6-1-2000

NITROGEN CYCLING IN A FOREST STREAM DETERMINED BY A 15N TRACER ADDITION

Patrick J. Mulholland
Oak Ridge National Laboratory

Jennifer L. Tank
University of Notre Dame

Diane M. Sanzone
University of Georgia

Wilfred M. Wollheim
University of New Hampshire, Durham, wil.wollheim@unh.edu

Bruce J. Peterson
Marine Biological Laboratory, Woods Hole, MA

See next page for additional authors

Ecological Monographs, 70(3), 2000, pp. 471–493

NITROGEN CYCLING IN A FOREST STREAM DETERMINED BY A ^{15}N TRACER ADDITION

PATRICK J. MULHOLLAND,[1] JENNIFER L. TANK,[2] DIANE M. SANZONE,[3] WILFRED M. WOLLHEIM,[4] BRUCE J. PETERSON,[4] JACKSON R. WEBSTER,[5] AND JUDY L. MEYER[3]

[1]*Environmental Sciences Division, Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, Tennessee 37831 USA*
[2]*Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, Illinois 61801 USA*
[3]*Institute of Ecology, University of Georgia, Athens, Georgia 30602 USA*
[4]*Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts 02543 USA*
[5]*Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061 USA*

Abstract. Nitrogen uptake and cycling was examined using a six-week tracer addition of ^{15}N-labeled ammonium in early spring in Walker Branch, a first-order deciduous forest stream in eastern Tennessee. Prior to the ^{15}N addition, standing stocks of N were determined for the major biomass compartments. During and after the addition, ^{15}N was measured in water and in dominant biomass compartments upstream and at several locations downstream. Residence time of ammonium in stream water (5–6 min) and ammonium uptake lengths (23–27 m) were short and relatively constant during the addition. Uptake rates of NH_4 were more variable, ranging from 22 to 37 $\mu g\ N \cdot m^{-2} \cdot min^{-1}$ and varying directly with changes in stream-water ammonium concentration (2.7–6.7 $\mu g/L$). The highest rates of ammonium uptake per unit area were by the liverwort *Porella pinnata*, decomposing leaves, and fine benthic organic matter (FBOM), although epilithon had the highest N uptake per unit biomass N.

Nitrification rates and nitrate uptake lengths and rates were determined by fitting a nitrification/nitrate uptake model to the longitudinal profiles of $^{15}N\text{-}NO_3$ flux. Nitrification was an important sink for ammonium in stream water, accounting for 19% of the total ammonium uptake rate. Nitrate production via coupled regeneration/nitrification of organic N was about one-half as large as nitrification of streamwater ammonium. Nitrate uptake lengths were longer and more variable than those for ammonium, ranging from 101 m to infinity. Nitrate uptake rate varied from 0 to 29 $\mu g \cdot m^{-2} \cdot min^{-1}$ and was ~1.6 times greater than assimilatory ammonium uptake rate early in the tracer addition. A sixfold decline in instream gross primary production rate resulting from a sharp decline in light level with leaf emergence had little effect on ammonium uptake rate but reduced nitrate uptake rate by nearly 70%.

At the end of the addition, 64–79% of added ^{15}N was accounted for, either in biomass within the 125-m stream reach (33–48%) or as export of $^{15}N\text{-}NH_4$ (4%), $^{15}N\text{-}NO_3$ (23%), and fine particulate organic matter (4%) from the reach. Much of the ^{15}N not accounted for was probably lost downstream as transport of particulate organic N during a storm midway through the experiment or as dissolved organic N produced within the reach. Turnover rates of a large portion of the ^{15}N taken up by biomass compartments were high (0.04–0.08 per day), although a substantial portion of the ^{15}N in *Porella* (34%), FBOM (21%), and decomposing wood (17%) at the end of the addition was retained 75 d later, indicating relatively long-term retention of some N taken up from water.

In total, our results showed that ammonium retention and nitrification rates were high in Walker Branch, and that the downstream loss of N was primarily as nitrate and was controlled largely by nitrification, assimilatory demand for N, and availability of ammonium to meet that demand. Our results are consistent with recent ^{15}N tracer experiments in N-deficient forest soils that showed high rates of nitrification and the importance of nitrate uptake in regulating losses of N. Together these studies demonstrate the importance of ^{15}N tracer experiments for improving our understanding of the complex processes controlling N cycling and loss in ecosystems.

Key words: ammonium uptake length; ^{15}N; gross primary production; nitrate uptake length; nitrification; nitrogen cycling; respiration; stable isotope; stream ecosystem; tracer addition.

INTRODUCTION

Nitrogen is a critical element whose availability can limit primary production in a wide variety of terrestrial and aquatic ecosystems (Elser et al. 1990, Vitousek and Howarth 1991). In recent decades, humans have had a large impact on the nitrogen cycle at global as well as local scales (Jordan and Weller 1996, Vitousek et al. 1997), increasing nitrogen inputs to most ecosystems. In excess, nitrogen can reduce forest growth rates (Aber

Manuscript received 28 September 1998; revised 12 May 1999; accepted 27 May 1999.

et al. 1995), initiate eutrophication and acidification, and impair drinking water supplies (Murdoch and Stoddard 1992, Nixon 1995). A more complete understanding of nitrogen cycling, retention, and fate is needed to better predict the effects of increased inputs of fixed N to the biosphere. For example, we need to know how rapidly inorganic forms of N are immobilized and which components of ecosystems are most important in the initial uptake and long-term retention of N.

In lotic ecosystems, low nitrogen concentrations can sometimes limit the growth of algae, either alone or in combination with phosphorus (Grimm and Fisher 1986, Hill and Knight 1988, Lohman et al. 1991, Rosemond et al. 1993). Our knowledge of N cycling in streams has largely come from input–output budgets (Meyer et al. 1981, Triska et al. 1984, Grimm 1987) and experimental additions of ammonium or nitrate (Richey et al. 1985, Triska et al. 1989, Munn and Meyer 1990, Marti and Sabater 1996). While these studies have provided important information on N dynamics, they have three major shortcomings. First, ambient rates of uptake, transformation, and retention could not be determined because either the simultaneous processes of uptake, release, and downstream transport could not be measured independently (budget studies) or were unavoidably altered by nutrient enrichment (N addition studies). Second, the role of specific biomass pools in N uptake and retention could only be indirectly inferred. Third, N turnover rates and the fate of N taken up could not be explicitly determined. These shortcomings can be overcome by using nutrient tracers, as shown by Newbold et al. (1981, 1983*a*) in their ^{32}P addition studies to determine rates and mechanisms of phosphorus spiraling.

Recently, a stable N isotope (^{15}N) field tracer approach has been developed to study nitrogen cycling at the ecosystem scale (Fry et al. 1995). Addition of ammonium or nitrate with high ^{15}N-enrichment levels results in negligible increase in ammonium or nitrate concentrations, avoiding the stimulatory effects of added nutrients. These ^{15}N tracer experiments allow the determination of N uptake, transformation, and turnover rates as well as consumer-food resource linkages within ecosystems.

Experiments involving whole-stream additions of ^{15}N-labeled ammonium have been conducted in the Kuparuk River, Alaska (Peterson et al. 1997) and in Hugh White Creek, North Carolina (Hall et al. 1998). This study at Walker Branch, Tennessee, was more comprehensive than these previous ^{15}N addition experiments in terms of the variety of ecosystem compartments studied and N transformation rates determined. The Walker Branch experiment is one of a series of identical ^{15}N-ammonium addition experiments conducted in streams throughout the United States as part of the lotic intersite nitrogen experiment (LINX) project.

Study Site

The study was conducted in a 125-m reach of the West Fork of Walker Branch, a first order, forested stream in the Ridge and Valley geophysical province of eastern Tennessee (latitude 35°58′ N, longitude 84°17′ W). The climate is typical of the humid Appalachian region of the southeastern United States, with a mean annual temperature of 14.5°C and mean annual precipitation of 140 cm. Although precipitation (almost exclusively as rain) is distributed relatively evenly throughout the year, stream discharge is highly seasonal because of high rates of evapotranspiration during the growing season. The West Fork of Walker Branch drains a 38.4 ha catchment on the U.S. Department of Energy's Oak Ridge National Environmental Research Park. The vegetation is dominated by oaks (*Quercus* spp.), hickory (*Carya* spp.), and red maple (*Acer rubrum*). A more detailed description of the catchment was given by Johnson and Van Hook (1989). The catchment is underlain by dolomite, and the stream arises in several springs discharging water from the bedrock aquifer (Mulholland 1992). The stream gradient is ~0.035. Stream chemistry is dominated by calcium, magnesium, and bicarbonate, and the pH is moderately basic (usually 8.0–8.3).

This reach of the West Fork of Walker Branch (hereafter referred to as Walker Branch) has been the site of numerous previous studies of phosphorus spiraling (Newbold et al. 1981, 1983*a*, Mulholland et al. 1985, 1997), periphyton–herbivore interactions (Rosemond 1993, Rosemond et al. 1993), nutrient dynamics (Mulholland 1992, Mulholland and Hill 1997), metabolism (Marzolf et al. 1994), and bryophytes (Steinman and Boston 1993). An earlier study indicated that algal growth and litter decomposition were phosphorus limited in Walker Branch (Elwood et al. 1981), although a more recent investigation has shown that algae are co-limited by N and P in spring (Rosemond et al. 1993).

Methods

Experimental design

The study consisted of extensive characterization of ecosystem parameters in a 125-m reach of stream followed by a six-week continuous addition of ^{15}N-labeled ammonium chloride to stream water beginning on 1 April 1997. The length of the ^{15}N addition (six weeks) was chosen to allow most ecosystem compartments to reach isotopic steady state. Approximately two weeks before the ^{15}N addition we measured a number of physical, chemical, and biological characteristics of the study reach. Samples of all major populations and organic matter pools were collected from several locations within the reach to determine background (natural abundance) levels of ^{15}N. At regular intervals during and following the ^{15}N addition, we measured ^{15}N levels in all major ecosystem compartments.

Sampling and analysis

Biomass (pre-^{15}N addition).—We measured mass of coarse benthic organic matter (CBOM, particles >1 mm in size) and fine benthic organic matter (FBOM, <1 mm) using a stratified random sampling design (n = 3–6 samples per habitat), based on measurements of the relative distribution of the primary habitat types in the study reach (57% riffle–run, 22% bedrock outcrop, 21% pool–backwater, as determined using a point-transect approach). An open-ended metal cylinder (0.07 m^2) was placed into the sediments as deeply as possible (generally ~10 cm) and coarse organic matter was removed by hand and separated into wood and leaves. The sediments were then vigorously agitated and pumped through a 1-mm mesh net into a 20 L container, and a representative subsample was taken from the carboy and filtered onto precombusted glass fiber filters (Whatman GFF, Whatman International, Maidstone, UK). The material collected in the net was added to the appropriate CBOM pool. The filters containing FBOM were dried at 60°C for 1 wk, weighed, combusted at 500°C for 4 h, rewetted, dried, and reweighed for calculation of dry mass and ash free dry mass (AFDM). Mean mass in the reach was determined from the means for each habitat per unit streambed area and the proportion of stream comprised by each habitat type.

We measured the concentration of suspended particulate organic matter (SPOM) by filtering 2–4 L of stream water at six locations through precombusted glass fiber filters (Whatman GFF) and determining AFDM. Abundance of SPOM per unit area was calculated from SPOM concentration and average stream water depth.

We measured epilithon mass and chlorophyll *a* content by randomly selecting rocks from riffle–run areas at 20-m intervals over the study reach (n = 12) and scraping material from a known area of rock surface using a wire brush into a small volume of water. Epilithon on bedrock outcrops was sampled by placing a 5 cm diameter cylinder with a neoprene gasket on bedrock at six random locations, dislodging the material with a stiff-bristled brush and pumping the loosened material into a cup. In the laboratory within two hours of collection, the epilithon slurries were filtered onto precombusted glass fiber filters (Whatman GFF). Half of the epilithon samples were processed for dry mass and AFDM as described for FBOM (except that they were not rewetted after combustion) and the other half were extracted in 90% acetone for chlorophyll *a* measurement (Wetzel and Likens 1991). Average stream reach epilithon mass and chlorophyll *a* were determined from the habitat averages (epilithon was considered absent from pools due to the predominance of fine sediments) and the proportion of stream comprised by each habitat.

We measured the abundance of bryophytes using transects across the stream at 5-m intervals over the study reach. Presence or absence and species were determined at 0.1-m intervals along each transect and percent cover was calculated. Samples of each species of bryophyte were scraped from 25-cm^2 areas of rock with complete bryophyte cover. To quantify the portion of actively growing material (and presumably that involved in nutrient uptake), the distal 1 cm of the frond was separated from the basal material and mass per unit area of each portion was determined. Bryophyte samples were processed for dry mass and AFDM as described for CBOM. Average mass in the reach for each bryophyte species was determined using percent cover and mass per unit area values.

Abundance of the snail, *Elimia clavaeformis*, the dominant consumer in Walker Branch (~95% of total consumer biomass, Newbold et al. 1983*a*), was determined by placing a 0.01-m^2 grid on the stream bottom at random locations within each habitat type and counting snails within the grid. Average snail density for the reach was determined from the habitat average densities and the proportion of stream comprised by each habitat type. Snail biomass was determined by randomly collecting 10 snails from each of five locations, removing the soft tissue by placing them in a microwave oven for 30 s, and measuring tissue dry mass (60°C for 48 h) and AFDM (500°C for 4 h). Average reach biomass was calculated from the density and average individual mass values.

We collected samples of other common macroinvertebrates by vigorously agitating sediments and capturing dislodged organisms in a net placed immediately downstream, and by collecting organisms from the undersides of rocks and from detritus accumulations. Macroinvertebrate sampling was designed to identify the most common populations and obtain specimens for chemical and isotopic analysis. To estimate biomass per unit area for macroinvertebrate populations other than snails we used results from macroinvertebrate sampling (0.1-m^2 Surber sampler) conducted during April in previous years (J. G. Smith, *unpublished data*).

We determined the nitrogen and carbon content and C:N ratios (mass basis) on subsamples (n = 2) of dried and pooled material from each of the dominant biomass compartments (wood and leaf CBOM, FBOM, SPOM, epilithon, and bryophytes) and each of the common macroinvertebrate taxa (Carlo Erba NA 1500 CN analyzer, Carlo Erba Instruments, Milan, Italy). These analyses were performed at the University of Georgia Institute of Ecology, Athens, Georgia, USA. Total standing stock of N in each biomass compartment or population per unit area was calculated using the measurements of dry mass per unit area and N content.

Microbial N.—We measured microbial N associated with detritus using the chloroform fumigation technique developed for soils (Brookes et al. 1985*a*, *b*). Briefly, this technique involves exposure of samples to chloroform to lyse microbial cells followed by mea-

surement of the inorganic nitrogen released. Samples of CBOM (wood and leaves separately) and surface FBOM were collected from six locations in the study reach on day 37 of the ^{15}N addition. CBOM-wood samples were scraped and only the outer layer used for this analysis. Moist subsamples of each type of material (two subsamples per location for a total of 12 subsamples of each material type) were placed in flasks in a desiccator and exposed to chloroform for 24 h at room temperature in the dark. Replicate subsamples ($n = 12$) were placed in a desiccator without chloroform to serve as nonfumigated controls. The fumigated and nonfumigated samples were then extracted in 0.5 mol/L potassium sulfate, the extracts were filtered, and the total N in each extract was determined by alkaline persulfate digestion (Cabrera and Beare 1993) followed by measurement of nitrate using automated colorimetry. Mass of microbial N was calculated as the difference between total N in extracts from fumigated and nonfumigated treatments divided by a factor of 0.54 as recommended by Brookes et al. (1985*b*) to account for incomplete release of microbial biomass N during a 24-h fumigation period. Microbial N mass was normalized to AFDM using wet mass/AFDM conversions determined for each sample type. Microbial N as a fraction of total N for each material type was calculated using AFDM-normalized microbial N and total N content per unit AFDM determined from the C:N analysis and dry mass/AFDM conversions.

Metabolism.—Whole-stream rates of gross primary production (GPP) and ecosystem respiration (R) were determined on three dates (1 April, 10 April, 7 May) using the diurnal upstream–downstream dissolved oxygen change technique (Marzolf et al. 1994), with the air–water exchange term modified as suggested by Young and Huryn (1998). Concentrations of dissolved oxygen and water temperature were measured at 1-min intervals at two locations in the study reach (10 m and 100 m downstream from the ^{15}N addition site) using an Orbisphere dissolved oxygen analyzer (model 2607 with model 2120 sensor, Orbisphere Laboratories, Emerson, New Jersey, USA) and averages logged at 5-min intervals over a 40-h period. Air–water oxygen exchange rates were determined using injections of propane and a conservative tracer during or immediately following the oxygen measurements as described by Marzolf et al. (1994). Photosynthetically active radiation (PAR) was monitored at a representative site near the middle of the study reach on each date that metabolism was measured using a Li-Cor quantum sensor and datalogger (LI-COR, Lincoln, Nebraska, USA).

^{15}N addition.—The addition of ^{15}N-enriched ammonium chloride (10% ^{15}N) to stream water was designed to achieve a 50% increase in the ^{15}N:^{14}N ratio in the dissolved ammonium pool (based on an assumed discharge rate of 10 L/s and NH_4 concentration of 3 μg N/L). The ^{15}N addition resulted in a negligible increase in the concentration of ammonium in stream water (~0.05 μg N/L). Thus, the added $^{15}NH_4$ served as an ammonium tracer and followed pathways identical to those followed by ammonium entering the stream from natural sources. The ^{15}N-enriched ammonium solution (made up weekly with distilled water) was pumped by a battery-powered peristaltic pump from a streamside carboy into a highly constricted section of the stream to achieve rapid mixing. The ^{15}N input was maintained at a rate of ~0.20 mg^{15}N/h throughout the six-week period, except for a 12–16 h period on 28–29 April when the pump malfunctioned and the ^{15}N supply was interrupted. The actual ^{15}N addition rate was determined weekly based on the volume remaining in the carboy when the $^{15}NH_4$ solution was replenished. Variation in stream discharge and ammonium concentration during the experiment, however, resulted in variation in the level of ^{15}N enrichment of stream water NH_4.

Sampling and analysis of ^{15}N.—Samples of water, organisms, and detritus were collected for ^{15}N analysis from an upstream station and stations located 10, 25, 50, 75, and either 100 or 125 m downstream from the ^{15}N addition point at various times during and after the addition. Constant rate injections of NaCl (lasting ~2 h) were also conducted each time water samples were collected to determine discharge at each station (Gordon et al. 1992). Two 4-L samples of stream water were collected from each station and filtered (0.45 μm polycarbonate membrane filter) within 1 h of collection for determination of N concentrations and ^{15}N in dissolved inorganic N pools. These samples were collected ~6 h after beginning the ^{15}N addition (day 0), on day 20, day 41, and 12 h after the end of the addition (day P1). In addition, water samples were collected at the 10-m station at ~2-d intervals, filtered immediately, and analyzed for N concentrations. Ammonium was determined by phenate colorimetry and nitrate plus nitrite by Cu-Cd reduction followed by azo dye colorimetry using a Bran Luebbe TRAACS 800 autoanalyzer (American Public Health Association 1992; Bran Luebbe GmbH, Norderstedt, Germany). Hereafter nitrate plus nitrite is referred to as nitrate because nitrite is negligible in this well-oxygenated stream (Mulholland 1992). Detection limits and precision for these analyses have been determined to be ~2 μg N/L and 0.5 μg N/L, respectively, for NH_4 and 1 μg N/L and 0.5 μg N/L, respectively, for NO_3 based on previous studies (Mulholland 1992; P. J. Mulholland, *unpublished data*).

We isolated ammonium for ^{15}N analysis in one of the 4-L filtered water samples from each station on each date using an ammonium diffusion procedure (Sorensen and Jensen 1991, Holmes et al. 1998). Briefly, this method involved addition of MgO to convert dissolved ammonium to ammonia, diffusion of the ammonia into the headspace of a sealed container, and sorption of the headspace ammonia onto a precombusted, acidified (25 μL of 2 mol/L H_2SO_4) glass fiber filter (Whatman GF/D) sealed within a packet formed by two Teflon filters

and floating on the surface of the water. Following 14 d of diffusion at 40°C on a shaker table, the filter packets were removed from the sample bottles and dried in a desiccator prior to ^{15}N analysis. The ^{15}N:^{14}N ratio in ammonium absorbed on each filter was determined by automated high temperature combustion with cryogenic separation followed by measurement of ^{15}N:^{14}N ratio using a Finnigan Delta S mass spectrometer (Finnigan MAT, San Jose, California, USA) at the Ecosystems Center laboratory, Marine Biological Laboratory, Woods Hole, Massachusetts, USA.

The second 4-L sample from each station was shipped immediately on ice to the Ecosystems Center laboratory, refrigerated, and prepared for analysis of ^{15}N in nitrate (within 1 wk of sample collection) based on the method of Sigman et al. (1997). Following addition of MgO and removal of ammonia by boiling 2 L of sample down to ~100 mL, the sample was transferred to a bottle and Devarda's alloy (EM Science, Gibbstown, New Jersey, USA) was added to reduce nitrate to ammonia. The sample was then treated as described above for headspace diffusion and trapping of ammonia and analysis of ^{15}N:^{14}N ratio by mass spectrometry.

All ^{15}N:^{14}N ratios are expressed as δ^{15}N values (units of ‰) according to the following equation:

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

where R_{sample} is the ^{15}N:^{14}N ratio in the sample and R_{standard} is the ^{15}N:^{14}N ratio in standard air (R_{standard} = 0.003663). All reported δ^{15}N values were corrected for background levels of ^{15}N as follows: δ^{15}N values of samples collected upstream from the ^{15}N addition point were subtracted from δ^{15}N values of similar type samples collected downstream from the ^{15}N addition. Therefore, all δ^{15}N values reported hereafter represent ratios of tracer ^{15}N:^{14}N only (i.e., do not include naturally occurring ^{15}N).

There are two potential sources of error in the streamwater δ^{15}N-NH_4 and δ^{15}N-NO_3 values obtained using the diffusion procedures described above. The first source of error is reagent N which acts to dilute the sample δ^{15}N values because the reagents are unenriched in ^{15}N relative to the sample. For the ^{15}N-NH_4 method, we measured a reagent blank of 3.2 μg N/sample for which we assumed a δ^{15}N value of 0‰. For the ^{15}N-NO_3 method, the reagent blank is derived from the Devarda's alloy. We measured a blank of 1.9 μg N/sample and a δ^{15}N value of −6‰ for the lot of Devarda's alloy used in this experiment (EM Science lot 34194, Sigman et al. 1997). All sample δ^{15}N-NH_4 and δ^{15}N-NO_3 values were corrected upward using the reagent blank mass, the appropriate blank δ^{15}N value, and the mass spectrometer estimate of total N and δ^{15}N for each filter. The second source of error for the streamwater δ^{15}N values is breakdown of dissolved organic N (DON) to either NH_4 or NO_3 during the sample processing period. Comparison of estimates of N recovered on filters with field measured concentrations of NH_4 and NO_3 indicates the possibility of a nonzero DON blank with the $^{15}NH_4$ method but not with the $^{15}NO_3$ method. The DON blank associated with the $^{15}NH_4$ could not be quantified because of the difficulty in measuring the true NH_4 concentration at such low levels. However, DON concentrations are generally low in Walker Branch (~20 μg N/L), making a large error due to the DON blank unlikely.

Ratios of ^{15}N:^{14}N were determined on samples of epilithon, bryophytes, CBOM (wood and leaves separately), FBOM, SPOM, and the dominant consumer organisms collected weekly at each station during the ^{15}N addition, and 1, 2, 3, 4, 8, 11, and 30 wk after the end of the addition. Sample collection and processing were as described above except that samples of each type were collected from 3–5 locations at each station and composited, and FBOM was collected only from surface deposits using a suction device. In the laboratory, the distal 1 cm of bryophyte fronds were separated from the basal material and only the distal portions analyzed for ^{15}N:^{14}N ratio. For wood, the outer surface was removed by scraping and only this material was analyzed for ^{15}N:^{14}N ratio. All samples were dried at 60°C for ≥2 d, finely ground using a Wiley mill (except for the samples collected on glass fiber filters), and subsamples analyzed for ^{15}N:^{14}N ratio using a Europa Model 20/20 isotope ratio mass spectrometer (PDZ Europa, Cheshire, UK) at the Ecosystems Center laboratory, Marine Biological Laboratory, Woods Hole, Massachusetts, USA. Usually only one subsample of each material type per station per date was analyzed for ^{15}N:^{14}N ratio. Analysis of replicates for a few samples indicated little variability (±0.5‰).

The tracer ^{15}N mass associated with each biomass compartment ($^{15}N_{\text{biomass}}$, units of mass/area) was determined from the background-corrected δ^{15}N values ($\delta^{15}N_{\text{biomass}}$) and the total N standing stock values (TN_{biomass}) for each compartment measured just prior to the ^{15}N addition as follows:

$$^{15}N_{\text{biomass}} = (\delta^{15}N_{\text{biomass}}/1000) \times 0.003663(TN_{\text{biomass}}). \quad (2)$$

At the end of the 42-d experiment, a mass balance of all tracer ^{15}N retained within all measured biomass compartments throughout the 125-m study reach was determined by calculating the tracer ^{15}N associated with each compartment at each station on day 42, determining the exponential rate of decline in tracer ^{15}N with distance for each biomass compartment, and integrating the tracer ^{15}N over distance.

Values of δ^{15}N for microbial biomass associated with CBOM and FBOM were determined on the samples collected on day 37 using the chloroform fumigation procedure as described above for microbial N analysis, followed by a nitrate reduction–alkaline diffusion procedure for isolating nitrogen in the digested extract samples for ^{15}N:^{14}N analysis (Brooks et al. 1989). This method has been used successfully to determine ^{15}N

content of soil microbes in ^{15}N additions to forest soils (Seely and Lajtha 1997, Stark and Hart 1997). The nitrate reduction–alkaline diffusion step and ^{15}N:^{14}N analysis was similar to that described above for streamwater nitrate, except that samples were placed in an oven at 60°C for 2 d and then removed and placed on a shaker table for an additional 5 d for diffusion and trapping of ammonia onto floating acidified filters.

Microbial $\delta^{15}N$ ($\delta^{15}N_{microbes}$) associated with decomposing leaves, wood, and FBOM was computed using the following equation

$$\delta^{15}N_{microbes} = [(\delta^{15}N_{fumigated} \times TN_{fumigated}) - (\delta^{15}N_{unfumigated} \times TN_{unfumigated})]/TN_{microbial} \quad (3)$$

where $\delta^{15}N_{fumigated}$ and $\delta^{15}N_{unfumigated}$ are the $\delta^{15}N$ measured in chloroform fumigated (microbial plus exchangeable N) and unfumigated (exchangeable N) samples, respectively, the $TN_{fumigated}$ and $TN_{unfumigated}$ are the total N values (mg N/g AFDM) determined for the fumigated and unfumigated samples, respectively, and $TN_{micriobial}$ is the extracted microbial N (determined as the difference between $TN_{fumigated}$ and $TN_{unfumigated}$) . To calculate the proportion of ^{15}N taken up by detritus that is in microbial biomass at the end of the experiment, tracer ^{15}N in microbial pools associated with detritus measured on day 37 was divided by ^{15}N in the bulk detritus measured on day 42. Mass of ^{15}N (normalized to AFDM) for microbial and bulk detritus pools was determined by Eq. 2 except that TN:AFDM ratios determined for microbes (described in the subsection on microbial N) and for each detritus compartment were used in the equation in place of $TN_{biomass}$ values.

Calculation of NH_4 uptake length and rates.—Ammonium uptake length was calculated as the inverse of the slope (k_1) of the regression of the natural logarithm of tracer ^{15}N vs. distance (Newbold et al. 1981). Longitudinal profiles of two tracer ^{15}N values were used to calculate ammonium uptake length: (1) ^{15}N-NH_4 flux in stream water, and (2) ^{15}N in biomass ($\delta^{15}N_{biomass}$) on the stream bottom. The tracer ^{15}N-NH_4 flux (μg ^{15}N/s) in stream water at each station i was calculated using the background-corrected measurements of ammonium $\delta^{15}N$, ammonium N concentration ([NH_4-N]) determined from total N recovered during mass spectrometer analysis of $\delta^{15}N$-NH_4 corrected for the reagent blank, and stream discharge (Q) using the equation:

$$^{15}N\text{-}NH_4 flux_i = (\delta^{15}N\text{-}NH_{4i}/1000) \times 0.003663 Q_i [NH_4\text{-}N]_i. \quad (4)$$

When using the tracer $\delta^{15}N_{biomass}$ data to compute uptake length, $\delta^{15}N_{biomass}$ values at downstream stations were increased by the fractional increase in discharge relative to the first station below the addition to account for dilution of tracer due to groundwater input. This method assumes that the $\delta^{15}N_{biomass}$ value at any station is a good representation of the average ^{15}N-NH_4 flux in stream water at that station.

Both methods for computing uptake length assume no release of tracer ^{15}N from biomass compartments back to water, an assumption likely true only during the very early stages of the ^{15}N addition (e.g., day 0). If regeneration of tracer ^{15}N-NH_4 back to water is significant relative to the total streamwater ^{15}N-NH_4 flux, then uptake lengths will be overestimated because some ^{15}N-NH_4 in water is regenerated ^{15}N. To correct the day 20 and day 41 NH_4 uptake lengths for regeneration of ^{15}N-NH_4 from biomass back to water, ^{15}N-NH_4 fluxes measured at each station on day P1 (12 h after termination of the ^{15}N addition) were used. One-half the day P1 ^{15}N-NH_4 fluxes at each station were subtracted from the day 20 ^{15}N-NH_4 fluxes, and the entire day P1 ^{15}N-NH_4 fluxes at each station were subtracted from the day 41 ^{15}N-NH_4 fluxes. These corrections assume that the rate of tracer ^{15}N-NH_4 regeneration from benthic biomass on day P1 was similar to the regeneration rate on day 41 (2 d earlier) and was twice the regeneration rate on day 20 (at approximately the midpoint of the tracer addition).

Whole-stream ammonium uptake rates per unit time (mg $N \cdot m^{-2} \cdot d^{-1}$) were computed for the three dates streamwater ^{15}N samples were collected by dividing the average reach flux of N-NH_4 in stream water (discharge·NH_4-N concentration) by the product of the NH_4 uptake length and the average wetted width of the stream (Newbold et al. 1981). Rates of ammonium uptake by each biomass compartment were calculated from tracer $^{15}N_{biomass}$ values on day 7 at individual sampling stations ($^{15}N_{biomass,7d}$) and the tracer ^{15}N:^{14}N ratios in streamwater NH_4 at those stations on day 0 ($^{15}N_{water\ ratio}$) as follows

$$\text{Ammonium uptake rate} = \frac{^{15}N_{biomass,7d}}{^{15}N_{waterratio} \times 7} \quad (5)$$

where $^{15}N_{biomass,7d}$ was calculated from Eq. 2 and $^{15}N_{water\ ratio}$ was calculated as (^{15}N-NH_4/1000)·0.003663. Because the tracer ^{15}N concentration in water declined sharply with distance, biomass at only the upper two stations (10 m, 25 m) acquired measurable tracer ^{15}N by day 7; thus, only data from these stations were used to calculate compartment-specific NH_4 uptake rates. To correct for turnover loss of ^{15}N from each biomass compartment during the first 7 d of the tracer addition (resulting in underestimation of ^{15}N uptake rates), values of $^{15}N_{biomass,\ 7d}$ were multiplied by correction factors determined from the measured turnover rates of ^{15}N in each compartment over the first 28 d following termination of the addition using the following tracer accumulation model

$$d^{15}N_{biomass,t}/dt = U - (k \times {}^{15}N_{biomass,t}) \quad (6)$$

where $^{15}N_{biomass,t}$ is the tracer ^{15}N accumulation in biomass at time t, U is the gross tracer ^{15}N uptake rate,

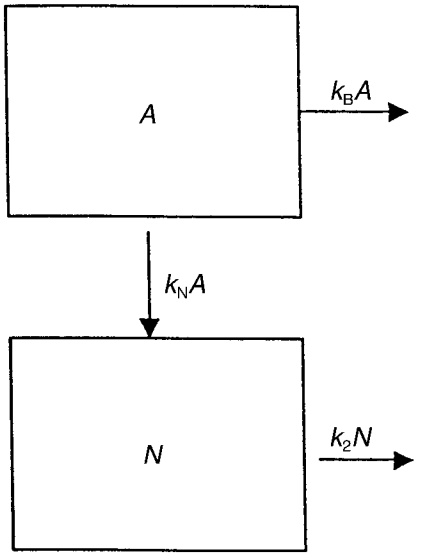

FIG. 1. Mass balance model of tracer ^{15}N-NH_4 (A) and ^{15}N-NO_3 (N) flux, where k_B is the assimilatory uptake rate of ^{15}N-NH_4 per unit distance, k_N is the nitrification rate, and k_2 is the ^{15}N-NO_3 uptake rate per unit distance. The sum of k_B and k_N (k_1) is the total uptake rate of ^{15}N-NH_4 with distance (equivalent to the inverse of the uptake length uncorrected for ^{15}N-NH_4 regeneration).

and k is the tracer ^{15}N turnover rate. The solution to Eq. 6 is

$$^{15}N_{biomass,t} = (^{15}N_{biomass,0} \times e^{-kt}) + [(U/k) \times (1 - e^{-kt})]. \quad (7)$$

Because at $t = 0$ there is no tracer ^{15}N accumulation in biomass ($^{15}N_{biomass,0} = 0$), Eq. 7 reduces to

$$^{15}N_{biomass,t} = (U/k) \times (1 - e^{-kt}). \quad (8)$$

Thus, the ^{15}N turnover correction factors used in the computation of gross compartment-specific ammonium uptake rates from measured rates of tracer ^{15}N accumulation in biomass on day 7 are equivalent to $7k/(1 - e^{-7k})$. The ^{15}N turnover correction factors ranged from 1.135 for *Porella* (with the lowest ^{15}N turnover rate) to 1.306 for leaves (with the highest ^{15}N turnover rate).

Calculation of nitrification rate and nitrate uptake rate and length.—Nitrification rate and nitrate uptake rate were determined by fitting a two-compartment mass balance model to the longitudinal profile for ^{15}N-NO_3 flux (μg ^{15}N/s) calculated from the measured values of δ^{15}N-NO_3, NO_3-N concentration, and stream discharge according to Eq. 4. The model describes the change in ^{15}N-NH_4 (A) and ^{15}N-NO_3 (N) flux over distance x (Fig. 1). The model equations are:

$$dA/dx = -k_1A \quad (9)$$

$$dN/dx = k_NA - k_2N \quad (10)$$

where k_1 (equal to $k_B + k_N$ in Fig. 1) is the measured rate of decline in streamwater ^{15}N-NH_4 flux with distance due to assimilatory uptake and nitrification (equivalent to the inverse of uptake length uncorrected for ^{15}N-NH_4 regeneration, in [meters]$^{-1}$), k_N is the nitrification rate per unit distance (in units of μg NO_3-N·[μg NH_4-N]$^{-1}$·m^{-1}, or per meter), and k_2 is the ^{15}N-NO_3 uptake rate per unit distance (in units of μg NO_3-N·[μg NO_3-N[$^{-1}$·m^{-1}, or per meter). The solution to Eq. 9 is:

$$A = A_0e^{-k_1x} \quad (11)$$

where A_0 is the flux of ^{15}N-NH_4 at the point of $^{15}NH_4$ addition (units of μg ^{15}N/s). Substituting for A into Eq. 10 we can solve for N (units of μg ^{15}N/s):

$$N = \frac{k_NA_0}{k_2 - k_1}(e^{-k_1x} - e^{-k_2x}) + N_0e^{-k_2x}. \quad (12)$$

Because there is no tracer ^{15}N-NO_3 at the point of $^{15}NH_4$ addition ($N_0 = 0$), the last term of Eq. 12 can be ignored.

We then used a least squares fitting procedure in conjunction with a spreadsheet model of Eq. 12 (using the Microsoft Excel optimization tool ''Solver''; Microsoft, Redmond, Washington, USA) to determine the values of the two unknowns (k_NA_0 and k_2) that best fit the observed longitudinal tracer ^{15}N-NO_3 flux distributions (i.e., k_NA_0 determines the rate of increase in ^{15}N-NO_3, and k_2 determines the rate of ^{15}N-NO_3 decline downstream). For day 0, A_0 is the experimental rate of ^{15}N-NH_4 addition at $x = 0$. Therefore, we were able to determine both the rate of direct nitrification, k_N (i.e., nitrification of ^{15}N-NH_4 in stream water per meter), and nitrate uptake rate, k_2, from the model fit to the day 0 data. On days 20 and 41, we do not know A_0 because indirect nitrification of tracer ^{15}N-NH_4 regenerated from biomass also contributed to the observed tracer ^{15}N-NO_3 flux profile. On these dates, we were able to estimate only the product k_NA_0 and k_2. Also, on days 20 and 41, we assumed that the total source of ^{15}N-NO_3 (i.e., ^{15}N-NH_4 in stream water plus ^{15}N-NH_4 regenerated from biomass) has the same rate of decline with distance downstream as water ^{15}N-NH_4 (i.e., the same k_1), uncorrected for regeneration. Estimates of k_1 derived from longitudinal $^{15}N_{biomass}$ profiles were similar to the k_1 determined from streamwater ^{15}N-NH_4 fluxes, uncorrected for regeneration, late in the addition making this a reasonable assumption.

The model used for the day P1 estimates of nitrification and nitrate uptake rate was slightly different. On this date (1 d after termination of ^{15}N-NH_4 addition) the only source of tracer $^{15}NO_3$ was biomass ^{15}N (indirect nitrification). To calculate the longitudinal rate of biomass ^{15}N decline (k_1, in units of [meters]$^{-1}$) and the biomass ^{15}N at the addition point (A_0, units of μg ^{15}N/m), we summed at each station the measured tracer ^{15}N (μg^{15}N/m stream length) in the biomass compart-

ments that could potentially contribute to indirect nitrification (i.e., epilithon, *Porella*, FBOM, leaves, and wood). The model was then fitted to the day P1 streamwater $^{15}N\text{-}NO_3$ data to determine k_N and k_2, except that for this case k_N had units of $(\text{seconds})^{-1}$ because the product $k_N A_0$ must have units of $\mu g\ ^{15}N \cdot m^{-1} \cdot s^{-1}$. For all dates, gross production rate of $^{15}N\text{-}NO_3$ over the reach (i.e., accumulation of $^{15}N\text{-}NO_3$ due to nitrification that would occur in the absence of $^{15}N\text{-}NO_3$ uptake) was determined by setting k_2 in Eq. 12 to zero and rerunning the simulation.

The flux of tracer $^{15}N\text{-}NO_3$ out of the study reach on each water sampling date (days 0, 20, 41) was determined from the $\delta^{15}N\text{-}NO_3$ values, NO_3-N concentrations, and the stream discharge at the 125 m sampling station according to Eq. 4. Loss of tracer $^{15}N\text{-}NO_3$ in stream water over the 42-d study period was determined by calculating average daily $^{15}N\text{-}NO_3$ fluxes by interpolating between the measured values on days 0, 20, and 41 and integrating the daily fluxes over time.

Calculation of NH_4 regeneration rate and N turnover rate.—Regeneration rate of tracer $^{15}N\text{-}NH_4$ (biomass to water) was determined for day P1 using a version of the two-compartment model (Fig. 1, Eq. 12) similar to that used to fit the day P1 $^{15}N\text{-}NO_3$ flux data. For this application, model compartment A is the biomass ^{15}N (sum of the ^{15}N in biomass compartments contributing to regeneration, units of $\mu g\ ^{15}N$/m stream length), N is streamwater $^{15}N\text{-}NH_4$ flux, k_A replaces k_N and is the production rate of $^{15}N\text{-}NH_4$ (per second), and k_2 is the uptake rate of regenerated $^{15}N\text{-}NH_4$ (per meter). The longitudinal rate of decline (k_1) of the source of $^{15}N\text{-}NH_4$ regenerated to water was assumed to be the longitudinal rate of decline in the sum of total biomass ^{15}N per unit area (epilithon, *Porella*, FBOM, leaves, and wood) measured on day 42. We determined A_0 by extrapolating the biomass ^{15}N upstream to the point of addition. The model was then fit to the day P1 longitudinal $^{15}N\text{-}NH_4$ flux profile to determine k_A and k_2 as described above for the day P1 $^{15}N\text{-}NO_3$ application. Gross production rate of $^{15}N\text{-}NH_4$ over the stream reach was determined by setting k_2 in Eq. 12 to 0 (no $^{15}N\text{-}NH_4$ uptake) and rerunning the simulation.

Turnover rates of tracer ^{15}N in specific biomass compartments (k in Eq. 6) were determined from the decline in biomass ^{15}N values at the 25-m station over the first 28 d after termination of the ^{15}N addition, assuming first-order dynamics [i.e., slope of the linear relationship between $\ln(^{15}N_{biomass})$ and time]. This approach assumes no reuptake of ^{15}N released to water upstream and thus may result in slight underestimates of turnover rate. Turnover times were calculated as the inverse of turnover rate.

Results

Ecological characteristics

Conditions in the West Fork of Walker Branch at the beginning of the $^{15}NH_4$ addition were typical for early spring (Table 1). Stream discharge was relatively stable but higher than typical baseflow values during summer and autumn. Nutrient concentrations in stream water were low and the ratio of dissolved inorganic N to soluble reactive phosphorus was ~17 (molar basis), suggesting potential colimitation of algae and microbes by N and P. Rates of GPP and GPP:R ratios were high relative to values measured at other times of the year because this is the period when light levels are greatest prior to emergence of leaves (Marzolf et al. 1994).

TABLE 1. Physical, chemical, and ecological characteristics of the West Fork of Walker Branch at or near the beginning of the ^{15}N addition.

Variable	Value
Physical (reach means):	
Discharge	9.6 L/s
Width	3.1 m
Depth	4.6 cm
Water velocity	6.8 cm/s
Water temperature (daily mean)	12.4°C
Water chemistry:	
pH	8.04
Alkalinity	2.0 meq/L
NH_4 concentration	2.7 μg N/L
NO_3 concentration	15.6 μg N/L
Soluble reactive phosphorus concentration	2.4 μg P/L
Metabolism:	
Gross primary production (GPP)	1.1 g $O_2 \cdot m^{-2} \cdot d^{-1}$
Ecosystem respiration (R)	6.3 g $O_2 \cdot m^{-2} \cdot d^{-1}$
GPP:R ratio	0.17

Stream biomass was dominated by the detritus compartments (leaves, wood, and FBOM), although N content of detritus was relatively low (Table 2). A leafy liverwort (*Porella pinnata*) was the dominant autotroph, both in terms of AFDM and N content, and covered 19.1% of the stream bottom. Epilithon biomass and N content were relatively low and habitat-weighted average epilithic chlorophyll was 12.2 mg/m^2. The snail *Elimia clavaeformis* dominated the consumer community. N content and C:N ratios were similar among consumer taxa, with N content substantially higher and C:N mass ratios substantially lower than for autotrophs and detritus. Total N standing stock for all biomass compartments together was 7700 mg/m^2.

Microbial N varied from 3% to 20% of detritus N, and was a greater fraction of the AFDM and total N of decomposing leaves than of wood and FBOM (Table 3). Microbial N associated with leaves and wood may be underestimated, however, because the chloroform fumigation technique appears to extract fungal N less effectively than N associated with bacteria and yeasts (Jenkinson 1976).

Discharge and NH_4-N concentration varied during the experiment (Fig. 2A, B). During the first 19 days of the addition, stream discharge was relatively stable,

TABLE 2. Abundance (AFDM; g/m^2), N content (percentage of dry mass), and C:N mass ratio of biomass compartments in Walker Branch.

Biomass compartment	AFDM	N content	C : N ratio
Primary uptake compartments			
Epilithon	3.8 (1.5)	0.35	22.9
Bryophytes			
Porella pinnata: tips	10.3 (3.1)	3.68	13.2
Porella pinnata: total	35.0 (9.7)	3.63	13.5
Amblystegium riparium: tips	2.3 (1.1)	2.20	17.8
Amblystegium riparium: total	5.0 (1.6)	1.88	19.0
CBOM			
leaves	76 (100)	0.98	47.5
wood	112 (68)	0.92	48.5
FBOM	197 (52)	0.32	18.6
SPOM	0.017	1.45	15.7
Primary consumers			
Elimia clavaeformis (soft tissue)	4.3 (1.5)	11.7	4.0
Gammarus minus	0.048	7.0	4.5
Psephenus herricki	0.041	10.3	4.9
Anchytarsus bicolor	0.032	11.6	4.6
Stenonema sp.	0.012	12.0	3.8
Diplectrona modesta	0.006	10.5	4.4
Baetis sp.	0.003	11.1	4.4
Secondary consumers			
Cambarus sp.	0.02	7.0	5.4
Eurycea sp.	0.08	11.5	4.3

Notes: Primary uptake compartments are those biomass pools involved in direct nutrient uptake from water. AFDM values for primary uptake compartments and snails are habitat-weighted means (pool, riffle, bedrock), and standard deviations are given in parentheses. SPOM standing stock (AFDM) is estimated from mean depth (4.6 cm) and measured SPOM concentration (0.38 mg AFDM/L). Standing stocks (AFDM) of primary consumers other than *Elimia* are based on wet mass measurements from samples collected during April 1985, 1986, and 1987 in a downstream reach and assuming dry mass is 17% of wet mass and AFDM is 90% of dry mass (J. G. Smith, *unpublished data*). Standing stocks (AFDM) of *Cambarus* and *Eurycea* are from Newbold et al. (1983*a*).

but increased sharply on the afternoon of day 20 in response to a large storm (but after the day 20 water samples were collected). Additional rainfall between day 27 and day 32 resulted in somewhat smaller increases in discharge. Stream NH_4-N concentrations ranged from <2 to 7 μg N/L, and were unrelated to variations in discharge, as has been observed in previous studies (Mulholland et al. 1990). Stream temperature varied from 12.2 to 14.7°C, with a slight warming trend over the period (Fig. 2B).

We added 182.1 mg of ^{15}N to the stream over the 42-day experiment. Although the input rate of ^{15}N was relatively constant throughout the experiment, tracer ^{15}N-NH_4 in stream water varied considerably because of changes in discharge and NH_4-N concentration (Fig. 2C). The expected ^{15}N-NH_4 in stream water at the 10 m station, computed from measurements of average daily discharge, NH_4-N concentration, ^{15}N-NH_4 input rate, and the rate of ^{15}N-NH_4 decline with distance (to account for uptake of ^{15}N between the point of ^{15}N addition and 10 m) varied from 40‰ to 750‰, although for most of the experiment the variation was considerably less (values were between 150‰ and 450‰ on two-thirds of all dates). Measured values of δ^{15}N-NH_4 at 10 m were higher than calculated values probably because the rate of ^{15}N-NH_4 decline in water over the first 10 m was overestimated due to incomplete mixing of tracer across the stream in the first few meters below the point of addition. Although the calculated δ^{15}N-NH_4 values at 10 m in Fig. 2C are likely underestimates, they provide a good indication of the relative variation in streamwater ^{15}N-NH_4 over the experiment.

TABLE 3. Microbial N normalized to AFDM (SD in parentheses, $n = 6$) and as a percentage of total N for the three detritus compartments.

Biomass compartment	Microbial N (mg N/g AFDM)	Microbial N (percentage of total N)
CBOM: leaves	2.19 (0.30)	20.2
CBOM: wood	1.01 (0.12)	10.1
FBOM	0.92 (0.012)	3.0

N uptake lengths and rates

Streamwater ^{15}N measurements.—Profiles of $^{15}NH_4$ in stream water declined exponentially with distance, as expected for a nutrient tracer (Fig. 3A). Semi-log plots of ^{15}N-NH_4 mass flux over the reach from 10 m

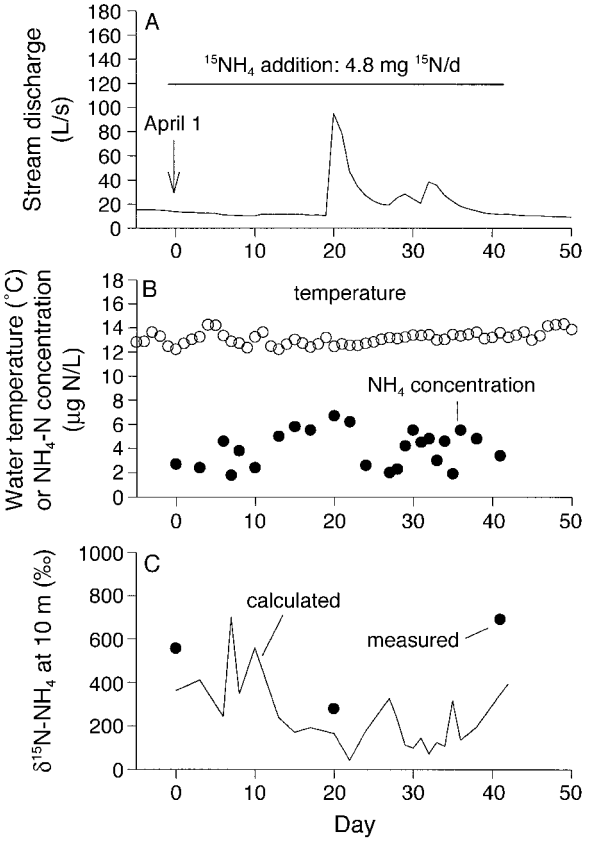

FIG. 2. Discharge at a weir located at the 200-m station (panel A), water temperature and NH_4-N concentration (panel B), and measured and calculated $\delta^{15}N$-NH_4 values in stream water at 10 m, the latter based on measurements of discharge, NH_4-N concentration, ^{15}N-NH_4 input rate, and rate of decline in ^{15}N-NH_4 flux per unit distance in water (panel C).

to 75 m indicated some variation in distance-normalized NH_4 uptake rate during the study (Fig. 3B). The uptake length of NH_4 (inverse of the distance-normalized uptake rate) was shortest on day 0 and longest on day 20 (Table 4). Much of the apparent increase in uptake length, however, resulted from its overestimation on days 20 and 41 because of ^{15}N-NH_4 regeneration from biomass back to water during the later portions of the study when biomass ^{15}N levels were high (violating the assumption of no tracer regeneration to water). Correcting for the effect of ^{15}N-NH_4 regeneration back to water, the revised NH_4 uptake lengths for day 20 (27 m) and day 41 (24 m) were only slightly longer than that on day 0 (23 m). There is somewhat greater uncertainty in the day 41 uptake length, however, because the regression was based on data from three rather than four stations across the study reach (the water sample from 75 m was lost). Residence time of ammonium in stream water was ~5 min, based on these uptake lengths and stream water velocity calculated at the beginning of the study (6.8 cm/s; Table 1).

Whole-stream NH_4 uptake rate per unit area was highest on day 20 despite the longer uptake length, because the streamwater NH_4-N concentration was considerably higher on this date (Table 4). Uptake rates of NH_4 on days 0 and 41 were similar, reflecting the similarity in the NH_4-N concentrations on these dates.

Nitrification rate (k_N) on day 0 was 0.00805 m^{-1}, based on the best fit of the two-compartment model to the ^{15}N-NO_3 flux data and using a ^{15}N-NH_4 addition rate (A_0) of 0.0556 μg ^{15}N/s (Fig. 4A). Thus, nitrification accounted for 19% of the total uptake rate of ^{15}N-NH_4 from stream water on day 0 (0.043 m^{-1}; Fig. 3B). Subtracting nitrification from total NH_4 uptake rate from water gives an assimilatory NH_4 uptake rate of 18 μg $N \cdot m^{-2} \cdot min^{-1}$ (Table 4).

The nitrification rate determined for day 0 was the direct nitrification rate only (i.e., nitrification of NH_4 in stream water) because at this time there was no tracer

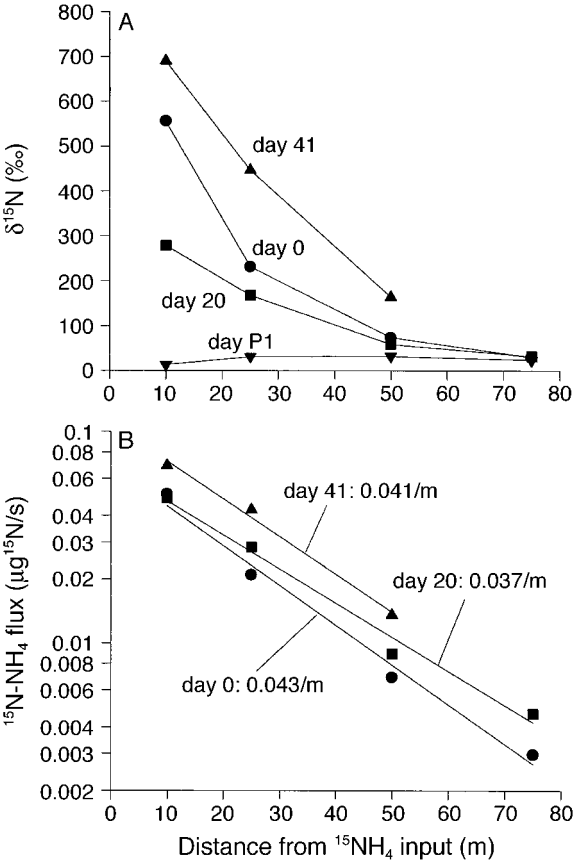

FIG. 3. Profiles of $\delta^{15}N$-NH_4 values in stream water over the study reach on three dates during the ^{15}N addition (days 0, 20, and 41) and one day after the experiment ended (day P1, panel A), and semi-log plots of tracer ^{15}N-NH_4 flux over distance (10–75 m) for each date (panel B). Values of $\delta^{15}N$-NH_4 at the upstream station vary over time primarily because of variation in NH_4-N concentration and discharge. Data from the 125-m station were not used to calculate the linear regressions in panel B because $\delta^{15}N$-NH_4 values were very low. The inverse of the regression slopes in panel B are the uptake lengths of NH_4 on each date. The day-0 data were also used in Mulholland et al. (2000*a*).

TABLE 4. Uptake lengths and uptake rates for NH_4 and NO_3 over the study period.

Variable	Day 0	Day 20	Day 41
Discharge, reach average (L/s)	9.6	7.6	8.5
NH_4			
Concentration (μg N/L)	2.7	6.7	3.4
Flux (μg N/min)	1 555	3 055	1 734
Uptake length (m)	23 (18–33)	31 (23–48)	29 (14–>100)
Uptake length (corrected for $^{15}NH_4$ regeneration [m])		27 (20–42)	24 (11–>100)
Uptake rate (μg $N \cdot m^{-2} \cdot min^{-1}$)	22	37	23
Assimilatory uptake rate (μg $N \cdot m^{-2} \cdot min^{-1}$)	18	30	19
NO_3			
Concentration (μg N/L)	15.6	40.7	28.2
Flux (μg N/min)	8 986	18 560	14 382
Uptake length (m)	101	∞	511
Uptake rate (μg $N \cdot m^{-2} \cdot min^{-1}$)	29	0	9
DIN			
Total uptake rate (μg $N \cdot m^{-2} \cdot min^{-1}$)	51	37	32
Assimilatory uptake rate (μg $N \cdot m^{-2} \cdot min^{-1}$)	47	30	28

Notes: The NH_4 uptake lengths were computed using the ^{15}N mass flux values for the reach from 10 m to 75 m, and corrections for ^{15}N regeneration were made using the day-P1 $^{15}NH_4$ values as described in text (*Results: N uptake lengths and rates*; 95% confidence intervals for NH_4 uptake lengths are given in parentheses). The assimilatory NH_4 uptake rate was calculated as total NH_4 uptake rate minus direct nitrification rate (assumed to be 19% of total NH_4 uptake rate on days 20 and 41 as determined for day 0).

^{15}N in biomass. On days 20 and 41, when both direct and indirect nitrification contributed to ^{15}N-NO_3 production, we could not determine nitrification rate from the model because A_0 (source of ^{15}N for nitrification) was a combination of ^{15}N-NH_4 in stream water and ^{15}N in benthic biomass and the two have different units. We were able to determine the relative contributions of direct and indirect nitrification from the asymptotes of the gross ^{15}N-NO_3 production rates determined from the model, however. Gross ^{15}N-NO_3 production rate increased from 0.010 μg ^{15}N/s on day 0 (Fig. 4A) to 0.014 and 0.016 μg ^{15}N/s on days 20 and 41, respectively (Fig. 4B, C). Assuming that the study reach was at or nearly at steady state with regard to biomass ^{15}N by day 41 (see Fig. 6B) and assuming that the direct nitrification rate was similar on days 0 and 41 (streamwater NH_4-N concentrations were similar), then the 1.6-fold increase in gross ^{15}N-NO_3 production rate from day 0 (direct nitrification only) to day 41 (direct plus indirect nitrification) suggests that indirect nitrification was ~60% as large as direct nitrification.

Nitrate uptake rate per unit distance (k_2) was 0.0099 m^{-1} on day 0 (Fig. 4A), but was considerably lower on days 20 and 41 (Fig. 4B, C). Thus, uptake lengths of NO_3 ranged from 101 m on day 0 to infinity on day 20, and whole-stream nitrate uptake rates ranged from 0 μg $N \cdot m^{-2} \cdot min^{-1}$ on day 20 to 29 μg $N \cdot m^{-2} \cdot min^{-1}$ on day 0 (Table 4). On day 0, the uptake rate of NO_3-N was 1.6 times higher than the assimilatory uptake rate of NH_4-N.

The assimilatory uptake rate of total dissolved inorganic N (DIN), calculated as the sum of assimilatory NH_4 uptake rate (assumed to be 81% of total ammonium uptake rate over the entire experiment as determined for day 0) and NO_3 uptake rate, declined from 47 μg $N \cdot m^{-2} \cdot min^{-1}$ on day 0 to 28–30 μg $N \cdot m^{-2} \cdot min^{-1}$ on days 20 and 41. The decline in assimilatory DIN uptake rate was the result of a decline in NO_3 uptake rather than NH_4 (Table 4, Fig. 5C). GPP also declined from 1.1–1.2 g $O_2 \cdot m^{-2} \cdot d^{-1}$ on days 2 and 10, to 0.3 g $O_2 \cdot m^{-2} \cdot d^{-1}$ on day 36 in response to an 80% decline in PAR reaching the stream as leaves emerged on riparian vegetation (Fig. 5A, B).

Biomass ^{15}N measurements.—Epilithon and *Porella* were more highly labeled with ^{15}N from water than the detritus compartments (Fig. 6A). The large storm on day 20 resulted in decline in $\delta^{15}N$ in *Porella* and detritus compartments at 25 m, probably from inadvertent sampling of material washed into the reach from upstream. The rapid return of *Porella* $\delta^{15}N$ to prestorm levels at 25 m and the absence of a large decline in *Porella* $\delta^{15}N$ on day 22 at 50 m suggests that the day 22 *Porella* sample at 25 m was an anomaly. Recovery of leaf and FBOM $\delta^{15}N$ at 25 m to prestorm values was slower, probably because these pools contained considerable amounts of material from upstream after the storm. The reason for the large decline in epilithon $\delta^{15}N$ at 25 m on day 35 is unknown and also probably an anomaly. Time course profiles for epilithon $\delta^{15}N$ at the 10-m (*data not shown*) and 50-m stations (Fig. 6A) indicated no decline on day 35.

Epilithon and *Porella* appeared to be approaching equilibrium with respect to tracer levels in water by the end of the 42-d ^{15}N addition, as indicated by relatively constant biomass $\delta^{15}N$:water $\delta^{15}N$ ratios if the anomalous day 22 *Porella* and day 35 epilithon values are excluded (Fig. 6B). It was not clear if the detritus compartments were approaching equilibrium given their very low $\delta^{15}N$ levels. The increase in biomass $\delta^{15}N$ values during the later portion of the experiment

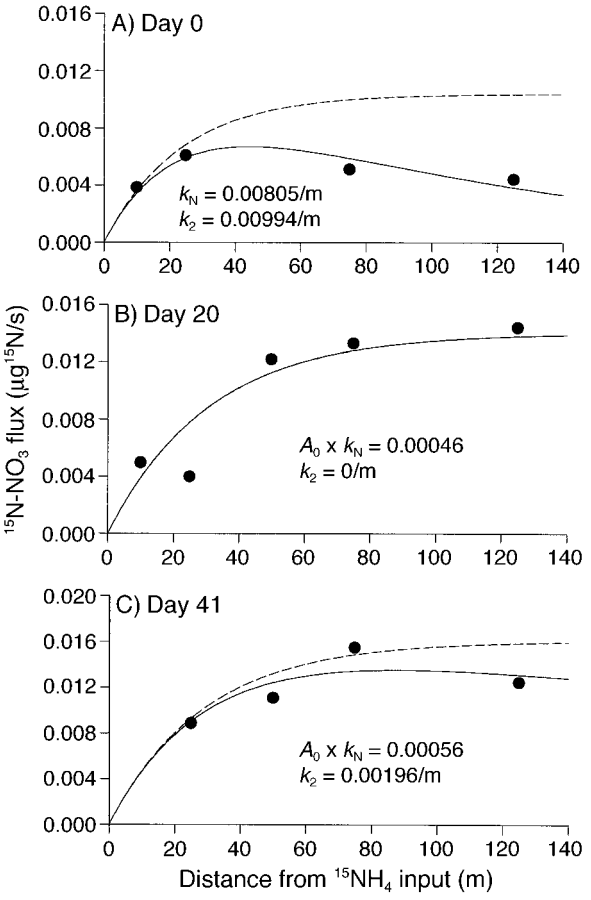

FIG. 4. Best fit of the two-compartment mass balance model (Fig. 1) to measured ^{15}N-NO_3 fluxes on day 0, day 20, and day 41. Values of the fitted parameters, nitrification rate (k_N) or k_NA_0 (when A_0 was unknown), and nitrate uptake rate (k_2) are indicated. Tracer ^{15}N-NO_3 flux values could not be determined at 10 m on day 41 because the δ^{15}N-NO_3 values were slightly negative (less than the value at the upstream background station). The calculated recovery of NO_3-N in the mass spectrometer analysis of this sample was very low (34%), and thus it was considered to be an outlier and was not used in the model fitting. The sample at 50 m on day 0 was lost. The dashed curves in each panel are the tracer ^{15}N-NO_3 fluxes that would have occurred given no uptake of nitrate, and the asymptote of each curve is the gross ^{15}N-NO_3 production rate within the reach. The data in panel A were also published in Mulholland et al. (2000*a*).

(days 28–42) evident in Fig. 6A probably reflected the increase in water δ^{15}N-NH_4 (Fig. 2C) resulting from decreasing stream discharge after day 32 (Fig. 2A). The biomass δ^{15}N:water δ^{15}N ratios were considerably less than 1.0 for all compartments, even at the end of the experiment, indicating either uptake of forms of streamwater N other than NH_4 (e.g., NO_3, DON) or that a large fraction of the N associated with these compartments was not the result of uptake from stream water (e.g., plant tissue N in detritus).

By the end of the ^{15}N addition tracer levels in microbes associated with detritus were substantially greater than those in the total detritus pool, especially for decomposing leaves and wood (Fig. 7). Nonetheless, microbial biomass δ^{15}N:water δ^{15}N ratios at all stations were substantially lower than 1.0, suggesting considerable uptake of forms of N other than NH_4 or extraction of some N from plant tissue by the chloroform fumigation method. It seems likely that microbial N had reached equilibrium with respect to tracer levels in water by day 37, given the relatively rapid growth rates of microbes.

Uptake length of NH_4 also can be computed using biomass δ^{15}N for stationary compartments because these reflect water δ^{15}N-NH_4 levels at each station over time. Uptake length of NH_4 calculated from epilithon δ^{15}N values on day 7 (23 m, 95% CI of 18–32 m) was identical to that calculated using the day 0 water ^{15}N-NH_4 flux (Table 4). This agreement lends confidence to the NH_4 uptake lengths calculated for the early part of the experiment. Biomass δ^{15}N data provide poorer estimates of NH_4 uptake lengths later in the experiment,

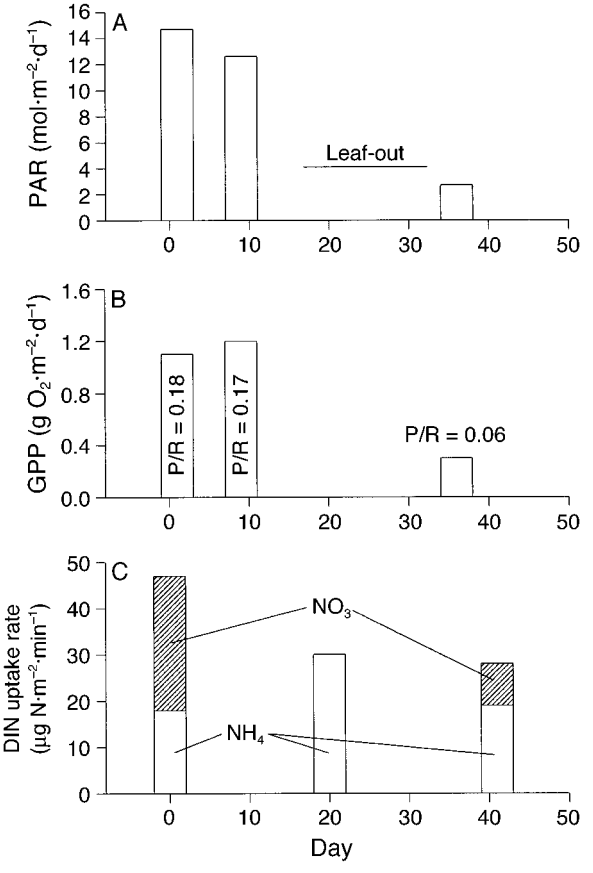

FIG. 5. Changes in PAR, GPP, and P:R ratio, and assimilatory DIN uptake rate over the study. Assimilatory DIN uptake rate is presented as the sum of the assimilatory ammonium-N uptake rate (total NH_4 uptake rate minus nitrification rate, the latter assumed to be 19% of total NH_4 uptake rate for all dates as determined for day 0) and nitrate-N uptake rate.

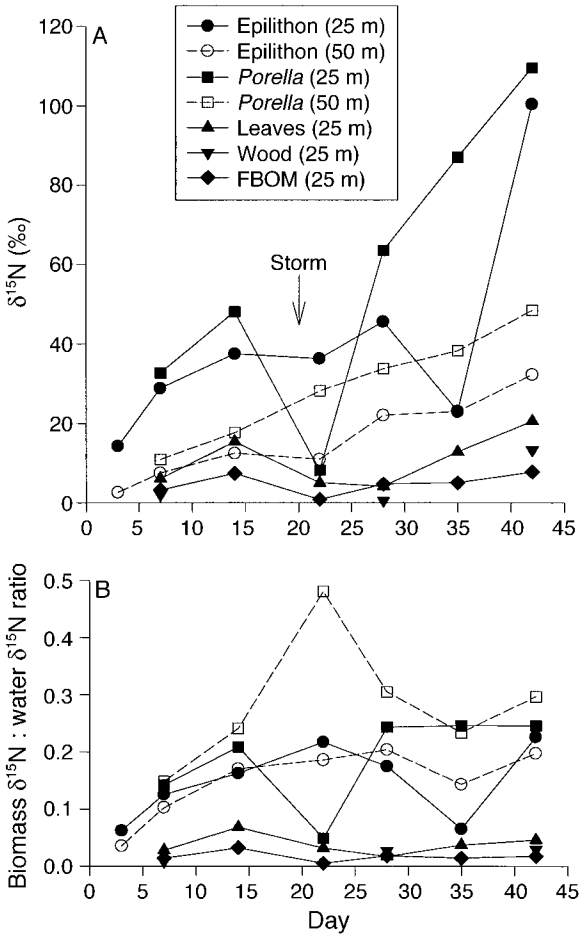

FIG. 6. Tracer δ^{15}N values for different organic matter compartments that take up ammonium from water over time at 25 m and at 50 m (epilithon and *Porella* only; panel A), and biomass δ^{15}N : streamwater δ^{15}N-NH_4 ratios over time at 25 m (panel B). On sampling dates when streamwater δ^{15}N-NH_4 values were not measured directly, they were estimated by linear interpolation between the values measured on days 0, 20, and 41. The data in this figure were also published in Mulholland et al. (2000*b*).

however, because regeneration of biomass ^{15}N back to water skews the biomass δ^{15}N profiles in the downstream direction.

Although the detritus compartments were the largest pools of N in the stream, *Porella* had the greatest NH_4 uptake rate per unit area (Table 5). Nitrification accounted for a considerable portion of the decline in streamwater ^{15}N-NH_4 flux despite low NH_4-N concentrations. In total, NH_4 uptake rate by all biomass compartments measured (including nitrification) amounted to 25 g $N \cdot m^{-2} \cdot d^{-1}$, or ~80% of the total NH_4 uptake rate calculated from the measured uptake length on day 0 (31.7 g $N \cdot m^{-2} \cdot d^{-1}$). The lower estimate of whole-stream NH_4 uptake rate determined by summing rates for individual biomass compartments is likely the result of uptake by unmeasured compartments (e.g., the moss *Amblystegium riparium*) and/or underestimation of correction factors applied to account for ^{15}N turnover in biomass (see below). The uptake rates computed for FBOM and wood, however, may be overestimates because they were based on δ^{15}N measurements on surface materials (surface FBOM, wood scraped from surfaces) that likely have higher tracer δ^{15}N values than the bulk detrital pools. We estimate that NH_4 uptake rates for FBOM and wood could be at least two times lower than those listed in Table 5. Uptake rates of NH_4, normalized to N content (N-specific uptake rates), were considerably higher for epilithon and *Porella* than for the detrital compartments (Table 5), probably because of large amounts of plant tissue N in detritus.

Fraction of total N uptake from water met by NH_4 uptake by each biomass compartment was calculated using the biomass δ^{15}N:water δ^{15}N ratios at the end of the experiment and a mixing model for ^{15}N-NH_4 and

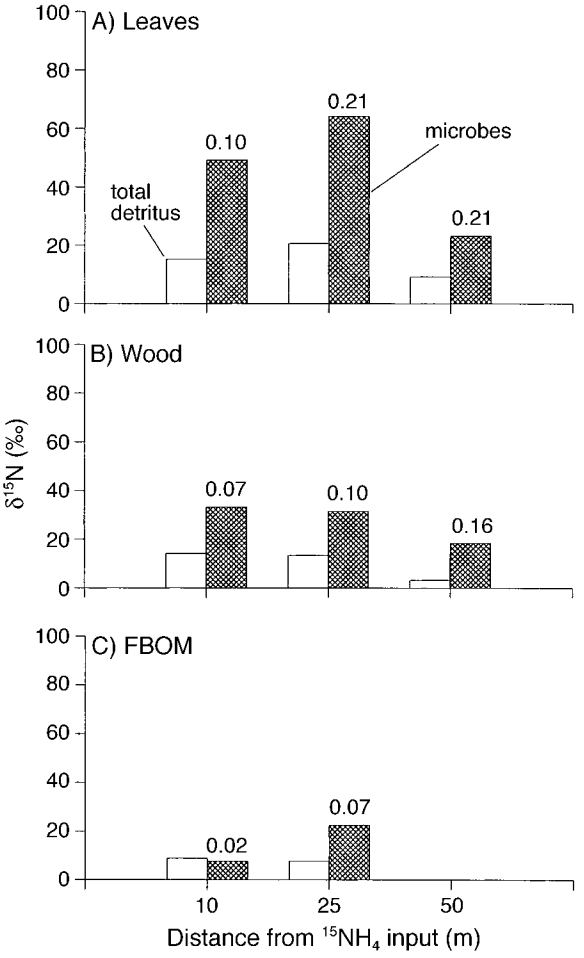

FIG. 7. Comparisons of tracer δ^{15}N for bulk detritus pools (sampled on day 42) and microbial N associated with decomposing leaves, wood, and FBOM at three stations (sampled on day 37). The ratios of δ^{15}N in microbial biomass to δ^{15}N-NH_4 in water (mean of day-20 and day-41 values) are indicated above the microbial δ^{15}N bars.

TABLE 5. N standing stock, NH_4 uptake rate (range in parentheses), and N-specific NH_4 uptake rate for different biomass compartments based on ^{15}N values on day 7 for the 10-m and 25-m stations.

Biomass compartment or process	N standing stock (g/m^2)	NH_4 uptake rate (mg $N \cdot m^{-2} \cdot d^{-1}$)	N-specific NH_4 uptake rate (d^{-1})
Epilithon	0.07	1.3 (1.1–1.5)	0.019
Porella (tips only)	0.46	8.4 (6.3–10.5)	0.018
CBOM: leaves	1.03	4.2 (3.1–5.3)	0.004
CBOM: wood	1.10	1.1 (0.6–1.6)	0.001
FBOM	2.71	4.1 (2.1–6.2)	0.001
Nitrification	···	5.9	
Total		25.0	
Total (based on uptake length)		31.7	

Notes: Total NH_4 uptake rates calculated as the sum of the individual compartments (including nitrification) and calculated on a whole-stream basis using the uptake length, NH_4-N concentration, and discharge (Table 4) are also given.

^{15}N-NO_3 uptake (Table 6). This calculation assumes that biomass compartments were at steady state relative to tracer ^{15}N levels in water and that all biomass N was supplied from streamwater NH_4 and NO_3. Based on this analysis, epilithon and *Porella* obtained 22% to 35% of their N from NH_4 uptake. Microbes associated with detritus obtained considerably less of their N from NH_4 in water, possibly reflecting uptake of organic N.

The longitudinal SPOM $\delta^{15}N$ distribution on day 42 was hump-shaped as a result of the gradual replacement of unlabeled SPOM from upstream by labeled particles entrained from below the ^{15}N addition point (Fig. 8). Comparison of the longitudinal $\delta^{15}N$ distributions for SPOM and FBOM indicated that SPOM was not generated only by entrainment of surface FBOM because peak $\delta^{15}N$ values in SPOM were considerably higher than the calculated $\delta^{15}N$ value for FBOM extrapolated back to the dripper (10.5‰). This result suggests that SPOM was generated from a compartment with greater tracer enrichment than bulk FBOM (e.g., epilithon, grazer feces, FBOM fractions with higher microbial biomass). We calculated an average SPOM travel distance (similar to an uptake length) of ~140 m from the decline in SPOM $\delta^{15}N$ between 50 and 125 m. This is likely to be an upper estimate, however, because it assumes no generation of ^{15}N-labeled SPOM below 50 m.

At the end of the 42-day ^{15}N addition, we could account for nearly one-half of the added tracer in biomass within the 125-m study reach, mostly in *Porella*, FBOM, and leaves (Table 7). Tracer retention by FBOM and wood likely was overestimated, however, because $\delta^{15}N$ was determined for surface materials only and these may have been more highly labeled than the total compartment N pool. Excluding ^{15}N retention by FBOM and wood from the analysis, 33% of added ^{15}N was accounted for in biomass on day 42. An additional 4% and 23% of added ^{15}N was lost as ammonium and nitrate, respectively, in stream water over the study period, based on linear interpolation of the measured fluxes of ^{15}N-NH_4 and ^{15}N-NO_3 at 125 m on day 0 (0.00008 and 0.0044 μg ^{15}N/s, respectively), day 20

TABLE 6. NH_4 uptake as a fraction of total N uptake from stream water for autotrophs and microbes associated with detritus.

Biomass compartment	NH_4 uptake as a fraction of total N uptake from water mean (SD)
Epilithon	0.22 (0.09)
Porella	0.35 (0.02)
Microbes	
Leaves	0.14 (0.05)
Wood	0.07 (0.01)
FBOM	0.03 (0.03)

Notes: Values were computed using data from the 10-m, 25-m, and 50-m stations and a mixing model that assumes all N uptake is from water NH_4 and NO_3 as follows: $(\delta^{15}N\text{-}NH_4 \times F_{NH_4}) + (\delta^{15}N\text{-}NO_3 \times F_{NO_3}) = \delta^{15}N_{biomass}$ where: $\delta^{15}N\text{-}NH_4$ and $\delta^{15}N\text{-}NO_3$ are the means of streamwater values on days 20 and 41 for each station, F_{NH_4} and F_{NO_3} are the fractions of total N uptake from streamwater NH_4 and NO_3 pools, respectively; and $^{15}N_{biomass}$ is the measured value for each biomass compartment at each station on day 42 (epilithon and *Porella*) or day 37 (microbes). Standard deviations are calculated using $n = 3$.

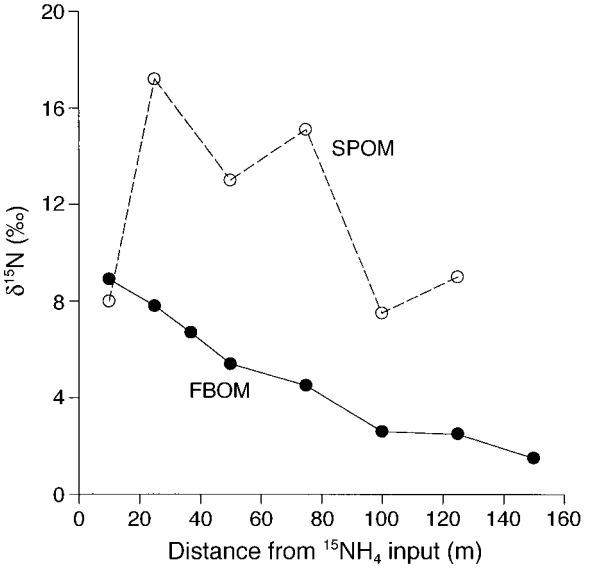

FIG. 8. Plot of tracer $\delta^{15}N$ in FBOM and SPOM over distance on day 42.

TABLE 7. Mass balance of ^{15}N at the end of the six-week experiment for the 125-m study reach.

Biomass compartment	^{15}N retained (mg ^{15}N)
Epilithon	3.3
Porella	36.0
FBOM	20
Leaves	15
Wood	6.7
Elimia	6.0
Stenonema	0.07
Psephenus	0.04
Baetis	0.03
Gammarus	0.02
Anchytarsus	0.02
Diplectrona	0.01
Crayfish	0.01
Salamanders	0.04
Total retained	87.2 (48% of added ^{15}N)
^{15}N-NH_4 export	6.5 (4% of added ^{15}N)
^{15}N-NO_3 export	41.7 (23% of added ^{15}N)
^{15}N-SPON export	7.6 (4% of added ^{15}N)

Note: Values for export of ^{15}N reflect baseflow conditions only because they are based on water samples collected on days 0, 20, and 41, all at baseflow.

(0.0021 and 0.014 μg ^{15}N/s, respectively), and day 41 (0.0032 and 0.012 μg ^{15}N/s, respectively). An additional 4% of the added tracer was accounted for via SPOM export, based on the calculated ^{15}N flux as SPOM at 125 m on day 42 (0.0021 μg^{15}N/s). This estimate may be high, however, because SPOM δ^{15}N probably was lower during the early part of the tracer addition than on day 42. Thus, ~21–36% of the added ^{15}N remains unaccounted for, presumably lost as DON or as particulate organic matter flushed out during storms.

Approximately 65% (SD = 31%, n = 3) of the ^{15}N in decomposing leaves was microbial at the end of the tracer addition, based on measurements of ^{15}N in the bulk material and in microbial biomass associated with leaves. Microbial ^{15}N constituted considerably lower proportions of ^{15}N in wood (25%, SD = 6%, n = 3) and FBOM (3%, SD = 2%, n = 3). Assuming that all ^{15}N taken up from water by detritus resulted from microbial processes, the detrital ^{15}N not accounted for as microbial biomass may be in N-containing extracellular materials produced by microbes or may be a result of incomplete extraction of some types of microbes (e.g., fungi, Jenkinson 1976) by the chloroform fumigation technique.

NH_4 regeneration rates and N turnover rates

The longitudinal profile of ^{15}N-NH_4 in stream water 12 h after the end of the ^{15}N addition (day P1) was hump-shaped, reflecting the simultaneous processes of $^{15}NH_4$ regeneration to water and its subsequent uptake downstream (Fig. 9A). Regeneration exceeded uptake in the upper portion of the study reach where biomass ^{15}N levels were greatest and uptake exceeded regeneration in the lower portions of the reach. Production rate of ^{15}N-NH_4 (k_A) was 1.2×10^{-7} s^{-1}, using an initial biomass ^{15}N (A_0) of 1826 μg ^{15}N/m calculated by extrapolating the sum of measured biomass ^{15}N levels back to the tracer addition point (Fig. 9B).

To estimate total NH_4 regeneration rate on day P1, the production of ^{15}N-NO_3 from biomass (indirect nitrification rate) must be added to the ^{15}N-NH_4 production rate. The indirect nitrification rate (k_N) on day P1 was 3.0×10^{-7} s^{-1}, again using an A_0 of 1826 μg ^{15}N/m (Fig. 9C). This estimate of indirect nitrification rate is considerably higher than that estimated two days earlier on day 41, because the gross ^{15}N-NO_3 production rate on day P1 (0.030 μg ^{15}N/s; Fig. 9C) was five times greater than the ^{15}N-NO_3 production rate on day 41 estimated to be the result of indirect nitrification (0.006 μg ^{15}N/s; Fig. 4A, C). Using the indirect nitrification rate determined for day P1, total NH_4 regeneration rate

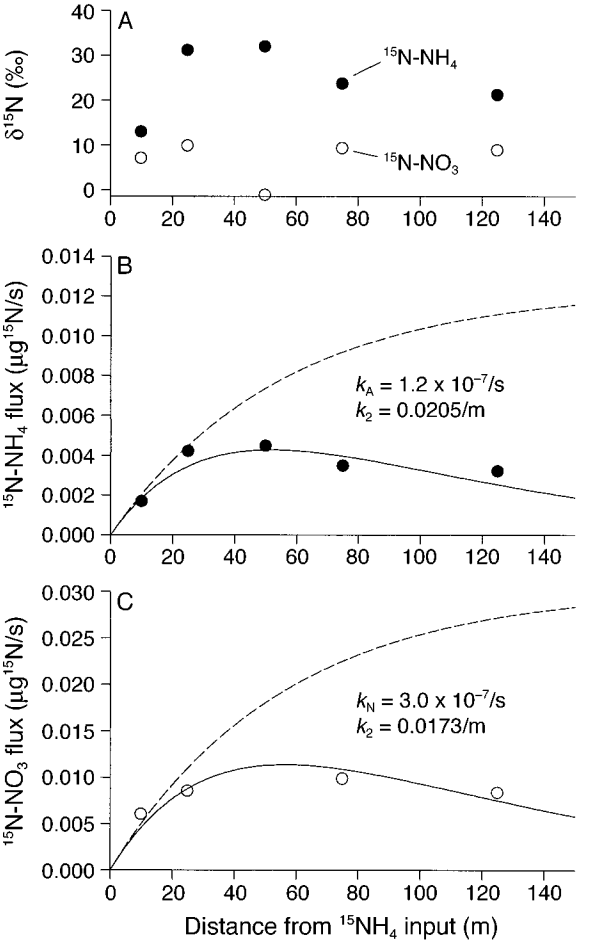

FIG. 9. Profiles of tracer δ^{15}N-NH_4 and δ^{15}N-NO_3 in stream water over the study reach on day P1, 12 h after termination of the $^{15}NH_4$ addition (panel A), and the best fit of the two-compartment mass balance model (Fig. 1) to the measured tracer ^{15}N-NH_4 (panel B) and ^{15}N-NO_3 fluxes (panel C) on day P1. The dashed curves in panels B and C represent the gross production fluxes of ^{15}N-NH_4 and ^{15}N-NO_3 that would have occurred given no uptake. Values for the fitted parameters k_2, k_A, and k_N are indicated.

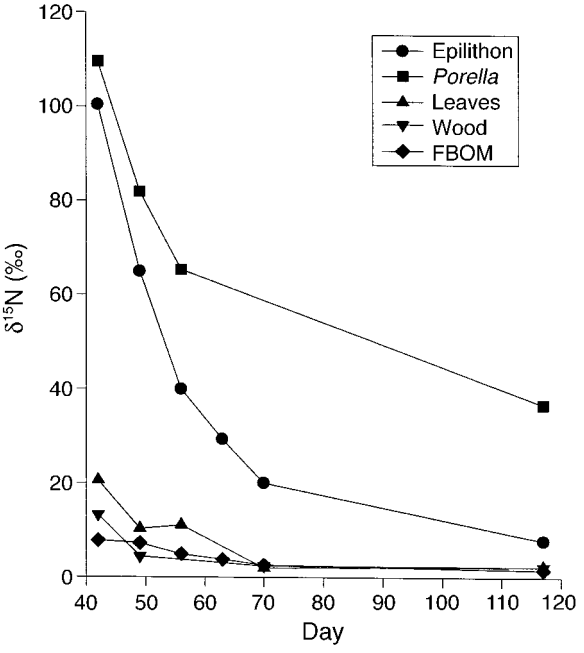

FIG. 10. Time course plots of tracer $\delta^{15}N$ in different organic matter compartments after the ^{15}N addition was terminated.

on this date (sum of k_A and k_N) was $\sim 4.2 \times 10^{-7}$ s^{-1} (3.6×10^{-2} d^{-1}). The inverse of this rate (28 d) is the average turnover time of NH_4 taken up by biomass from water. Using the lower estimate of indirect nitrification rate determined for day 41, total NH_4 regeneration rate was 1.8×10^{-7} s^{-1} and average turnover time of NH_4 taken up from water was 62 d.

There also were sizeable discrepancies between the uptake rates determined for day P1 and those determined for day 41. The day P1 ammonium uptake rate per unit distance (0.0205 m^{-1}; k_2 in Fig. 9B) was one-half the regeneration-corrected uptake rate determined from the water ^{15}N-NH_4 fluxes measured on day 41 (0.041 m^{-1}, Fig. 3B). The day P1 nitrate uptake rate per unit distance (0.0173 m^{-1}, k_2 in Fig. 9C) was ~nine times greater than that determined for day 41 (0.00196 m^{-1}, k_2 in Fig. 4C). Stream discharge and ammonium and nitrate concentrations were similar on days 41 and P1, consequently large differences in uptake rates would not be expected. The discrepancies in uptake rates between these dates may be a result of overestimation of the day P1 rates if the longitudinal distribution of the source of ^{15}N available for ammonium regeneration and indirect nitrification declined more sharply with distance than the longitudinal distribution of the sum of measured biomass ^{15}N used in the day P1 calculations. This situation is plausible because biomass ^{15}N in the lower portions of the study reach was likely to have a greater proportion of slower turnover ^{15}N pools associated with FBOM transported from upstream.

Levels of ^{15}N in biomass compartments declined rapidly following the termination of the ^{15}N addition, although epilithon and *Porella* $\delta^{15}N$ were still well above background levels 75 d (day 117) after the end of the addition (Fig. 10). N turnover rates calculated from the decline in biomass $\delta^{15}N$ over the first 28 days following termination of ^{15}N addition (day 42 to day 70 in Fig. 10) varied from 0.037 d^{-1} for *Porella* to 0.08 d^{-1} for leaves, with corresponding N turnover times of 27 d and 12.5 d, respectively (Table 8). These values may slightly underestimate actual ^{15}N turnover rates because of uptake of ^{15}N regenerated to water after termination of the addition (Fig. 2B). Alternatively, turnover rates determined in this way include the effects of resuspension and transport, which could have resulted in higher turnover rates for highly transportable compartments (e.g., FBOM, leaves) if material from unlabeled areas upstream was deposited at the 25 m station. Stream discharge was relatively constant over the first 28 d after the tracer addition (only one small storm on day 61 that resulted in a peak daily discharge ~three times baseflow). Thus, resuspension and transport may not have had a large effect on measured ^{15}N turnover rate. Growth dilution of tracer also could have resulted in overestimation of turnover rates for epilithon and *Porella*, although low GPP levels suggested that there was little accumulation of new biomass during the four weeks following the ^{15}N addition.

Despite the relatively rapid N turnover rate calculated for *Porella*, a substantial portion of the N taken up from water by this species appears to be partitioned into a relatively slow turnover pool. Based on $\delta^{15}N$ values at 25 m, *Porella* still retained 34% of its day 42 ^{15}N (6.7% of the ^{15}N added during the experiment) 75 d after the tracer addition ended (Fig. 10), and 23% of its day 42 ^{15}N (4.6% of the ^{15}N added) 30 wk after the addition ended (*data not shown*). In contrast, epilithon retained only ~8% of its day 42 ^{15}N after 75 d and only ~1% after 30 weeks. Transfer of ^{15}N to *Elimia clavaeformis*, by far the most important consumer in terms of biomass (Table 2), also was an important mechanism for long-term retention of N taken up from water. Approximately 77% of the ^{15}N in *Elimia* at the end of the tracer addition (3.3% of the ^{15}N added, Table

TABLE 8. Mean turnover rates (95% confidence interval in parentheses) of ^{15}N for individual biomass compartments determined from the decline in $\delta^{15}N_{biomass}$ over the first 28 days after termination of the ^{15}N addition, and turnover times of biomass N calculated as the inverse of the ^{15}N turnover rate.

Biomass compartment	^{15}N turnover rate (per day)	^{15}N turnover time (d)
Epilithon	0.057 (0.049–0.065)	17.5
Porella tips	0.037 (0.003–0.048)	27.0
Leaves	0.080 (0.005–0.154)	12.5
Wood	0.054 (0–0.382)	18.5
FBOM	0.041 (0.027–0.054)	24.7

7) was still retained 75 d later, and 19% was retained 30 wk after the experiment ended (0.8% of the ^{15}N added).

Discussion

The value of using in situ, tracer additions to study ecosystem nutrient dynamics is that processes can be examined under ambient conditions, without the artifacts resulting from stimulation of process rates by temporarily increasing nutrient concentrations (nutrient enrichment studies), or artifacts associated with the use of enclosures (microcosm studies). In this ^{15}N tracer experiment, the concentration of NH_4 was increased by a negligible amount (0.05 μg N/L) relative to ambient concentrations (<2–7 μg N/L). Several other recent studies involving in situ, tracer-level $^{15}NH_4$ additions to study stream N dynamics have been reported, including those in the Kuparuk River, Alaska (Peterson et al. 1997), Hugh White Creek, North Carolina (Hall et al. 1998), and Upper Ball Creek, North Carolina (J. L. Tank, *unpublished data*). Although the Kuparuk River is considerably larger in size than Walker Branch (100 times greater discharge), the other streams were not greatly different in size (Hugh White Creek discharge was slightly lower and Upper Ball Creek discharge was about five times greater than in Walker Branch) and all have low NH_4 concentrations (<5 μg N/L). Comparisons of our data with these other stream ^{15}N additions are highlighted in the discussion of our results below.

Whole-stream N dynamics

Uptake rates and lengths.—The short uptake lengths (23–27 m) and residence time in water (5 min) for NH_4 in Walker Branch indicate tight cycling of this nutrient. Further, the nearly twofold greater NH_4 uptake rate at twofold higher NH_4 concentration on day 20 compared with values on days 0 and 41 indicates that NH_4 uptake rate was strongly dependent on concentration as expected for a limiting nutrient in high demand relative to its supply rate. The relative constancy of NH_4 uptake length despite large variation in uptake rate over the experiment highlights the fact that nutrient uptake length is a measure of nutrient uptake efficiency (uptake/supply) rather than uptake rate per se.

Walker Branch NH_4 uptake length and residence time in water were considerably shorter than values determined for the Kuparuk River (1 km and 60 min, respectively), but were similar to values determined for Hugh White Creek (30 m and 10 min, respectively) and at the lower end of the range in Upper Ball Creek (29–126 m and 5–20 min, respectively). The differences in uptake lengths and residence times of NH_4 in water between Walker Branch and the Kuparuk River reflect the much lower average water depth and water velocity in Walker Branch. The differences in uptake length and residence time also reflect differences in biotic nutrient demand. The total uptake rate of ammonium on days 0 and 41 in Walker Branch (22–23 μg $N \cdot m^{-2} \cdot min^{-1}$) was nearly three times greater than the ammonium uptake rate in the Kuparuk River (8.4 μg $N \cdot m^{-2} \cdot min^{-1}$, computed from the values for average NH_4-N concentration, discharge, stream width, and NH_4 uptake length given by Peterson et al. 1997), despite the similarity in NH_4-N concentrations in the two streams (2–4 μg N/L in Walker Branch vs. 3 μg N/L in the Kuparuk). Although water temperatures in Walker Branch (12°–13°C) were higher than in the Kuparuk (8–10°C, Peterson et al. 1993), the considerably greater NH_4 uptake rate in Walker Branch probably resulted mostly from larger plant and microbial biomass and consequently greater biological demand. Epilithic algal biomass was ~2.5 times greater in Walker Branch (1.2 μg chlorophyll a/cm^2) than in the Kuparuk (0.5 μg/cm^2), and bryophyte biomass in Walker Branch (50 g dry mass/m^2) was about three times greater than that in the Kuparuk River (17 g dry mass/m^2, Bowden et al. 1994). In addition, the Kuparuk River lacks decomposing leaves, an important component of NH_4 uptake in Walker Branch, and the peat-derived FBOM in the Kuparuk appears to be of lower quality than FBOM in Walker Branch. A chironomid bioassay for food quality of FBOM (Meyer et al., 2000) indicated no growth on FBOM from the Kuparuk River, in contrast to a growth rate of 0.05 d^{-1} on FBOM from a headwater stream at Coweeta (J. Meyer and C. Hax, *unpublished data*).

Although NH_4 uptake lengths were similar, NH_4 uptake rates differed considerably among the smaller streams (Walker Branch, Hugh White Creek, and Upper Ball Creek). The differences in NH_4 uptake rates likely reflect seasonal variations in biological nutrient demand in streams draining deciduous forests in the Southern Appalachians. The total NH_4 uptake rate in Walker Branch in spring (22–37 μg $N \cdot m^{-2} \cdot min^{-1}$) was up to two times greater than that in Hugh White Creek in summer (17 μg $N \cdot m^{-2} \cdot min^{-1}$, computed from data in Hall et al. 1998), possibly as a result of the larger biomass and N demand by plants in Walker Branch during the period of high light availability. Epilithon biomass was about two times greater and bryophyte biomass was about 10 times greater in Walker Branch than in heavily shaded Hugh White Creek (Hall et al. 1998). In Walker Branch, NH_4 uptake by epilithon and bryophytes accounted for ~31% of the total NH_4 uptake rate, whereas in Hugh White Creek epilithon and bryophytes accounted for only ~8% of total NH_4 uptake rate (Hall et al. 1998). In contrast, Walker Branch NH_4 uptake rates during spring were considerably lower than those in Upper Ball Creek in autumn (66–172 μg $N \cdot m^{-2} \cdot min^{-1}$; J. L. Tank, *unpublished data*), probably reflecting the large recent input of leaf litter to Upper Ball Creek and high rates of NH_4 uptake by microbes using this labile carbon source. Mulholland et al. (1985) have shown that uptake rates of PO_4 in Walker Branch increase sharply in autumn after leaffall as a result of increased uptake by heterotrophic

microbes. Thus, comparisons of the ^{15}N results from these three forested, Southern Appalachian streams suggest that total nitrogen demand is greatest in autumn after leaf-fall and lowest in summer when leaf detritus and light availability are lowest, a finding similar to that of Mulholland et al. (1985) for phosphorus in Walker Branch.

Several lines of evidence suggest that the uptake rates of NH_4 measured in Walker Branch were the result of biotic rather than physicochemical sorption processes. First, sampling on day 0 did not commence until 6 h after the ^{15}N addition had begun, an interval that should have been long enough to allow for isotopic exchange with adsorbed NH_4 pools to reach steady state. Second, NH_4 concentrations were not measurably increased by the ^{15}N addition, precluding concentration-driven increases in net adsorption rates. In the absence of a continuous supply of new materials with available sites for adsorption, net NH_4 removal from water by adsorption should be negligible. In a previous study involving NH_4 additions to Walker Branch, Newbold et al. (1983*b*) reported that uptake of NH_4, even at substantially increased concentrations, was largely biotic.

The NH_4 uptake length in Walker Branch was considerably shorter than uptake lengths of PO_4 (73–156 m) measured using P radiotracers in the same reach under similar discharges during April and early May of previous years (Mulholland et al. 1985; Mulholland et al., *unpublished data*). Newbold et al. (1982) suggested that the uptake length of the limiting nutrient should be shorter than those of nonlimiting nutrients in streams. Based on this criterion, it would appear that N is the primary limiting nutrient in Walker Branch at this time of year. Our results indicated that nitrate was also an important source of N for biota, however, and the uptake length of nitrate at the beginning of the experiment (101 m) was within the range of those for PO_4. Thus, both N and P appear to be important limiting nutrients in Walker Branch in early spring based on spiraling measurements, with proximate limitation probably switching between N and P depending on small changes in nutrient supply ratios.

Uptake lengths of NO_3 in Walker Branch were considerably longer than those for NH_4. Nonetheless, uptake of NO_3 was a significant portion of total assimilatory DIN uptake on two of the three dates it was measured (62% on day 0 and 32% on day 41), presumably because uptake of NH_4 could not meet the demand for DIN at low NH_4 concentrations. Estimates of the relative importance of nitrate vs. ammonium uptake derived from biomass $\delta^{15}N$ values at the end of the tracer addition also indicated the importance of NO_3 uptake, suggesting that as much as 65–78% of total N demand by epilithon and bryophytes was met by uptake of nitrate (Table 6). The nitrate uptake rate measured on day 0 of this study (29 $\mu g\ N \cdot m^{-2} \cdot min^{-1}$) was considerably higher than uptake rates determined in previous years during early spring and based on declines in nitrate concentration in stream water over the same reach (8–13 $\mu g\ N \cdot m^{-2} \cdot min^{-1}$; Mulholland 1992). The discrepancy in nitrate uptake rates is not unexpected, however, because the earlier measurements provided estimates of net uptake rate whereas gross uptake rate was determined in this ^{15}N tracer study. The day 0 gross nitrate uptake rate in Walker Branch was also somewhat higher than nitrate uptake rates reported for forested streams in western North Carolina and Oregon (3.9–11.9 $\mu g\ N \cdot m^{-2} \cdot min^{-1}$; Munn and Meyer 1990), determined from 20 μg N/L nitrate addition experiments, but lower than the maximum uptake rate reported for Sycamore Creek (125 $\mu g\ N \cdot m^{-2} \cdot min^{-1}$; Grimm 1987), determined from mass balance calculations during intense algal growth following flash floods. Comparisons of our ^{15}N-tracer based nitrate uptake rates with those reported for other streams must be made with caution, however, because the latter usually have been determined from nitrate addition experiments (which may overestimate ambient rates if uptake is stimulated by added nitrate) or from mass balance measurements (which may underestimate gross uptake rate).

The most important determinant of nitrate uptake rate in Walker Branch appeared to be ammonium uptake rate which was controlled by NH_4 concentration. Although N demand can be met either by NH_4 or NO_3 in water, NH_4 is favored because of the lower energy requirements for assimilation. Uptake of NH_4, however, is often limited by its low concentration in stream water. At these times uptake of NO_3 is also required to meet total N demand. The uptake rate of NH_4 increased sharply with an increase in NH_4 concentration from 2.7 μg N/L on day 0 to 6.7 μg N/L on day 20, and this was accompanied by a sharp decline in NO_3 uptake rate despite much higher NO_3 concentrations on day 20 (Table 4). Ammonium uptake rate declined between days 20 and 41 as NH_4 concentration declined, and NO_3 uptake rate increased despite a decline in NO_3 concentration. The day 41 nitrate uptake rate was not as high as that on day 0, however, presumably because of lower demand for DIN by photosynthetic autotrophs as light levels and GPP declined after leaves developed in the forest canopy (Fig. 5).

Nitrate dynamics in Walker Branch appears to be controlled both by the total biological demand for N and by the availability of ammonium to meet that demand. Our results suggest that stream plants and the seasonal variation in GPP have a much greater impact on nitrate dynamics than on ammonium dynamics. Mulholland (1992) previously showed that diel variations in NO_3 concentrations in Walker Branch were large during early spring (April) but were absent in summer. Mass balance and nitrate addition studies in other streams have also demonstrated the importance of algal uptake in establishing temporal and spatial variations in nitrate concentrations (Grimm 1987, Triska et al. 1989).

Nitrification.—Our results indicate that nitrification can be a significant sink for ammonium and a substantial source of nitrate in low N streams. Direct nitrification rate accounted for nearly 20% of the total NH_4 uptake from water on day 0. This is a surprisingly high value given the low concentration of ammonium (2.7 μg N/L) and the relatively high demand for NH_4 by plants and heterotrophic microbes in Walker Branch. As stream water flowed through the 125-m study reach, direct nitrification consumed ~3 μg N/L of ammonium (gross consumption), producing a similar amount of nitrate-N which was equivalent to ~20% of the ambient nitrate concentration. Thus, in-stream nitrification was a significant control on stream nitrate concentration.

Some studies involving experimental NH_4 additions to streams have shown similar or much higher nitrification rates than in Walker Branch. For example, Richey et al. (1985) reported that nitrification accounted for one-third to two-thirds of the uptake of added NH_4 to Bear Brook, New Hampshire, or ~50–100 μg N/L over a 150-m reach. In their fertilization study in the Kuparuk River, Peterson et al. (1993) reported ~40% of the 100 μg N/L of added ammonium was nitrified over a 2-km reach of stream, about the same fractional rate of direct nitrification per meter as in Walker Branch. These NH_4 addition studies, however, almost certainly overestimated ambient nitrification rates because of stimulation from the enrichments. The nitrification rates we report here using a N tracer are, to our knowledge, the first measurements of nitrification rate in a stream with low ambient levels of ammonium.

Indirect nitrification rate (coupled regeneration of biomass N and nitrification) also appeared to be a significant contributor to the total nitrification rate, as suggested by the model results showing 1.6-fold higher gross ^{15}N-NO_3 production on day 41 than on day 0. Newbold (1992) noted that nitrification in streams is primarily associated with sediments (i.e., mostly indirect nitrification). Our data suggest that both nitrification of NH_4 in water and coupled remineralization/nitrification of biomass N on the stream bottom are significant contributors to stream NO_3 concentrations, even in streams with low NH_4 concentrations.

Our nitrification results are consistent with results from recent ^{15}N tracer experiments in forest soils indicating high nitrification rates even at sites with low NH_4 availability (Davidson et al. 1992, Stark and Hart 1997). Because nitrification and nitrate uptake are often closely coupled, ^{15}N tracer studies are needed to accurately determine nitrification and nitrate uptake rates in ecosystems. Together, these ^{15}N tracer experiments suggest that nitrification rates in terrestrial and aquatic ecosystems may be considerably higher than previously thought.

Uncertainty in modeling results.—There are several reasons for higher levels of uncertainty in our estimates of nitrate uptake lengths and rates, nitrification rates, and N regeneration rates derived from the model than those calculated directly from the data. These include: (1) low tracer δ^{15}N values (generally 5–15‰) relative to the variation (~2‰) in background δ^{15}N levels, (2) a relatively small number of data points (2–3) on the ascending and descending portions of the longitudinal ^{15}N flux profiles reducing the power of the model fitting procedure, and (3) uncertainty in the longitudinal distribution of biomass ^{15}N that serves as a source for ^{15}N-NH_4 and ^{15}N-NO_3 regeneration on day P1. We can estimate the level of uncertainty in the modeling results by comparing rates for day 41 with those for day P1 which we would expect to be similar because conditions were similar (i.e., stream discharge, streamwater N concentrations) and measurements were only two days apart. Uptake rates of NH_4 (Figs. 3B and 9B), uptake rates of NO_3, and gross production rates of ^{15}N-NO_3 (Figs. 4C and 9C) on these dates differed by factors ranging from ~2 to 9, suggesting considerable uncertainty in the model-derived rates. Because of the uncertainties in the longitudinal distribution of the ^{15}N source and the low tracer ^{15}N-NH_4 and ^{15}N-NO_3 fluxes measured on day P1, we have lower confidence in the modeling results for this date than for the earlier dates. Nonetheless, the application of the model to the longitudinal ^{15}N tracer data represents a new and potentially powerful approach for assessing rates of nitrate uptake, nitrification, and N regeneration under ambient conditions in streams.

N dynamics for specific compartments

Among the largely autotrophic compartments in Walker Branch, *Porella* appeared to play a much greater role in NH_4 uptake and N retention than epilithon. While epiphytes may have contributed to the NH_4 uptake rates measured for *Porella,* their effect was probably minor because of the relatively high δ^{15}N values attained by *Porella* despite its high total N content. Adsorption also was unlikely to account for the high NH_4 uptake rates by *Porella,* based on the lack of a sharp decline in its δ^{15}N immediately after termination of the ^{15}N addition (Fig. 10). Uptake by *Porella* appeared to be an important mechanism for long-term, in-place N retention, with nearly one-fourth of the ^{15}N in *Porella* at the end of the experiment (5% of ^{15}N added during the experiment) still present 30 wk later. Peterson et al. (1997) also reported considerable ^{15}N retention in mosses in the Kuparuk River a year after their experiment ended. These results suggest that bryophytes may be important in long-term nutrient retention in streams where they are abundant.

Decomposing leaves are a significant contributor to the standing stock of N in Walker Branch, even during spring, well after the annual peak in leaf-fall (early November) and high winter flows that remove leaves from much of the wetted channel. Among the detritus compartments, leaves had the highest rates of total and N-specific NH_4 uptake, the highest rate of N turnover, and the highest fraction of microbial N. Approximately

two-thirds of the ^{15}N retained by leaves at the end of the experiment was in microbial biomass. These results indicate that decomposing leaves play an important role in N dynamics in Walker Branch, even during the spring. They are also consistent with results of a previous P radiotracer study showing that leaves play the dominant role in P uptake in Walker Branch during all seasons but summer (Mulholland et al. 1985).

One interesting difference between the ^{15}N experiment results from Walker Branch and those from the North Carolina streams, Hugh White Creek (Hall et al. 1998) and Upper Ball Creek (J. L. Tank, *unpublished data*), involved the tracer enrichment in decomposing leaves. In the North Carolina streams, $\delta^{15}N$ values for decomposing leaves were 2–3 times higher at stations with similar water $\delta^{15}N$-NH_4 values to those in Walker Branch. This comparison suggests a greater dependence on nitrate as an N source for heterotrophic microbes in Walker Branch, where, unlike the other streams, nitrate concentrations were considerably higher than those of ammonium. We estimate that only 14% of the N demand by microbes colonizing decomposing leaves was met by ammonium in water, based on their $\delta^{15}N$ values near the end of the experiment, the remainder presumably met by uptake of streamwater nitrate and DON and use of organic N within the leaf. A similar analysis for Upper Ball Creek indicated that microbes on leaves obtained >50% of their N from streamwater ammonium (J. L. Tank, *unpublished data*).

In Walker Branch, FBOM is by far the largest pool of N and accounted for a moderate fraction of total NH_4 uptake from water. However, the estimate of NH_4 uptake by FBOM is likely an overestimate because sampling of FBOM for ^{15}N included surface material only. Several measures suggested that much of the N associated with FBOM is relatively refractory. Microbial N, calculated as a faction of the total N pool, was lower for FBOM than the other detritus pools (Table 3) as was the degree of ^{15}N labeling of FBOM at the end of the experiment (Fig. 6). Only ~3% of the ^{15}N retained by FBOM at the end of the experiment was in microbial pools, suggesting that much of the NH_4 uptake by FBOM microbes may have been partitioned into noncellular pools that may be of lower nutritional quality for consumers. Together, our results suggest that much of the N in FBOM may be associated with biologically refractory materials. The results of the ^{15}N experiments in Hugh White Creek (Hall et al. 1998) and Upper Ball Creek (J. L. Tank, *unpublished data*) also indicated low levels of ^{15}N labeling of FBOM. Several studies of metabolism or microbial activity associated with stream FBOM have indicated that this detrital pool may be of lower quality than CBOM (Peters et al. 1989, Webster et al. 1999), although other studies have shown the opposite relationship as well (e.g., Bott et al. 1985).

Synthesis

To summarize our findings of N dynamics in Walker Branch, we present a flow diagram of N standing stocks measured just prior to the ^{15}N addition and N flux rates calculated from the ^{15}N data during the first week of the experiment (Fig. 11). Thus, these standing stocks and fluxes reflect N dynamics during daylight early in the experiment. Several key points are highlighted by this flow diagram: (1) the importance of nitrification and uptake by *Porella* as sinks for ammonium in water; (2) the substantial contribution of nitrate uptake in satisfying the N demand by most benthic organic matter compartments; and (3) the importance of *Porella*, decomposing leaves, FBOM (although probably overestimated), and the snail *Elimia clavaeformis* for N retention within the ecosystem. Relative to its N standing stock, however, uptake and accumulation of ^{15}N by epilithon was disproportionately large (much larger per unit N standing stock than for other compartments).

Our study shows that Walker Branch is quite retentive of ammonium, with one-third to one-half of the ^{15}N-NH_4 added to streamwater retained within the first 125 m of stream at the end of the 42-day experiment. The average residence time (5 min) and travel distance (~25 m) of an ammonium ion in water before it is taken up by biota or nitrified was quite short. Despite the low concentrations of ammonium and high demand relative to its supply, nitrification rates were substantial and nearly one-fourth of the added ^{15}N-NH_4 was lost from the 125-m reach as ^{15}N-NO_3. In total, the results of our ^{15}N tracer experiment showed that ammonium retention and nitrification were both high in Walker Branch, and that the downstream loss of N was primarily as nitrate and was determined largely by nitrification, the assimilatory demand for N, and the availability of ammonium to meet that demand.

The latter two determinants of nitrate loss were variable over time. The demand for N is a function of primary productivity (which varies with light availability) and productivity of heterotrophic microbes (which varies with availability of labile organic carbon in the form of terrestrial detritus). Ammonium availability is a function of streamwater ammonium concentration which varies unpredictably over time, probably due to variation in organic N mineralization and ammonium inputs from the catchment. Although nitrate is sometimes taken up at relatively high rates in Walker Branch (at times greater than the uptake rate of ammonium), it is less preferred as a source of N due to higher energy requirements for assimilation and thus its uptake is the result of the inability of organisms to meet their demand for N by uptake of ammonium.

The cycling of N in ecosystems is complex, involving many different processes and several different forms of N. Measurement of N concentrations alone over time often provides little information on process rates because of simultaneous production and con-

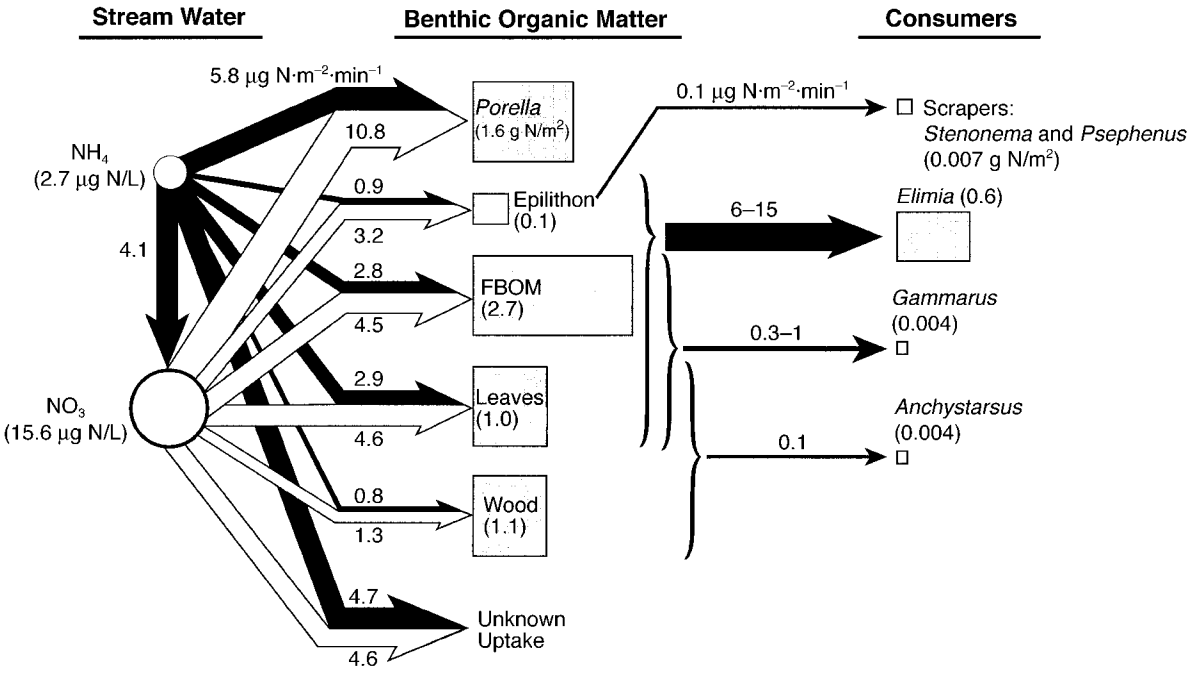

FIG. 11. N standing stocks (boxes, values in parentheses all g N/m^2) and fluxes (arrows, values all μg N·m^{-2}·min^{-1}) in Walker Branch representative of the early part of the experiment. Standing stocks were calculated from measurements made just prior to the ^{15}N addition (Table 2). Fluxes of NH_4-N from water to benthic organic matter compartments and nitrification are from Table 5. The unknown uptake flux was calculated as the difference between total NH_4 uptake rate on day 0 (Table 4) and the compartment-specific uptake rates. Fluxes of NO_3-N from water to epilithon and *Porella* were calculated from the NH_4 uptake rates and NH_4-N uptake as a fraction of total N uptake (Table 6). Fluxes of NO_3-N from water to the detritus compartments were calculated as the NH_4 uptake rate times 1.6 (the ratio of total NO_3-N uptake to total assimilatory NH_4-N uptake calculated for day 0). The unknown flux of NO_3-N from water to benthic organic matter was calculated as the difference between NO_3-N uptake by known compartments and the total NO_3-N uptake determined for day 0 (Table 4). The fluxes of N from benthic organic matter compartments to consumers (only those with standing stocks ≥0.1 g N/m^2) were determined from the measured tracer δ^{15}N for consumers on day 7 (Eq. 2) and the average δ^{15}N of their presumed food over the first seven days (day-7 value divided by 2). For *Elimia,* which is thought to feed primarily on epilithon at this time of year but may feed on FBOM and leaves as well, N flux rate was calculated based on consumption of epilithon only (lower value) and equal consumption of epilithon, FBOM, and leaves (upper value). For *Gammarus,* flux rates were calculated based on feeding on either leaves (lower value) or FBOM (upper value). For *Anchytarsus,* the calculated flux rate was similar regardless of whether it fed on leaves or wood. Regeneration fluxes of organic N back to water are not shown for simplicity.

sumption of the different forms of N. The development of ^{15}N tracer techniques for ecosystem studies has allowed the determination of N cycling rates under ambient conditions. For stream ecosystems, ^{15}N tracer addition experiments have shown that uptake lengths of ammonium are short (tens of meters; Peterson et al. 1997, Hall et al. 1998; this study; J. L. Tank, *unpublished data*), indicating tight spiraling of N, and that bryophytes can be very important in ammonium uptake and retention (Peterson et al. 1997; this study). Substantial gaps in our knowledge still remain, however, particularly with regard to the production and export of DON and rates of denitrification.

Some of the findings from ecosystem-scale ^{15}N experiments have been quite surprising and are changing our perception of N dynamics in ecosystems, particularly the production and retention of nitrate. Tracer ^{15}N addition experiments conducted in a coastal forest in Massachusetts (Seely and Lajtha 1997) have demonstrated that nitrate uptake rates by soil microbes are high and provide an important mechanism for long-term N retention. Tracer-level ^{15}N experiments in soil cores from coniferous forests in the western United States also have demonstrated that rates of nitrification and microbial assimilation of nitrate are much higher than formerly thought (Davidson et al. 1992, Stark and Hart 1997). Results from the ^{15}N study reported here demonstrate the importance of nitrification as a sink for ammonium in stream ecosystems, even those with low ammonium concentrations, with the balance between nitrification and nitrate uptake controlling the loss of N from the ecosystem via hydrologic transport.

ACKNOWLEDGMENTS

Melanie Carter and Norm Leonard assisted with field sampling and sample processing. Ramie Wilkerson performed the water nutrient analysis and Kris Tholke the mass spectrometry. John Smith provided unpublished data on macroinvertebrate abundance from previous years in Walker Branch. Allan Fife helped identify bryophyte taxa. Walter Hill, Michael Huston, Val Smith, and two anonymous reviewers pro-

vided helpful reviews of earlier drafts of the manuscript. This research was part of the Lotic Intersite Nitrogen Experiment (LINX) supported by a grant from the Ecosystems Program, National Science Foundation, to Virginia Tech (DEB-9628860). The research was conducted on the Oak Ridge National Environmental Research Park, Environmental Sciences Division, Office of Biological and Environmental Research, U.S. Department of Energy under contract DE-AC05–96OR22464 with Lockheed Martin Energy Research Corp. Publication 4901, Environmental Sciences Division, Oak Ridge National Laboratory.

Literature Cited

Aber, J. D., A. Magill, S. G. McNulty, R. D. Boone, K. J. Nadelhoffer, M. Downs, and R. Hallett. 1995. Forest biogeochemistry and primary production altered by nitrogen saturation. Water, Air, and Soil Pollution **85**:1665–1670.

American Public Health Association. 1992. Standard methods for the examination of water and wastewater. 18th edition. American Public Health Association, Washington D.C., USA.

Bott, T. L., J. T. Brock, C. S. Dunn, R. J. Naiman, R. V. Ovink, and R. C. Petersen. 1985. Benthic community metabolism in four temperate stream ecosystems: an interbiome comparison and evaluation of the river continuum concept. Hydrobiologia **123**:3–45.

Bowden, W. B., J. C. Finlay, and P. E. Maloney. 1994. Long-term effects of PO_4 fertilization on the distribution of bryophytes in an arctic river. Freshwater Biology **32**:445–454.

Brookes, P. C., J. F. Kragt, D. S. Powlson, and D. S. Jenkinson. 1985*a*. Chloroform fumigation and the release of soil nitrogen: the effects of fumigation time and temperature. Soil Biology and Biochemistry **17**:831–837.

Brookes, P. C., A. Landman, G. Pruden, and D. S. Jenkinson. 1985*b*. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry **17**:837–842.

Brooks, P. D., J. M. Stark, B. B. McInteer, and T. Preston. 1989. Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. Soil Science Society of America Journal **53**:1707–1711.

Cabrera, M. L., and M. H. Beare. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Science Society of America Journal **57**: 1007–1012.

Davidson, E. A., S. C. Hart, and M. K. Firestone. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. Ecology **73**:1148–1156.

Elser, J. J., E. R. Marzolf, and C. R. Goldman. 1990. Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: a review and critique of experimental enrichments. Canadian Journal of Fisheries and Aquatic Sciences **47**:1468–1477.

Elwood, J. W., J. D. Newbold, A. F. Trimble, and R. W. Stark. 1981. The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. Ecology **62**:146–158.

Fry, B., D. E. Jones, G. W. Kling, R. B. McKane, K. J. Nadelhoffer, and B. J. Peterson. 1995. Adding ^{15}N tracers to ecosystem experiments. Pages 171–192 *in* E. Wada et al., editors. Stable isotopes in the biosphere. Kyoto University Press, Kyoto, Japan.

Gordon, N. D., T. A. McMahon, and B. L. Finlayson. 1992. Stream hydrology: an introduction for ecologists. Wiley, Chichester, UK.

Grimm, N. B. 1987. Nitrogen dynamics during succession in a desert stream. Ecology **68**:1157–1170.

Grimm, N. B., and S. G. Fisher. 1986. Nitrogen limitation in a Sonoran Desert stream. Journal of the North American Benthological Society **5**:2–15.

Hall, R. O., Jr., B. J. Peterson, and J. L. Meyer. 1998. Testing a nitrogen-cycling model of a forest stream by using a nitrogen-15 tracer addition. Ecosystems **1**:283–298.

Hill, W. R., and A. W. Knight. 1988. Nutrient and light limitation of algae in two northern California streams. Journal of Phycology **24**:125–132.

Holmes, R. M., J. W. McClelland, D. M. Sigman, B. Fry, and B. J. Peterson. 1998. Measuring $^{15}N\text{-}NH_4^+$ in marine, estuarine, and fresh waters: an adaptation of the ammonium diffusion method for samples with low ammonium concentrations. Marine Chemistry **60**:235–243.

Jenkinson, D. S. 1976. The effects of biocidal treatments on metabolism in soil. IV. The decomposition of fumigated organisms in soil. Soil Biology and Biochemistry **8**:203–208.

Johnson, D. W., and R. I. Van Hook. 1989. Analysis of biogeochemical cycling processes in Walker Branch watershed. Springer-Verlag, New York, New York, USA.

Jordan, T. E., and D. E. Weller. 1996. Human contributions to terrestrial nitrogen flux. BioScience **46**:655–664.

Lohman, K., J. R. Jones, and C. Baysinger-Daniel. 1991. Experimental evidence for nitrogen limitation in a northern Ozark stream. Journal of the North American Benthological Society **10**:14–23.

Marti, E., and F. Sabater. 1996. High variability in temporal and spatial nutrient retention in Mediterranean streams. Ecology **77**:854–869.

Marzolf, E. R., P. J. Mulholland, and A. D. Steinman. 1994. Improvements to the diurnal upstream–downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. Canadian Journal of Fisheries and Aquatic Sciences **51**:1591–1599.

Meyer, J. L., C. Hax, J. B. Wallace, S. L. Eggert, and J. R. Webster. 2000. Terrestrial litter inputs as determinants of food quality of organic matter in a forest stream. Verhandlungen Internationale Vereingung Limnologie **27**. *In press.*

Meyer, J. L., G. E. Likens, and J. Sloane. 1981. Phosphorus, nitrogen, and organic carbon flux in a headwater stream. Archiv fur Hydrobiologie **91**:28–44.

Mulholland, P. J. 1992. Regulation of nutrient concentrations in a temperate forest stream: roles of upland, riparian, and instream processes. Limnology and Oceanography **37**: 1512–1526.

Mulholland, P. J., J. W. Elwood, J. D. Newbold, L. A. Ferren, and J. R. Webster. 1985. Phosphorus spiralling in a woodland stream: seasonal variations. Ecology **66**:1012–1023.

Mulholland, P. J., and W. R. Hill. 1997. Separating catchment flowpath and instream effects on stream nutrient and DOC concentrations: evidence from long-term data records. Water Resources Research **33**:1297–1306.

Mulholland, P. J., E. R. Marzolf, J. R. Webster, D. R. Hart, and S. P. Hendricks. 1997. Evidence that hyporheic zones increase heterotrophic metabolism and phosphorus uptake in forest streams. Limnology and Oceanography **42**:443–451.

Mulholland, P. J., J. L. Tank, D. M. Sanzone, J. R. Webster, W. Wollheim, B. J. Peterson, and J. L. Meyer. 2000*a*. Ammonium and nitrate uptake lengths in a small forested stream determined by ^{15}N tracer and short-term nutrient enrichment experiments. Verhandlungen Internationale Vereingung Limnologie **27**. *In press.*

Mulholland, P. J., J. L. Tank, D. M. Sanzone, W. M. Wollheim, B. J. Peterson, J. R. Webster, and J. L. Meyer. 2000*b*. Food web relationships in a forested stream determined by natural abundance and experimental ^{15}N-tracer studies. Journal of the North American Benthological Society **19**:145–157.

Mulholland, P. J., G. V. Wilson, and P. M. Jardine. 1990. Hydrogeochemical response of a forested watershed to

storms: effects of preferential flow along shallow and deep pathways. Water Resources Research **26**:3021–3036.

Munn, N. L., and J. L. Meyer. 1990. Habitat-specific solute retention in two small streams: an intersite comparison. Ecology **71**:2069–2082.

Murdoch, P. S., and J. L. Stoddard. 1992. The role of nitrate in the acidification of streams in the Catskill Mountains of New York. Water Resources Research **28**:2707–2720.

Newbold, J. D. 1992. Cycles and spirals of nutrients. Pages 379–408 *in* P. Calow and G. E. Petts, editors. The rivers handbook. Volume 1. Blackwell, Oxford, UK.

Newbold, J. D., J. W. Elwood, R. V. O'Neill, and A. L. Sheldon. 1983*a*. Phosphorus dynamics in a woodland stream ecosystem: a study of nutrient spiralling. Ecology **64**:1249–1265.

Newbold, J. D., J. W. Elwood, R. V. O'Neill, and W. Van Winkle. 1981. Measuring nutrient spiraling in streams. Canadian Journal of Fisheries and Aquatic Sciences **38**:860–863.

Newbold, J. D., J. W. Elwood, M. S. Schulze, R. W. Stark, and J. C. Barmeier. 1983*b*. Continuous ammonium enrichment of a woodland stream: uptake kinetics, leaf decomposition, and nitrification. Freshwater Biology **13**:193–204.

Newbold, J. D., R. V. O'Neill, J. W. Elwood, and W. Van Winkle. 1982. Nutrient spiralling in streams: implications for nutrient limitation and invertebrate activity. American Naturalist **120**:628–652.

Nixon, S. W. 1995. Coastal marine eutrophication: a definition, social causes, and future consequences. Ophelia **41**: 199–219.

Peters, G. T., E. F. Benfield, and J. R. Webster. 1989. Chemical composition and microbial activity of seston in a southern Appalachian headwater stream. Journal of the North American Benthological Society **8**:74–84.

Peterson, B. J., M. Bahr, and G. W. Kling. 1997. A tracer investigation of nitrogen cycling in a pristine tundra river. Canadian Journal of Fisheries and Aquatic Science **54**: 2361–2367.

Peterson, B. J., et al. 1993. Biological responses of a tundra river to fertilization. Ecology **74**:653–672.

Richey, J. S., W. H. McDowell, and G. E. Likens. 1985. Nitrogen transformations in a small mountain stream. Hydrobiologia **124**:129–139.

Rosemond, A. D. 1993. Interactions among irradiance, nutrients, and herbivores constrain a stream algal community. Oecologia **94**:585–594.

Rosemond, A. D., P. J. Mulholland, and J. W. Elwood. 1993. Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. Ecology **74**:1264–1280.

Seely, B., and K. Lajtha. 1997. Application of a ^{15}N tracer to simulate and track the fate of atmospherically deposited N in the coastal forests of the Waquoit Bay Watershed, Cape Cod, Massachusetts. Oecologia **112**:393–402.

Sigman, D. M., M. A. Altabet, R. Michener, D. C. McCorkle, B. Fry, and R. M. Holmes. 1997. Natural abundance-level measurement of the nitrogen isotopic composition of oceanic nitrate: an adaptation of the ammonia diffusion method. Marine Chemistry **57**:227–242.

Sorensen, P., and E. S. Jensen. 1991. Sequential diffusion of ammonium and nitrate from soil extracts to a polytetrafluoroethylene trap for ^{15}N determination. Analytica Chimica Acta **252**:201–203.

Stark, J. M., and S. C. Hart. 1997. High rates of nitrification and nitrate turnover in undisturbed coniferous forests. Nature **385**:61–64.

Steinman, A. D., and H. L. Boston. 1993. The ecological role of aquatic bryophytes in a woodland stream. Journal of the North American Benthological Society **12**:17–26.

Triska, F. J., V. C. Kennedy, R. J. Avanzino, G. W. Zellweger, and K. E. Bencala. 1989. Retention and transport of nutrients in a third-order stream in northwestern California: channel processes. Ecology **70**:1877–1892.

Triska, F. J., J. R. Sedell, K. Cromack, S. V. Gregory, and F. M. McCorison. 1984. Nitrogen budget for a small coniferous forest stream. Ecological Monographs **54**:119–140.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and G. D. Tilman. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications **7**:737–750.

Vitousek, P. M., and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: how can it occur? Biogeochemistry **13**:87–115.

Webster, J. R., E. F. Benfield, M. A. Schaeffer, T. P. Ehrman, J. L. Tank, J. J. Hutchens, and D. J. D'Angelo. What happens to allochthonous material that falls into streams: synthesis of new and published information from Coweeta. Freshwater Biology **41**:687–705.

Wetzel, R. G., and G. E. Likens. 1991. Limnological analyses. Second edition. Springer-Verlag, New York, New York, USA.

Young, R. G., and A. D. Huryn. 1998. Comment: further improvements to the diurnal upstream–downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. Canadian Journal of Fisheries and Aquatic Sciences **55**:1784–1785.