 September 1998). Detection limit for NH_4^+ is 5 μg N/L. Mean concentrations of other nutrients were: dissolved organic nitrogen (DON), 50 μg N/L; soluble reactive phosphorus (SRP), 13 μg P/L; total phosphorus (TP), 19 μg P/L. Mean pH was 7.5.

Standing stocks

Standing stocks of organic material in Mack Creek were dominated by large wood (Table 1). However, the portion interacting with stream water is limited to a thin surface biofilm (Aumen et al. 1985), the epixylon. Nitrogen standing stocks, excluding large wood, totaled 4.0 g N/m^2 and were dominated by detrital components; FBOM alone comprised 77% of the total (Table 1). Bryophytes had higher nitrogen stocks than epilithon (Table 1); these in-stream primary producers accounted for 8.8% of the total N standing stock. Consumer nitrogen standing stock was similar to that of primary producers, 0.35 g N/m^2 (8.9%). Vertebrates comprised the majority of the consumer AFDM and nitrogen storage, with insects contributing only 8% of consumer N.

Biomass estimates of terrestrial riparian herbaceous (leaves, stems, and roots), shrub, and woody (leaves only) vegetation were derived from earlier summer surveys in the study reach (Gregory et al. 1991). Standing stocks for herbaceous and deciduous species were 28.6 g AFDM/m^2; when conifer needles were included this increased to 1298 g AFDM/m^2. N content ranged from

TABLE 1. Abundance (ash-free dry mass, AFDM), N content, and standing stocks of biomass components in Mack Creek in the western Cascade Mountains of Oregon, USA.

Component	Representative sampled	AFDM (g/m^2)	%N (dry mass)	C:N	N standing stock (g N/m^2)
Primary uptake					
Riparian plants					
Herbaceous	*A.f., O.o., S.c., T.m.*	28.6	2		0.62
Total riparian		1298.1	1–2		24.53
Epilithon		2.9	1.5	6.7	0.08
Bryophytes		16.2	1.2	21.3	0.24
Conifer needles		0.4	1.1	45.9	0.01
Small wood (<5 cm diameter)		3.9	0.8	59.6	0.22
Large wood (>10 cm diameter)		9600.0	0.8	59.6	79.87
Epixylon		0.8	1.4	23.7	0.64
FBOM		108.6	0.9	27.2	3.04
SPOM (g/L)		<0.0003	0.4	8.2	
Primary consumers					
Invertebrate shredders	Peltoperlidae	0.02	10.6	4.5	0.002
Invertebrate scrapers	Heptageneidae	0.09	10.5	4.4	0.011
Invertebrate filterer	*Parapsyche*	0.02	11.2	4.0	0.002
Invertebrate gatherers	Baetidae	0.02	10.8	4.6	0.003
Vertebrate scraper	*A. truei* tadpole	0.44	10.7	4.3	0.052
Secondary consumers					
Invertebrate predator	*Calineuria* sp.	0.09	11.3	4.2	0.011
Vertebrate predator	*O. clarki* fry (age 0+ yr)	0.09	12.2	3.9	0.015
Vertebrate predator	*O. clarki* adult (age 1+ yr)	0.58	10.3	5.5	0.080
Vertebrate predator	*D. tenebrosus*	1.25	12.7	3.7	0.177
Total stream		9735.4			83.8
Without large wood		135.4			3.9
Total stream and riparian		11,033.5			108.4
Without large wood		1433.5			28.5

Notes: Representative riparian plants: *Athyrium filix-femina* (*A.f.*), *Oxalis oregona* (*O.o.*), *Stachys cooleyae* (*S.t.*), and *Tolmiea menziesii* (*T.m.*). Large wood biomass was calculated from annual surveys (S. V. Gregory, *unpublished data*). FBOM, fine benthic organic material; SPOM, suspended particulate organic material.

1% to 2%, depending on species (coniferous vs. deciduous) and plant part (leaf vs. root) (Gholz et al. 1985). The amount of N stored in this component is high due to these large standing stocks (Table 1), but does not include stem biomass of large conifers.

DIN dynamics

The $\Delta^{15}NH_4^+$ in the water column increased at the upstream-most sites on consecutive sample days due to declining discharge and constant ^{15}N addition rate (Fig. 2A). Ammonium uptake lengths, computed from the decline in $^{15}NH_4^+$ flux (Fig. 3A–D) and corrected for regeneration of $^{15}NH_4^+$, declined over time, from 54.8 to 35.6 m, as would be expected with diminishing stream flows (Table 2). Reach uptake rates also decreased, from 0.72 to 0.27 $\mu g\ N \cdot m^{-2} \cdot s^{-1}$ with the greatest differences between day 0 and day 20. The mass transfer coefficient, V_f, which controls for the changes in discharge by removing the areal component (Peterson et al. 2001, Webster et al. 2003), indicates uptake was similar on all dates (Table 2). Residence times for $^{15}NH_4^+$ in water were short (<12 min) and also declined over time.

Nitrification was detectable despite low NH_4^+ concentrations: $^{15}NO_3^-$ was measured only six hours after the isotope addition began (Figs. 2B, 3A–D). The percentage of total $^{15}NH_4^+$ removed from the water column and attributed to nitrification averaged 45.7% (Table 2). ^{15}N-labeled nitrate was still present in the stream 14 hours after the dripper was turned off (day 43, Fig. 3D), probably from regenerated $^{15}NH_4^+$ that was nitrified. Fluxes of $^{15}NH_4^+$ in water were lower than those for $^{15}NO_3^-$ after the tracer was stopped (day 43), reflecting both rapid uptake of regenerated NH_4^+ and conversion to NO_3^-. We cannot explain the high $^{15}NH_4^+$ flux at the most downstream site (Fig. 3D); it may indicate either sample contamination or slow release of labeled NH_4^+ from a perched side channel immediately upstream. The higher NO_3^- concentrations during and after the addition (Fig. 1) were reflected in long uptake lengths (>1 km; Table 2). Residence times for NO_3^- were greater than those for NH_4^+, ranging from 327 to 244 min, whereas uptake rates were relatively constant. However, uptake velocities (V_f) declined slightly over time, also reflecting the increase in ambient NO_3^- concentrations.

Total uptake of DIN was calculated as the sum of assimilatory NH_4^+ uptake rate (i.e., total NH_4^+ uptake rate minus nitrification) and NO_3^- uptake rate (Mulholland et al. 2000*a*). DIN uptake rates declined from 1.1 to 0.6 $\mu g\ N \cdot m^{-2} \cdot s^{-1}$ between days 0 and 20 (Table 2), then showed little change between days 20 and 41.

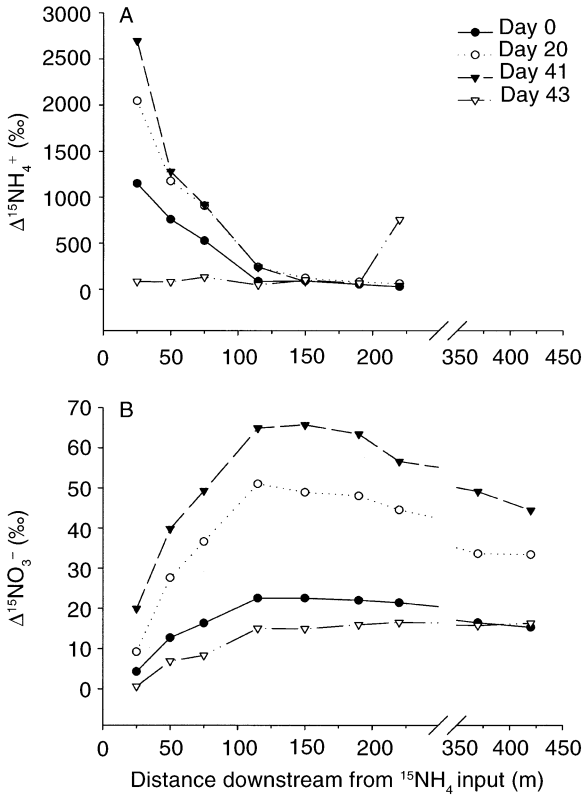

FIG. 2. $\Delta^{15}N$ labels for (A) NH_4^+ and (B) NO_3^- within the study reach on the four days sampled. The first sample (day 0) was collected 6 hours after start of tracer addition; the last sample (day 43) was collected 12 hours after addition ended. Note two additional downstream sites for nitrate.

This pattern reflects the decline in NH_4^+ uptake rather than a change in NO_3^- uptake rates.

Biotic uptake: aquatic ecosystem

Spatial patterns of ^{15}N enrichment in aquatic components reflected longitudinal abundances of dissolved $^{15}NH_4^+$ (Fig. 4A–C), with $\Delta^{15}N$ declining rapidly as distance from the dripper increased. Aquatic primary producers labeled quickly during the addition (Figs. 4, 5A), with bryophytes becoming more enriched than epilithon (maximum $\Delta^{15}N$ for bryophytes 1500‰, for epilithon 400‰) and showing higher uptake rates (bryophytes totaled 10.2 mg $N \cdot m^{-2} \cdot d^{-1}$, epilithon 1.0 mg $N \cdot m^{-2} \cdot d^{-1}$; Table 3). When adjusted for biomass, bryophytes also had greater N-specific uptake of $^{15}NH_4^+$ (Table 3). The ^{15}N signal for all primary producers declined rapidly once the addition was stopped (Fig. 5A): most species lost 75% of their maximum label in 1 mo, indicating rapid turnover rates (Table 3).

^{15}N-labeling of biota and organic material associated with the detrital-based food web varied by component (Fig. 5B). FBOM and epixylon had rapid uptake over the first nine days (Table 3), with epixylon the most enriched (Fig. 5B). In contrast, uptake rates (and $\Delta^{15}N$ levels) for small wood chunks and conifer needles were lower (Table 3). The high uptake rates of FBOM resulted from its large standing stock: when adjusted for biomass, N-specific uptake rates were lower than for all other primary uptake components except conifer needles. With the exception of epixylon, the detrital components were not as enriched as the primary producers, suggesting large portions of their nitrogen pool were relatively inactive and turnover rates low (Table 3).

All aquatic consumers, both invertebrate and vertebrate, showed increases in $\Delta^{15}N$ by the end of the addition (Figs. 4, 5). Grazers were more enriched than detritivores, reflecting the greater $\Delta^{15}N$ of primary producers compared to detrital materials (Fig. 5A–C). In the grazing food web, both baetid and heptageneid mayflies were more strongly labeled than their food sources (i.e., epilithon, epixylon, and FBOM). The collector/filterer, the caddisfly *Parapsyche*, showed ^{15}N incorporation by day 42, but remained less enriched than its presumed food source, SPOM (Fig. 5B), whereas the shredder, a peltoperlid stonefly, was much richer in ^{15}N than any of its potential food resources. Predators were less enriched than any of their potential prey (Fig. 5C). The stonefly *Calineuria* and young-of-the-year cutthroat trout were similar in their temporal and spatial patterns of ^{15}N uptake, suggesting they feed on similar prey (Fig. 5C). Slight (<10‰) ^{15}N enrichment was observed for the larger vertebrate predators, adult cutthroat trout and salamanders.

Biotic uptake: riparian areas

After six weeks of tracer addition, the four species of plants sampled in the riparian areas adjacent to each stream sampling station showed ^{15}N enrichment in leaves, roots, and stems, with species-specific differences in the pattern of tissue enrichment (Table 4). Three species (all except *Stachys*) had their greatest $\Delta^{15}N$ label 75 m or more away from the tracer addition (Fig. 6) and were among the most highly labeled of the terrestrial plants sampled (Table 4). Among ferns, *Adiantum pedatum* was more enriched than the other three species, all of which grow in similar habitats. Most striking was the variation in maples, with *Acer macrophyllum* measurably enriched and *A. circinatum* not. Among woody shrubs, *Vaccinium* species had no enrichment, while *Ribes* and *Rubus* did. Surprisingly, the stream-dwelling perennial *Petasites fridgidus* had less ^{15}N label than the other perennials. Ouzels, small stream-feeding birds, also showed slightly elevated $\Delta^{15}N$ (Table 4).

Mass balance and carryover

On day 42 we were able to account for approximately 81% of the ^{15}N added (Table 5). Most was exported during the addition as $^{15}NO_3^-$ (42.7%), with small amounts of $^{15}NH_4^+$, $DO^{15}N$, and suspended particulates accounting for an additional 6.3%. The $DO^{15}N$ values are likely an underestimate and should be interpreted

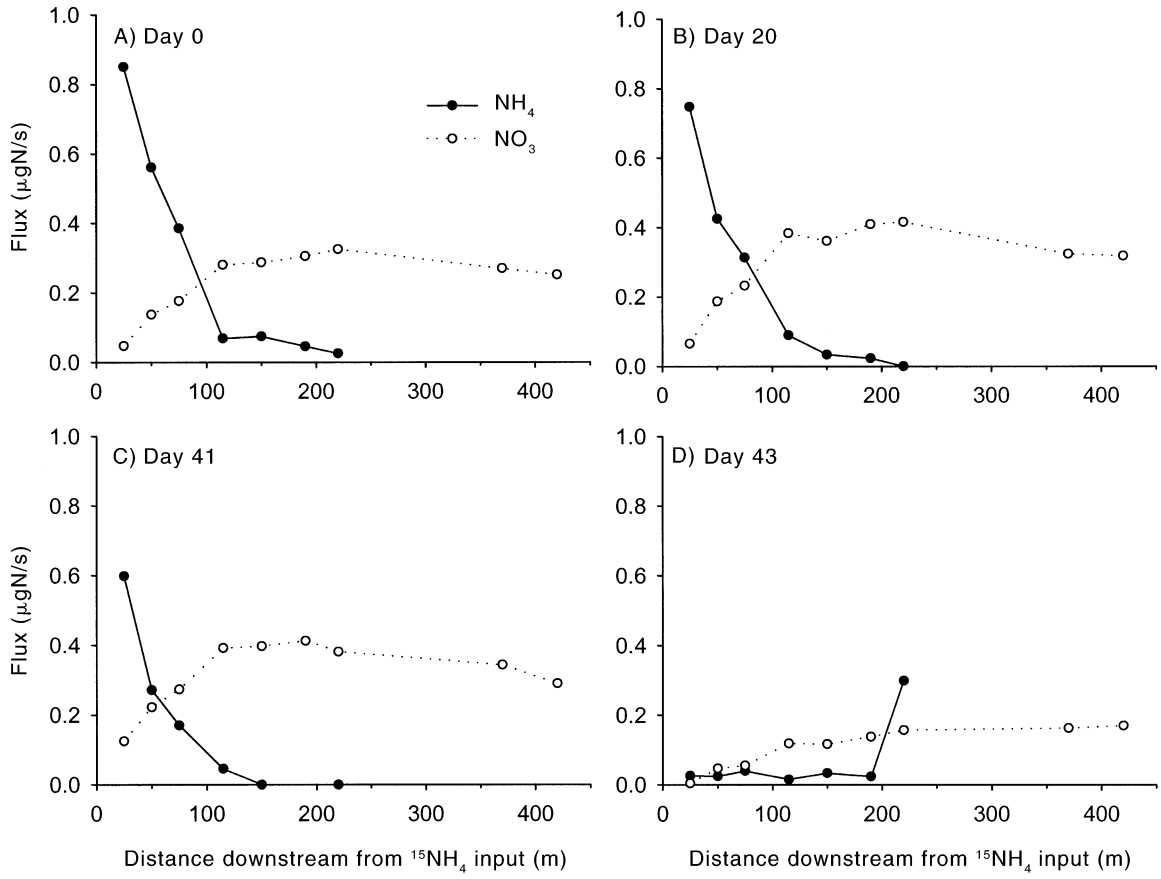

FIG. 3. Fluxes of ^{15}N-labeled ammonium and nitrate below the tracer addition site for the four sample dates. Days (A) 0, (B) 20, and (C) 41 are during the tracer addition; (D) day 43 is 12 hours after the addition stopped. Fluxes on days 20 and 41 are corrected for regeneration of NH_4 from the stream bottom; day-43 fluxes show regeneration and subsequent nitrification NH_4 to NO_3. Negative fluxes are graphed as zero.

with caution, since they are based solely on samples from the final day of ^{15}N addition. We calculated the biota retained 32.3% of the added ^{15}N label, mostly in primary uptake components. Bryophytes and epixylon were highly retentive (8.9% each) and were exceeded in storage only by FBOM (10.6%). Riparian plants had greater storage than in-stream epilithon, needles, and small wood. Consumers, both invertebrate and vertebrate, retained only a small fraction of the label, reflecting their comparatively low N standing stocks and the slow transfer of N to higher trophic levels.

One year after the tracer addition ceased, we sampled a limited number of components above the addition site and within the study reach. Of the aquatic biota, only epilithon, bryophytes, and the predaceous stonefly *Calineuria* showed evidence of tracer ^{15}N. Bryophytes at the uppermost station (25 m) had the highest Δ^{15}N levels, 30‰, with other components less enriched (Δ^{15}N for epilithon 10‰, stonefly 5‰). *Oxalis* roots and all tissues of *Tolmiea* were 2–3‰ above background, the only riparian plants to retain tracer ^{15}N.

DISCUSSION

Ecologists have examined nutrient retention and fluxes as key measures of ecosystem function (Vitousek and Reiners 1975). Basin-level studies of nitrogen dynamics and retention in terrestrial ecosystems have contributed substantially to our understanding of patterns of nutrient export (e.g., Likens and Bormann 1974, Sollins et al. 1980, Vitousek et al. 1982). However, these basin-level export measures may not be adequate indicators of total ecosystem fluxes, because aquatic ecosystems, particularly small streams, can modify nutrient form and availability (Alexander et al. 2000, Peterson et al. 2001). This study demonstrates the importance of in-stream processes on nitrogen dynamics in a pristine stream surrounded by old-growth forest, in a basin with no significant atmospheric deposition or history of land use change.

A controlled, continuous release of ^{15}N-NH_4^+ allowed us to determine rates of nitrification, uptake rates, and fluxes through multiple trophic levels of the aquatic food web (Fig. 7). Most added ^{15}N was con-

TABLE 2. Reach-scale dissolved inorganic nitrogen dynamics derived from $\Delta^{15}N$ measurements (see Figs. 1 and 3 for concentrations and fluxes).

Variable	Day 0	Day 20	Day 41
NH_4			
Uptake length (m)	54.8	50.5	46.6
Uptake length, regeneration corrected (m)	···	44.3	35.6
Uptake rate ($\mu g\ N \cdot m^{-2} \cdot s^{-1}$)	0.72	0.28	0.27
Mass transfer coefficient (V_f) (m/s)	0.00020	0.00019	0.00018
Residence time (min)	12.0	9.7	7.8
Direct nitrification			
Uptake length (m)	122.4	85.5	87.7
Uptake rate ($\mu g\ N \cdot m^{-2} \cdot s^{-1}$)	0.322	0.145	0.110
Mass transfer coefficient (V_f) (m/s)	0.00009	0.00010	0.00007
Percentage of NH_4 nitrified	44.8	51.8	40.6
Assimilatory NH_4 (nitrification corrected)			
Uptake length (m)	99.2	92.0	59.9
Uptake rate ($\mu g\ N \cdot m^{-2} \cdot s^{-1}$)	0.397	0.135	0.160
Mass transfer coefficient (V_f) (m/s)	0.00011	0.00009	0.00011
NO_3			
Uptake length (m)	1491.2	1261.4	1111.1
Uptake rate ($\mu g\ N \cdot m^{-2} \cdot s^{-1}$)	0.398	0.363	0.392
Mass transfer coefficient (V_f) (m/s)	7.3×10^{-6}	6.6×10^{-6}	5.8×10^{-6}
Residence time (min)	327.0	276.6	243.7
DIN			
Total uptake rate ($\mu g\ N \cdot m^{-2} \cdot s^{-1}$)	1.117	0.644	0.662
Percentage of assimilatory NH_4 uptake	49.9	27.1	29.0
Percentage of assimilatory NO_3 uptake	50.1	72.9	71.0

Note: DIN, dissolved inorganic nitrogen.

verted to nitrate (shading of arrows, Fig. 7) and exported from the study reach (Table 5). Primary producers showed the greatest relative increases in ^{15}N label (shading of boxes) and high retention of ^{15}N (width of boxes) despite low standing stocks (height of boxes). The detrital portion of the food web, particularly FBOM, dominated biotic nitrogen uptake and retention because standing stocks of fine detritus are larger than other biological pools. However, bryophytes and biofilms on large wood and stones showed the greatest ^{15}N incorporation and biomass-specific uptake rates (Table 3 and Fig. 7). Macroinvertebrate consumers showed different responses to enrichment: scrapers and collector-gatherers were more labeled than their food sources, while shredders and filterers were not. Enrichment at higher trophic levels was low, reflecting the short duration of the tracer addition relative to the life spans of these organisms. Enrichment was detected in riparian vegetation (Fig. 7, Table 4), suggesting terrestrial and aquatic ecosystems may be coupled in a complex manner.

Low ambient NH_4^+ concentrations, short uptake lengths, low residence times, and minimal export indicate a high biological demand for ammonium in Mack Creek. In contrast, nitrate concentrations were relatively high, resulting in lower uptake rates and longer uptake lengths and residence times. Uptake lengths for both nitrogen species declined during the release, as did discharge. As we hypothesized, despite this strong demand for ammonium, 40–50% of the $^{15}NH_4^+$ added was nitrified to $^{15}NO_3^-$ (Table 2). Nitrification rates in Mack Creek were higher than those reported from streams in the Southeast (Mulholland et al. 2000*a*, Tank et al. 2000) and similar to those found in the tropical stream in Puerto Rico (Merriam et al. 2002). Like Mack, nitrification in the Puerto Rican stream accounted for 50–60% of the labeled ammonium uptake. Rates were lower in a Michigan deciduous-forest stream (Hamilton et al. 2001), but still made up 30–50% of the total $^{15}NH_4^+$ uptake. In contrast, Tank et al. (2000) found virtually no nitrification in a North Carolina stream during autumn leaf-fall. Rapid nitrification rates indicate that, at least in forested ecosystems, this process may be an important sink for ammonium as well as a source of atmospheric N_2O (Peterson et al. 2001).

Contrary to our expectations, epilithon in Mack Creek had one of the lowest uptake rates observed for primary uptake components (Table 3). The ratios of uptake to post-decay turnover rates indicate only 25% of epilithic biomass was composed of actively cycling nitrogen, which may account for these low uptake rates. When compared to epilithon at all other LINX sites regardless of biome, the uptake rates in Mack Creek were among the lowest seen (Dodds et al. 2000, Mulholland et al. 2000*a*, Tank et al. 2000, Hamilton et al. 2001, Merriam et al. 2002).

The greatest biomass-specific uptake rates in Mack Creek were observed for bryophytes and the biofilm on large wood, epixylon. Although epilithon is the

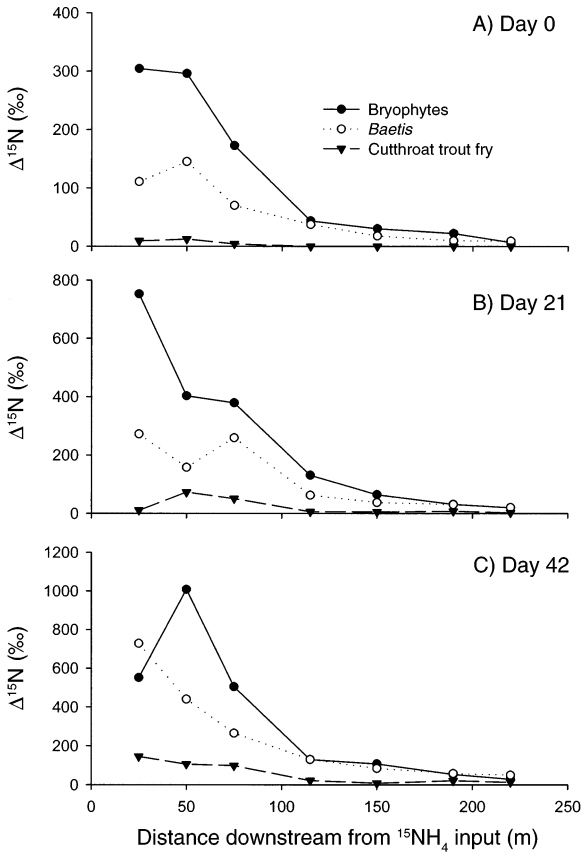

FIG. 4. Spatial trends in Δ^{15}N label in three different trophic components for sample days (A) 0, (B) 21, and (C) 42. Moss is a primary producer, *Baetis* is an herbivore, and trout fry are predators.

more frequently measured primary producer, aquatic bryophytes had greater standing stocks, uptake rates, and retention of nitrogen in this heavily shaded coniferous forest ecosystem (Fig. 7). The ratio of N uptake to post-decay turnover rates (Table 3) suggests almost all bryophyte biomass is actively cycling nitrogen. The importance of bryophytes to stream nutrient dynamics has been noted in tundra, alpine (e.g., Arscott et al. 1998), and temperate deciduous biomes (e.g., Steinman and Boston 1993), but they have been little studied in coniferous forest streams (Stream Bryophyte Workgroup 1999). Bryophytes in an open, deciduous forest stream also showed higher uptake rates and retention of ^{15}N than other primary uptake components (Mulholland et al. 2000*a*). The results from these two forested streams suggest that when present, bryophytes can play a major role in aquatic cycling of nitrogen.

The high biomass-specific uptake rates and retention observed for epixylon (Tables 3 and 5, Fig. 7) indicate this biofilm may be critical to nitrogen processing in forested streams. All LINX sites sampled small fragments of decayed wood, but epixylon was collected only at Mack Creek. At all sites, uptake rates and percentage of retention for wood fragments were among the lowest seen for any primary uptake component (Tables 3 and 5). These results are a distinct contrast to the results we found for epixylon and suggest it is essential to sample the biologically active portion of in-stream wood. The surface area created by wood of all size fractions creates a template for colonization by microbial communities (Aumen et al. 1985). Chamber studies have demonstrated rapid assimilation of nitrogen by the surfaces of wetted wood (Aumen et al. 1985). However, large wood has been assumed to be metabolically unimportant due to relatively small surface areas compared to smaller in-stream fragments of wood (Hall et al. 1998). These patterns are more likely in streams within recently disturbed forests where large wood is now scarce. However, for streams in mature forests, large wood provides an extensive surface area for microbial colonization. Measurement of epixylon uptake rates and retention at additional forested streams could provide a more complete picture of watershed nitrogen cycles.

Small invertebrate scrapers and collectors were consistently more enriched than their presumed food sources, epilithon and FBOM (Fig. 5A). This enrichment pattern was seen in a range of other invertebrate consumers at other LINX sites, including grazing mayflies in southern temperate forest streams (Mulholland et al. 2000*b*), suspension-feeding bivalves in northern temperate forest streams (Raikow and Hamilton 2001), and caddisflies and grazing shrimp in a tropical stream (Merriam et al. 2002). The relatively low percentage of actively cycling nitrogen in epilithon and FBOM at all of these sites indicates a high level of dead or refractory material in these components. Evidently the invertebrate consumers can selectively consume or assimilate a more biologically active (and thus more enriched) fraction of their food than we can sample (Mulholland et al. 2000*b*, Tank et al. 2000, Merriam et al. 2002). In contrast, invertebrate predators and vertebrates were less enriched than their food sources, presumably due to their comparatively large body mass, slow turnover rates, and longer time required to reach isotopic equilibrium.

Aquatic subsidies of terrestrial ecosystems have generally focused on food web transfers to higher trophic levels (e.g., Grimm 1988, Nakano and Murakami 2001); in Mack Creek some terrestrial predators such as ouzels and spiders were measurably enriched (Table 4; Sanzone 2001). To our surprise, however, most transfer of aquatic ^{15}N to terrestrial organisms was observed in the plant community. Our exploratory findings of enriched plant tissues in the riparian zone imply ^{15}N was being transported out of the stream. Some terrestrial plants may assimilate nitrate more rapidly than ammonium (Waring and Schlesinger 1985), so the ^{15}N enrichment in plant tissues may have reflected patterns of Δ^{15}N in nitrate rather than ammonium (Fig. 2B). However, the level of enrichment in these species

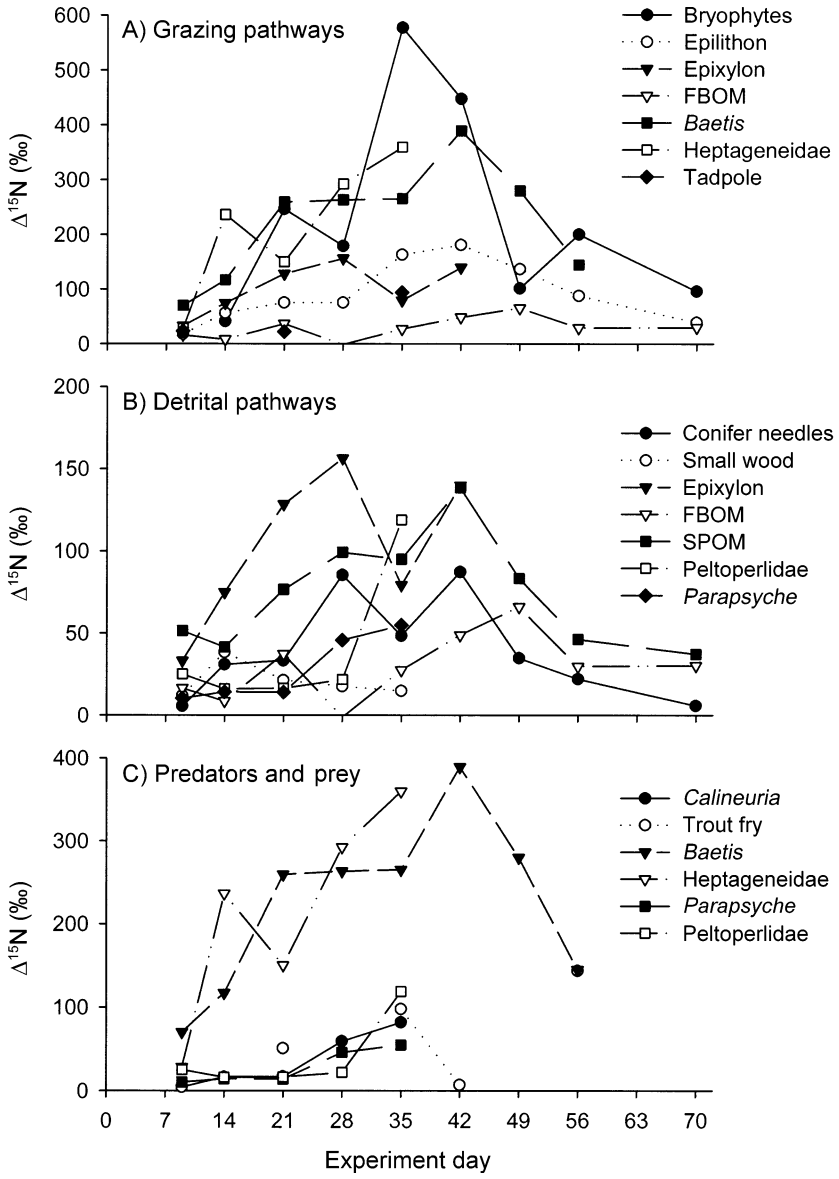

FIG. 5. Food web components 75 m below the site of $^{15}NH_4$ addition. (A) Grazing pathways: heptageneid mayflies and tadpoles are both scraping herbivores. (B) Detrital pathways: peltoperlid stoneflies feed on decaying leaves and wood; *Parapsyche* is a filter-feeding caddisfly. (C) Predators and prey: *Calineuria* is a predaceous stonefly. Abbreviations: FBOM, fine benthic organic material; SPOM, suspended particulate organic material.

ranged from 10 to 40‰ while nitrate maxima were approximately 60‰, suggesting plants were more likely using ammonium from the stream. Had plants directly used surface or subsurface water, the ^{15}N in their tissues would have declined with distance downstream, similar to the $\Delta^{15}N$ for ammonium (Fig. 2A) and aquatic organisms (Fig. 4C). However, the enrichment pattern in three of the four species sampled along the length of the study reach was higher 75 m or more downstream of the injection point (Fig. 6). This lag in ^{15}N enrichment suggests some riparian plants may use longer longitudinal subsurface flow paths that can run parallel to the stream (Wondzell and Swanson 1996). Our preliminary results suggest the interface between hyporheic waters and plant uptake would be an intriguing area for additional study.

Most nitrogen studies in forested streams have focused on within-stream processes (see reviews by Dahm et al. [1998], Fisher et al. [1998]) and have tended to view riparian vegetation primarily as an input (e.g., Vannote et al. 1980). Other studies have viewed riparian vegetation as an interceptor of nutrients in surface and subsurface hillslope water (e.g., Schlosser and Karr 1981, Peterjohn and Correll 1984). Our preliminary results of elevated ^{15}N in streamside vegetation suggest that, in this old-growth forest, riparian plants

TABLE 3. Uptake rates and turnover times for primary uptake components.

Component	Uptake rate (mg N·m^{-2}·d^{-1})	AFDM-specific uptake rate (mg N·mg AFDM^{-1}·d^{-1})	Post-drip decay turnover	
			Rate (1/d)	Time (d)
Epilithon	1.01 (0.40–1.88)	0.35	0.055	18.04
Bryophytes				
Liverworts	5.65 (3.32–10.44)	1.34	0.042	24.07
Mosses	4.53 (3.12–6.49)	1.19	0.027	37.57
Epixylon	5.76 (1.57–13.72)	7.12	0.032	31.67
FBOM	8.84 (−0.79–23.68)	0.08	0.023	43.08
Conifer needles	0.01 (−0.01–0.02)	0.02	0.094	10.65
Small wood (<5 cm diameter)	0.88 (0.38–1.69)	0.23	0.031	31.83
Total mean uptake	31.49			

Notes: Uptake rates are means of all seven sample sites below the tracer input (ranges are given in parentheses). Note relative rates of uptake when corrected for standing stocks. See Table 1 for ash-free dry mass (AFDM) and N standing stocks. Abbreviation: FBOM, fine benthic organic material.

are retaining a portion of stream nitrogen. Due to their comparatively large standing stocks (Table 1, Fig. 7), riparian plants may play a sizeable role in aquatic nutrient cycling, but this process presumably works in more than one direction: riparian plants not only remove nitrogen from surface and/or subsurface waters, they also return it in the form of leaves and wood.

We were able to account for ~81% of the tracer ^{15}N added over the six-week injection period (Table 5). Forty-nine percent was transformed into $^{15}NO_3^-$ and exported with lesser amounts of $^{15}NH_4^+$ and DO^{15}N also leaving the study reach (Table 5 and Fig. 7). We computed a total export of ~49% of the ^{15}N added, comparable to that seen in other temperate forested streams (Table 5). However, the form of the export varied among sites. For three LINX sites, Mack Creek, an eastern deciduous forest stream prior to leaf-out, and a tropical stream, most export was in the form of nitrate, reflecting the high rates of nitrification (Table 5; Mulholland et al. 2000*a*, Merriam et al. 2002). In contrast, for a Michigan stream with high ambient ammonium concentrations, most ^{15}N was exported as ammonium (Hamilton et al. 2001), while for a North Carolina stream during autumnal leaf fall, most export was suspended particulate material (Tank et al. 2000). At Mack Creek, the stream and riparian biota retained ~32.5% of the $^{15}NH_4^+$, within the range seen for the other LINX sites (Table 5). As we had anticipated, the biotic component that retained the most isotope was fine benthic organic material, a pattern seen at all LINX sites regardless of biome. FBOM had low biomass-adjusted uptake rates across all sites; however, it may still func-

TABLE 4. Δ^{15}N labeling of terrestrial species from the 75-m site on day 42.

Species	Tissue	Δ^{15}N (‰)
Conifers		
Taxus brevifolia	Leaf	1.8
Thuja plicata	Leaf	1.4
Perennials		
Oxalis oregona	Leaf	28.3
	Root	28.6
	Stem	24.8
Petasites fridgidus	Leaf	7.4
Stachys cooleyae	Leaf	18.1
	Stem	10.9
Tolmiea menziesii	Leaf	41.0
	Root	7.5
	Stem	40.3
Vertebrate		
Cinclus mexicanus	Blood	5.0
Deciduous trees and shrubs		
Acer circinatum	Leaf	−0.3
Acer macrophyllum	Leaf	4.6
Oplopanax horridum	Leaf	4.7
Ribes bracteosum	Leaf	1.2
Rubus spectabilis	Leaf	3.0
Vaccinium alaskaense	Leaf	−2.6
Vaccinium parvifolium	Leaf	−3.1
Ferns		
Adiantum pedatum	Leaf	4.7
Athyrium filix-femina	Leaf	1.9
	Root	51.7
	Stem	3.8
Blechnum spicant	Leaf	1.8
Polystichum munitum	Leaf	2.3

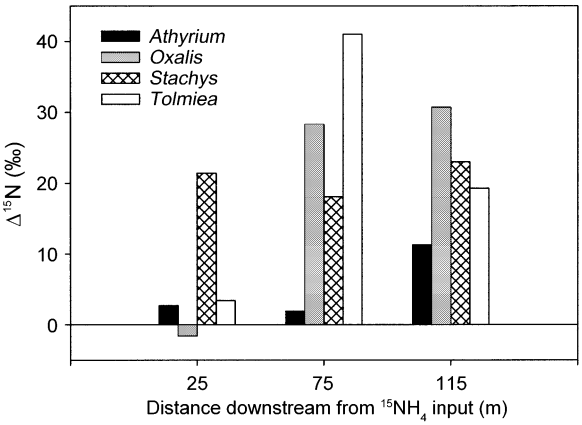

FIG. 6. Spatial patterns of Δ^{15}N accumulation in leaves of four herbaceous perennials. Data are from day 42, the final day of addition.

TABLE 5. Comparison of mass balance among forested Lotic Intersite Nitrogen eXperiment (LINX) project sites, a comparative study of stream nitrogen dynamics across different biomes in North America.

Component	Site				
	Mack	Oak Ridge	Luquillo	Coweeta	Kellogg
Stored in reach					
Epilithon	1.2	1.8	6.3	0.3	2.2
Bryophytes	8.9	19.8	···	0.1	···
Riparian plants	2.0	···	···	···	···
Small wood	0.4	3.7	0.2	···	2.2
Epixylon	9.0	···	···	···	···
Leaves/needles	0.0	8.2	0.4	9.3	0.0
FBOM	10.6	11.0	10.2	1.1	2.1
Invertebrates	0.08	3.43	0.7	1.4	0.16
Vertebrates	0.03	0.02	···	···	0.02
Total retained	32.3	47.9	17.8	12.2	6.7
Export					
SPOM and storm detritus	0.002	4.2	6.0	11.5	8.5
Ammonium	2.2	3.6	1.2	16.5	65.5
Nitrate	42.7	23.0	50.2	6.9	13.0
DON	4.2	···	7.7	6.0	10.2
Total export	49.0	30.8	65.1	40.7	97.2
Total accounted for	81.3	78.7	82.9	53.0	103.9

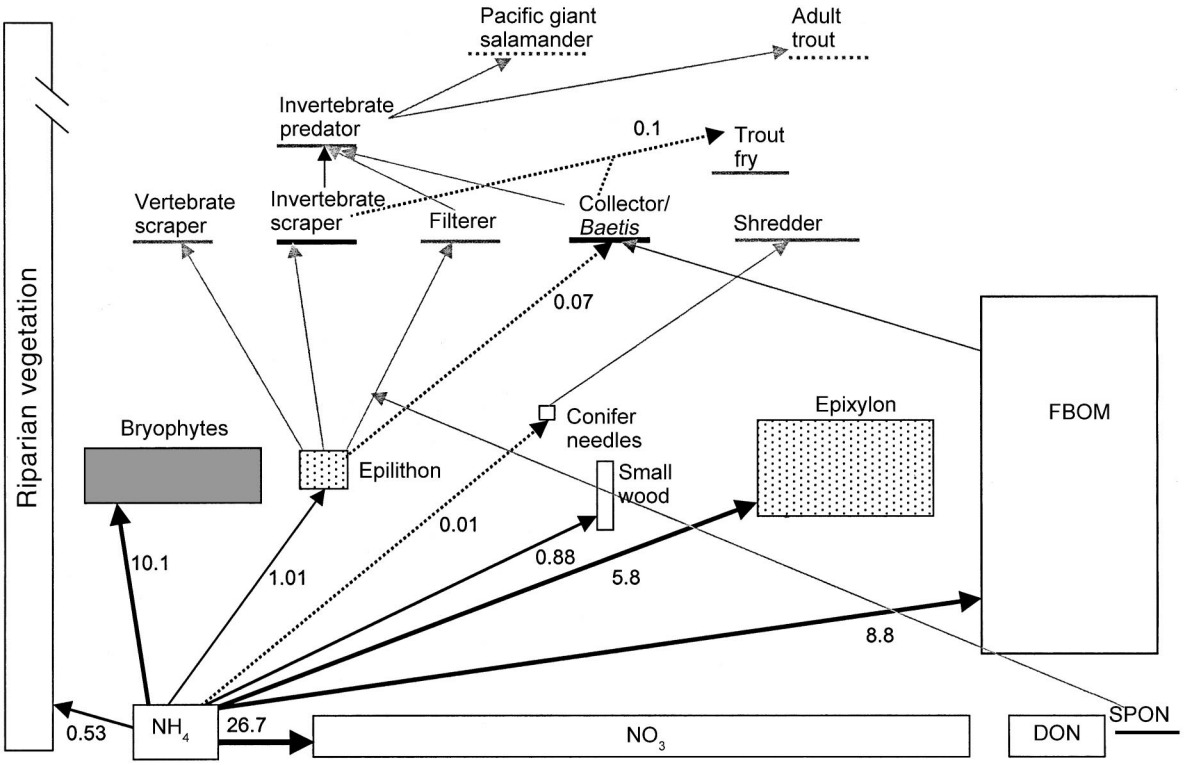

FIG. 7. The stream and riparian food web in Mack Creek. Arrows represent mean uptake rates (mg $N \cdot m^{-2} \cdot d^{-1}$) for the first nine days of tracer addition (42 days for riparian vegetation); width and density of lines are proportional to rates. The lack of a value adjacent to an arrow indicates that the rate of transfer to the next trophic level is unknown. Boxes represent sampled food web components: box height is proportional to standing stock of N; box width is proportional to percentage of ^{15}N retained after 42 days, and shading density is proportional to maximum $\Delta^{15}N$ label. Note that the ''riparian plants'' box is not to scale for N standing stock, and large in-channel wood is omitted for reasons of scale (see Table 1). Uptake rates for baetid mayflies and trout fry were derived from box models (Dodds et al. 2000). Key to abbreviations: DON, dissolved organic nitrogen; FBOM, fine benthic organic material; SPOM, suspended particulate organic material.

tion as a nitrogen sink due to high standing stocks, especially in forested streams.

We could not account for ~19% of the $^{15}NH_4^+$ we added to Mack Creek. Changes in stream discharge can affect rates of uptake, retention, and export. For example, increasing stream flows can increase export rates but also enlarge surface areas available for nitrogen uptake (Stream Solute Workshop 1990, Webster et al. 2003). However, discharge in Mack Creek declined slowly over the duration of the release, unlike many other LINX sites that had more variable flows. Underestimates of standing stocks would cause overall retention at the end of the study to be underestimated as well (Tank et al. 2000). Changes in standing stocks of some components, such as leaves in the stream, likely changed over the duration of the tracer release and would not have been detected by our methods. Tracer ^{15}N could have been lost via denitrification, which may occur in wet organic riparian soils (Ostrom et al. 2002) or in hyporheic areas (Duff and Triska 1990). We likely underestimated export of DO^{15}N due to sampling and analytical constraints. DON export has been demonstrated to be significant in undisturbed watersheds (Sollins et al. 1980, Triska et al. 1984, Perakis and Hedin 2002, Vanderbilt et al. 2002) . Finally, despite our attempts to measure all possible sources of nitrogen uptake and retention, we undoubtedly missed critical components or processes, such as the role played by hyporheic waters.

Conclusions

This stable isotope tracer study of nitrogen dynamics and food webs in a 500-year-old coniferous forest ecosystem documents the in-stream dynamics of nitrogen, its rate of uptake and transformation, and estimates of retention efficiency in a nearly pristine environment (summarized in Fig. 7). Uptake, transformation, and retention of N appear to be highly efficient over a comparatively short distance in this stream ecosystem, highlighting that streams are not simply nutrient conduits, but sites of important transformations. A large proportion of tracer $^{15}NH_4^+$ was nitrified to $^{15}NO_3^-$ and exported, despite comparatively low ammonium and high nitrate concentrations. Additional research on the role of bryophytes could help further define their role in nitrogen processing. The high rates of uptake and retention by biofilms on large wood and the presence of ^{15}N in streamside plants from a range of taxa point to the importance of the age and structure of riparian vegetation to basin N cycles.

The tracer Δ^{15}N found in streamside vegetation suggests a coupling of aquatic and terrestrial systems and indicates a topic that would benefit from additional research. Classic watershed nutrient analyses (e.g., Sollins et al. 1980) tend to view streams as conduits and to view weir chemistry as the primary contribution of streams to watershed budgets. Conversely, stream ecologists have been inclined to view riparian vegetation as a nutrient and energy source for stream biota and have focused on the processes occurring within the aquatic ecosystem. Studies on hyporheic zones compliment these two paradigms, but plant uptake of a limiting nutrient from aquatic surface or subsurface flow paths has not been examined as closely. Regardless of the characteristics of the terrestrial ecosystem, future studies of nutrient cycling should account for the uptake, transformation, and storage of nutrients within both stream and adjacent terrestrial ecosystems.

Acknowledgments

We thank Cody Fleece, Norm Leonard, Pat Mulholland, Diane Sanzone, Jen Schaefer, and Jack Webster for field assistance, Kris Tholke and Marshall Oetter for mass spectrometer analyses, and Cam Jones for water nutrient analyses. Pat Mulholland, Steve Perakis, and two anonymous reviewers provided insightful comments on earlier drafts. This research was part of the Lotic Intersite Nitrogen eXperiment (LINX), funded by the National Science Foundation to Virginia Tech (DEB 96-28860). The LINX ''community'' of researchers supplied a supportive and thought-provoking study environment. Logistical and laboratory support was provided by the H. J. Andrews Experimental Forest, funded by the U.S. Forest Service and National Science Foundation (DEB 96-32921).

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