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Analysis of nitrogen cycling in a forest stream during autumn using a ^{15}N-tracer addition

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Abstract

We added $^{15}NH_4Cl$ over 6 weeks to Upper Ball Creek, a second-order deciduous forest stream in the Appalachian Mountains, to follow the uptake, spiraling, and fate of nitrogen in a stream food web during autumn. A priori predictions of N flow and retention were made using a simple food web mass balance model. Values of $\delta^{15}N$ were determined for stream water ammonium, nitrate, dissolved organic nitrogen, and various compartments of the food web over time and distance and then compared to model predictions.

Ammonium uptake lengths were shortest at the beginning of the tracer addition (28 m) and increased through time (day 20 = 82 m, day 41 = 94 m), and ammonium residence time in stream water ranged from 4 min on day 0 to 15 min on day 41. Whole-stream ammonium uptake rates, determined from the decline in $^{15}NH_4$ in water over the stream reach, decreased from 191 mg N m^{-2} d^{-1} on day 0 to 83.2 mg N m^{-2} d^{-2} on day 41. Temporal trends in the NH_4 mass transfer coefficient (v_f) were similar to uptake rates; v_f was highest on day 0 (7.4×10^{-4} m s^{-1}) and lower on days 20 and 41 (2.7 and 2.8×10^{-4} m s^{-1}, respectively). Rates of nitrification were estimated to be very low throughout the tracer addition and accounted for <3% of $^{15}NH_4$ uptake on day 0.

It appears that most of the N in epilithon was actively cycling based on comparisons of ^{15}N in stream water and biomass at the end of the experiment. In contrast, for allochthonous organic matter, we found that microbial ^{15}N represented 69% of the label in wood, 20% in leaves, and 31% in fine benthic organic matter (FBOM). Despite higher $\delta^{15}N$ values in primary producers, $^{15}NH_4$ uptake rates per unit stream bottom area were generally lower in epilithon compared to the detrital compartments, a result of the lower biomass of epilithon. Turnover times were

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similar for epilithon (47 d), leaves (38 d), and FBOM (53 d) based on the decline in ^{15}N tracer over the first 28 d after the addition stopped.

Incorporation of ^{15}N varied among biomass compartments involved in ammonium uptake from water. Primary producers were more highly labeled than allochthonous organic matter. Epilithon $\delta^{15}N$ values were higher than leaves or FBOM, appeared to reach isotopic equilibrium by day 42, and followed model-predicted trends. The grazing mayfly *Stenonema* was more highly labeled than the epilithon, which suggests selective feeding or assimilation of the more highly labeled algal-bacterial portion of the epilithon. Leaves had very low $\delta^{15}N$ values, and $\delta^{15}N$ values for the shredding stonefly *Tallaperla* were close to model predictions and followed labeling in leaves.

Total retention of ^{15}N at the end of the experiment by the nine largest biomass compartments within the study reach accounted for only 12.3% of added ^{15}N, with leaves and FBOM representing the largest portions. Export of ^{15}N by suspended particulate and dissolved N accounted for an additional 11% and 30% of added ^{15}N, respectively.

Results from the ^{15}N-tracer addition in Upper Ball Creek demonstrate the high ammonium demand associated with microbes colonizing leaf detritus and the resultant linkage to invertebrate shredders. In Upper Ball Creek in autumn, spiraling of NH_4 is very tight, NH_4 residence time in water is short, and uptake rates are very high. Analyses of N spiraling in unimpacted streams provide an ecological foundation for assessment of spiraling in high-N streams.

Nitrogen can control the productivity and dynamics of stream ecosystems, and many streams are limited by the supply of biologically reactive nitrogen (e.g., Grimm and Fisher 1986). Anthropogenic perturbations of the nitrogen cycle through fossil fuel combustion, fertilizer production, and waste disposal have caused a several-fold increase in nitrogen loading to streams (Howarth et al. 1996). These human activities are changing biological processes in streams, rivers, lakes, estuaries, and ultimately coastal habitats worldwide (Vitousek et al. 1997). We currently lack a sound predictive understanding of the consequences of this nitrogen loading because we know little about processes controlling nitrogen uptake, retention, and spiraling within stream ecosystems. Quantifying these processes in unimpacted streams provides a baseline by which we can assess changes that have occurred or will result from human impacts.

This study represents the first experiment in the multisite Lotic Intersite Nitrogen eXperiment (LINX). In the 2-yr study, identical 6-week $^{15}NH_4$ tracer additions were conducted in 10 streams located in different biomes. Streams were chosen to represent variability in ecological characteristics (primary productivity, hydraulic retention, food web complexity, etc.) while holding constant other variables such as stream size (<100 L s^{-1}), low levels of human impact, and the availability of long-term ecological data from years of previous study. Using $^{15}NH_4$ as a tracer, we were able to follow the uptake, spiraling, and fate of N in a stream food web without significantly increasing background ammonium levels (Fry et al. 1995; Peterson et al. 1997).

Upper Ball Creek, a deciduous forest stream at Coweeta Hydrologic Laboratory in the mountains of western North Carolina, was chosen to represent a heterotrophic headwater stream with very low primary production, high leaf fall, and very low nutrient concentrations. We estimated N uptake and fluxes through this detritus-based stream ecosystem using a simple compartment model to predict N flow and retention (Hall et al. 1998; Wollheim et al. 1999) and then tested our predictions using data from the 6-week $^{15}NH_4$ tracer addition.

Use of stable isotope tracers to track N in streams is a relatively recent technique, and methods for tracer additions were pioneered in studies of the autotrophic Kuparak River, Alaska (Peterson et al. 1997; Wollheim et al. 1999), focusing particularly on the fate of added $^{15}NH_4$ through the stream food web. Work by Hall et al. (1998) on Hugh White Creek, North Carolina used the ^{15}N-tracer technique to characterize the food web in a headwater stream, during summer, and tested field results against model-generated predictions. The Upper Ball Creek study builds on the theoretical framework from previous work by implementing recently developed techniques for measuring the ^{15}N content in streamwater N (NH_4, NO_3, dissolved organic N), allowing us to quantify rates of dissolved nitrogen uptake and release (Sorenson and Jensen 1991; Sigman et al. 1997; Holmes et al. 1998). This is the first comprehensive study of NH_4 uptake and N cycling in a stream where ecosystem function is dominated by autumn inputs of allochthonous organic matter (leaves); autumn is a time of peak heterotrophic activity in temperate forested streams. The Upper Ball Creek study provides a contrast to a similar study conducted in Walker Branch, Tennessee, another forested stream and LINX study site, but one in which primary production played a more significant role in the energy base of the food web (Mulholland et al. 2000*a*). By comparing model predictions with results from the field tracer addition, we identify areas of nutrient cycling in need of further research.

Methods

Site description—Upper Ball Creek is a second-order stream at Coweeta Hydrologic Laboratory, Macon County, North Carolina. The riparian forest consists of oaks, hickory, poplar, and dogwood and a dense understory of rhododendron, which heavily shades the stream all year. Upper Ball Creek is a low-nutrient, soft-water stream with a mixed substratum of cobble-pebble and sand-gravel and some bedrock outcrops (Table 1). Our study site was located at an elevation of approximately 922 m, where stream gradient was 0.17 m m^{-1}.

Stream characterization—Two weeks before we began the ^{15}N-tracer addition, we characterized stream ecosystem processes in Upper Ball Creek to assist in interpreting the ^{15}N-tracer data. We measured hydrodynamic properties using a conservative tracer release, short-term nutrient injections to determine nutrient uptake lengths, whole-stream metabolism, and nitrogen microbial content. A detailed description of methods used in stream characterization is given in Mulholland et al. (2000*a*).

Table 1. Stream characteristics for Upper Ball Creek at beginning of ^{15}N addition.

Parameter	Value
Physical (reach average)	
Discharge at Day 0	55.6 L s^{-1}
Mean width	2.7 m
Mean depth at day 0	10 cm
Temperature (daily mean)	7.2°C
$A_s:A$	0.37
Water chemistry	
NH_4 concentration	3 μg N L^{-1}
NO_3 concentration	2 μg N L^{-1}
SRP concentration	3 μg P L^{-1}
DON concentration	18.5 μg N L^{-1}
Nutrient uptake lengths*	
NH_4	65 m
NO_3	199 m
SRP	151 m
Metabolism	
Epilithon standing stock	0.73 mg Chl *a* m^{-2}
Epilithon Chl *a*: organic ratio	0.58 mg Chl *a* g $AFDM^{-1}$
GPP	0.06 gO_2 m^{-2} d^{-1}
R	32.3 gO_2 m^{-2} d^{-1}
P:*R* ratio	0.002

* Measured with short-term enrichment experiments.

Solute dynamics: We measured the hydrodynamic properties of our 250-m stream reach by injecting a conservative hydraulic tracer (chloride) and analyzing these data using a transient storage model (Bencala and Walters 1983; Hart 1995; Webster and Ehrman 1996).

In addition to the conservative tracer, we simultaneously added one of each of three inorganic nutrients (ammonium chloride, potassium nitrate, or potassium phosphate) at concentrations necessary to raise stream levels 5–10 times background levels to determine uptake lengths of ammonium, nitrate, and phosphate using the short-term enrichment approach (Webster and Ehrman 1996). Nitrate (reported as NO_3-N) was measured using the cadmium reduction method (APHA 1995), and ammonium (NH_4-N) and soluble reactive phosphorus (SRP) concentrations were analyzed spectrophotometrically (Wetzel and Likens 1991).

Metabolism and chlorophyll *a*: The rates of whole-stream respiration and gross primary production were measured using the upstream–downstream diurnal dissolved oxygen change technique (Marzolf et al. 1994, 1998; Young and Huryn 1998) using Orbisphere dissolved oxygen meters. To complement measurements of whole-stream primary production, we also measured chlorophyll *a* (Chl *a*) in epilithon by placing a 5-cm diameter PVC cylinder sealed with neoprene on a rock surface, scrubbing with a brush, rinsing the material into a vial, and filtering onto ashed GF/F filters. These were extracted at 4°C in the dark for 24 h in 20 ml of 90% acetone. Extracts were then analyzed on a spectrophotometer at 664 and 750 nm before and after acidification for determination of Chl *a* and phaeophytin (APHA 1995).

Microbial nitrogen associated with organic matter: We measured microbial N associated with leaves, wood scrapings, and fine benthic organic matter (FBOM) from six locations along the study reach on day 38 of the ^{15}N-tracer release using a modification of the chloroform fumigation technique (Brookes et al. 1985*a*,*b*) as detailed by Mulholland et al. (2000*a*). Four subsamples (100 g wet weight) of each of three material types from six locations were placed in individual 125-ml Erlenmeyer flasks. Half of the replicates were fumigated with chloroform for 24 h to lyse microbial cells, and half were not exposed to chloroform and were designated as the "unfumigated" samples. All subsamples were extracted with 0.5 M K_2SO_4, filtered, and analyzed for total N using alkaline persulfate digestions (Cabrera and Beare 1993) then analyzed for nitrate produced from the digestions (described previously). The difference in N content between fumigated and unfumigated K_2SO_4 extracts represents microbial N (expressed as mg N g ash-free dry $mass^{-1}$), which was then divided by a factor of 0.54 to account for incomplete extraction of microbial biomass using the chloroform fumigation technique (Brookes et al. 1985*b*).

Calibration data for N cycle model and calculation of predicted ^{15}N—Before beginning the ^{15}N-tracer addition to Upper Ball Creek, we ran a 15-compartment stream N model that simulated the 6-week tracer addition to a 200 m reach and predicted the ^{15}N values in the food web over time and distance (Wollheim et al. 1999). The model assumes that the system is at steady state with regard to N (i.e., flows into each compartment equal flows out). The model was partitioned into 10-m stream segments, and all stocks were converted to g N $segment^{-1}$; fluxes were in g N $segment^{-1}$ d^{-1}. The model calculates $\delta^{15}N$ for each compartment in each segment for each day after the start of the $^{15}NH_4$ addition. Model inputs of N from upstream were varied daily by incorporating variations of stream discharge into the model.

The model included three dissolved N pools: ammonium, nitrate, and DON (Table 2). Ammonium and nitrate concentrations were measured as described above. DON was calculated as the difference between total dissolved N (determined using persulfate digestion method; Ameel et al. 1993) and inorganic N concentrations. Compartments taking up dissolved N in the model were leaves, wood, seston, FBOM, epilithon, and moss. We used the best available estimates for N standing stocks and fluxes (either measured directly or derived from previous studies on Upper Ball Creek) to parameterize the model. Stocks of particulate N were determined by multiplying standing stocks (g AFDM m^{-2}) by the percentage of N measured for each compartment (Carlo Erba NA 1500 CN Analyzer). Standing stocks used to parameterize the model are summarized in Table 2.

Standing stocks for both benthic and suspended organic matter were obtained from a year-long study on Upper Ball Creek (Benfield et al. in press) and verified for this study by sampling 1 week before the ^{15}N-tracer release. At 12 locations, we placed a metal cylinder (0.07 m^2) into the sediments and removed course benthic organic matter (CBOM), separating it into wood and leaves. Fine benthic organic matter (FBOM) was sampled by sealing a metal cylinder on the stream bottom, mixing the sediments to 5 cm, pumping

Table 2. Compartmental summary of C and N standing stocks in Upper Ball Creek.

Compartment	Biomass (g AFDM m^{-2})	% Nitrogen	C:N ratio	C:AFDM ratio	N standing stock (g m^{-2})
NO_3	—	—	—	—	1–4 $\mu g\ L^{-1}$
NH_4	—	—	—	—	3–5 $\mu g\ L^{-1}$
DON	—	—	—	—	20 $\mu g\ L^{-1}$
Seston	0.0004 g L^{-1}*	0.66	19.65	0.465	9.4 $\mu g\ L^{-1}$
FBOM	43.64	0.66	19.65	0.465	1.028
Leaves	62.50*	0.93	50.21	0.642	0.810
Wood	2.80*	0.84	45.53	0.389	0.024
Epilithon	1.26	0.23	4.43	0.078	0.021
Moss	0.055	1.22	21.28	0.905	0.003
Grazers	0.128†	10.96	4.57	0.540	0.017
Collectors	0.228†	9.59	3.97	0.593	0.026
Filterers	0.176†	10.71	5.34	0.573	0.022
Shredders	0.208†	9.65	5.12	0.583	0.024
Invertebrate Predators	0.192†	12.12	3.89	0.556	0.028
Fish	0.063‡	11.39	3.65	0.549	0.009

* Webster and Benfield unpubl. data.
† Houston (1993).
‡ Grossman unpubl. data.

streamwater and particles into a bucket, measuring total volume, subsampling, filtering onto a GF/F filter, drying, weighing, ashing, and reweighing to determine FBOM standing stock in g AFDM m^{-2} streambed. Epilithon standing stock was determined using 12 randomly selected rock scrapings of known area and filtering the epilithon slurry from each rock onto an ashed preweighed GF/F filter. Filters were processed as for FBOM. Bryophytes were measured by drying and weighing material removed from a 25-cm^2 area of rock surface with 100% bryophyte cover. Bryophyte biomass over the entire stream reach was determined by surveying percentage moss cover on 5-m transects over the 200-m study reach.

The consumer compartments were macroinvertebrates, salamanders, and fish. Qualitative samples were taken along the 200-m reach using a D-frame kick net. From these samples we identified the most common taxa in five functional feeding groups: grazers, collectors, filterers, shredders, and invertebrate predators. We collected organisms for C:N and background isotope analysis. Biomass estimates (g AFDM m^{-2}) were taken from a study at this site conducted by Houston (1993) and converted to N standing stocks using measured % N values, which varied relatively little across taxa (Table 2). Surveys of fish (primarily young of the year brook trout, *Salvelinus fontinalis*) in Upper Ball Creek have been conducted regularly by G. Grossman (University of Georgia, unpubl. data), and we used a winter biomass average of 0.063 g AFDM m^{-2}.

Fluxes of N into and out of compartments were calculated using methods and transfer rates described in detail by Hall et al. (1998) for a nearby stream. Briefly, uptake of dissolved N into primary uptake compartments (FBOM, leaves, epilithon, moss) was determined using the equation:

$$U(\text{g N seg}^{-1}\ \text{d}^{-1}) = F \times L/S, \quad (1)$$

where U is the uptake rate, F is the downstream flux of nutrient entering the segment (g N d^{-1}), L is length of segment (m segment^{-1}), and S is uptake length (m) calculated from the short-term nutrient addition experiments described above. We assumed DON uptake would approximate DOC uptake (Hall et al. 1998), and we used a previously measured uptake length of DOC for another stream at Coweeta (Munn 1989; Munn and Meyer 1990). Seston uptake length was determined to be 60 m based on releases of corn pollen (seston surrogate) in Hugh White Creek (Webster et al. 1999). We then apportioned uptake of the three forms of dissolved N to each compartment based on proportional substrate respiration rates using the following formula (Hall et al. 1998):

$$F_{x,y} = U_x \times (R_y/R_{\text{total}}), \quad (2)$$

where x is the dissolved N compartment, y is the primary uptake compartment (FBOM, leaves, epilithon, moss), and R = (respiration rate x standing stock) for each uptake compartment. Leaf respiration rate in Upper Ball Creek was 1.8 mg AFDM g AFDM^{-1} d^{-1} (Tank et al. 1993); wood respiration was 0.18 mg AFDM g AFDM^{-1} d^{-1} (Tank et al. 1993); FBOM respiration was 1.01 mg AFDM g AFDM^{-1} d^{-1} (Webster et al. 1999); and epilithon respiration was 7.5 mg AFDM g AFDM^{-1} d^{-1} (Tank and Webster unpubl. data). Respiration rates for moss have not been measured at Coweeta, but we assumed a rate of 1 mg AFDM g AFDM^{-1} d^{-1} (Hall et al. 1998). The model assumed that the entire N biomass of each uptake compartment was uniformly involved in N cycling (e.g., no separation was made between the rapid-turnover microbial compartment and the noncycling detrital N).

Fluxes from primary uptake compartments to invertebrates were based on previous research of gut contents from invertebrates in a similar Coweeta stream. Shredders consumed 85% leaves and 15% FBOM; gatherers consumed 100% FBOM; filterers consumed 100% seston; scrapers consumed 70% epilithon and 30% FBOM; and invertebrate predators consumed 26% filterers, 30% gatherers, 9% scrapers, and 13% invertebrate predators (Hall et al. 1998). Secondary production was previously determined by Houston

(1993) on Upper Ball Creek. Using data from Grimm (1988), we estimated ingestion, egestion, and excretion rates: secondary production was 26% of ingested N, 24% was excreted as ammonia and 50% egested as FBOM in the form of feces.

Field $^{15}NH_4$ tracer addition, sampling and analysis—We released 0.6131 g ^{15}N (as 10% enriched $^{15}NH_4Cl$) over 42 d from 3 November 1996 to 15 December 1996 using a Watson-Marlow peristaltic pump. This rate of enrichment was designed to increase the $\delta^{15}N$ of the stream water ammonium pool to 500‰ at the addition site, based on an estimated discharge of 50 L s^{-1} and an NH_4 concentration of 2 μg N L^{-1}. The tracer solution drip rate of 2 ml min^{-1} was checked daily, batteries exchanged every 48 h, and the carboy of addition solution was changed weekly. The ^{15}N addition rate raised the background concentration of ammonium by less than 1%; hence the $^{15}NH_4$ addition was truly a tracer addition. The actual δ value of the ammonium pool in stream water varied as a result of variation in discharge and slight variations in ammonium concentration throughout the 6-week experiment (Fig. 1A,B).

Eight sampling stations were selected at −10 m (reference site), 15, 27, 50, 70, 115, 150, and 200 m downstream of the ^{15}N addition site to examine ^{15}N labeling of food web compartments and downstream transport. Particulate compartments were sampled on days 3, 7, 14, 21, 28, 35, and 42 during the release and days 6, 16, 28, and 90 after the release. Dissolved compartments were sampled at the eight stations on days 0 (8 h), 20, 41, and post day 1.

The ^{15}N in ammonium was measured using the ammonium diffusion method followed by mass spectrometry (Sorenson and Jensen 1991; Sigman et al. 1997; Holmes et al. 1998). Two filtered 4-liter water samples were collected at each sampling site and shipped on ice to the Marine Biological Laboratory (Woods Hole, Massachusetts) after initiating the ammonium sorption process. One sample was used for determination of $^{15}NH_4$ by adding MgO, converting NH_4^+ to NH_3, which then diffuses and is trapped on an acidified GF/F filter sandwiched between two Teflon filters floating on the sample surface. After shaking for 2 weeks at 40°C, filters were removed, dried, and analyzed for ^{15}N on a mass spectrometer. The second 4-liter sample was shipped on ice to the Marine Biological Laboratory and used for $^{15}NO_3$ analysis using the method of Sigman et al. (1997). Ammonium was removed by boiling down a 2-liter sample to 100 ml under basic conditions (with MgO). DeVarda's Alloy was added to convert NO_3 to NH_4^+, which was sorbed onto an acidified filter and analyzed as described above. DO^{15}N was analyzed by UV oxidizing a subsample of water collected above (70 ml) for 24 h, then converting all N in the sample (NH_4^+ and DON) to NO_3. Again, DeVarda's Alloy and MgO were added to convert NO_3 to NH_4^+, and NH_4^+ to NH_3, respectively, which was sorbed onto an acidified filter and analyzed as described above.

Particulate compartments for ^{15}N analysis were sampled using several methods. Seston was sampled by filtering stream water through a 25-mm GF/F filter using a 12 V Geopump until the filter clogged. FBOM slurries were collected using suction, and samples from 4–5 different locations at each station were pooled to achieve a representative station sample. FBOM slurries were filtered onto an ashed GF/F filter. Seston and FBOM filters were dried at 55°C. A random selection of leaves was hand-collected from various debris dams at each station, rinsed, dried, and ground using a Wiley mill. Small sticks were collected at each station, returned to the lab, scraped with a razor blade to collect surface wood colonized by microbes, dried, and ground. Epilithon was scrubbed with a wire brush from three rocks at each station, pooled, filtered onto an ashed GF/F filter, and dried. Moss was scraped from rock surfaces with a knife and tips were rinsed, dried, and ground.

Since all stream consumers could not be collected each week for ^{15}N analysis because of concern for depletion and disturbance, the most common and easily collected taxon from each functional feeding group was chosen to represent that group for ^{15}N analysis. Our chosen shredder was *Tallaperla maria* (Peltoperlidae, Plecoptera), the scraper was *Stenonema* spp. (Heptageneiidae, Ephemeroptera), the collector–gatherer was *Diplectrona* spp. (Hydropsychidae, Trichoptera), the filterer was *Parapsyche* spp. (Trichoptera), and the invertebrate predator was *Acroneuria* spp. (Plecoptera). Approximately 5–10 individuals of each taxon were collected from each station on each date, held in streamwater and allowed to clear their guts overnight, dried, weighed, and ground using a small spatula. Vertebrate predators (small brook trout *Salvelinus fontinalis,* and salamanders *Desmognathus* and *Eurycea* spp.) were sampled at only the end of the 42-d release because of concern with depletion.

All mass spectrometry was conducted at The Ecosystems Center, Marine Biological Lab, Woods Hole, Massachusetts. Samples were analyzed using an automated sample combustion system and a Finnigan Delta S isotope ratio mass spectrometer. All ^{15}N values are expressed as $\delta^{15}N$ values calculated from the following equation:

$$\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1{,}000, \quad (3)$$

where $R = {}^{15}N:{}^{14}N$ ratio. The $\delta^{15}N$ of samples collected before the tracer addition, as well as all samples collected at the −10-m reference station, were used to define natural abundance of ^{15}N ($\delta^{15}N_{backround}$) and were subtracted from $\delta^{15}N$ values of samples collected after the tracer addition began. Thus, all reported $\delta^{15}N$ values from the tracer experiment are background-corrected and include only tracer ^{15}N, unless otherwise noted.

Description of calculations using $\delta^{15}N$ data—$^{15}NH_4$ uptake length: We calculated $^{15}NH_4$ uptake length for samples collected on days 0, 20, and 41 by regressing the natural log of the $^{15}NH_4$ flux in water at each station vs. distance (m) downstream from addition site. The slope of the regression is the distance-normalized NH_4 uptake rate (m^{-1}), and the inverse of the slope is the NH_4 uptake length (m; Newbold et al. 1981; Mulholland et al. 2000*a*). ^{15}N-NH_4 flux (μg ^{15}N s^{-1}) at each station was calculated using the following equation:

$$^{15}NH_4 \text{ flux} = (\delta^{15}N/1000) \times 0.003663 \times Q \times [NH_4] \quad (4)$$

where 0.003663 is the ratio of ^{15}N to ^{14}N in air. This method

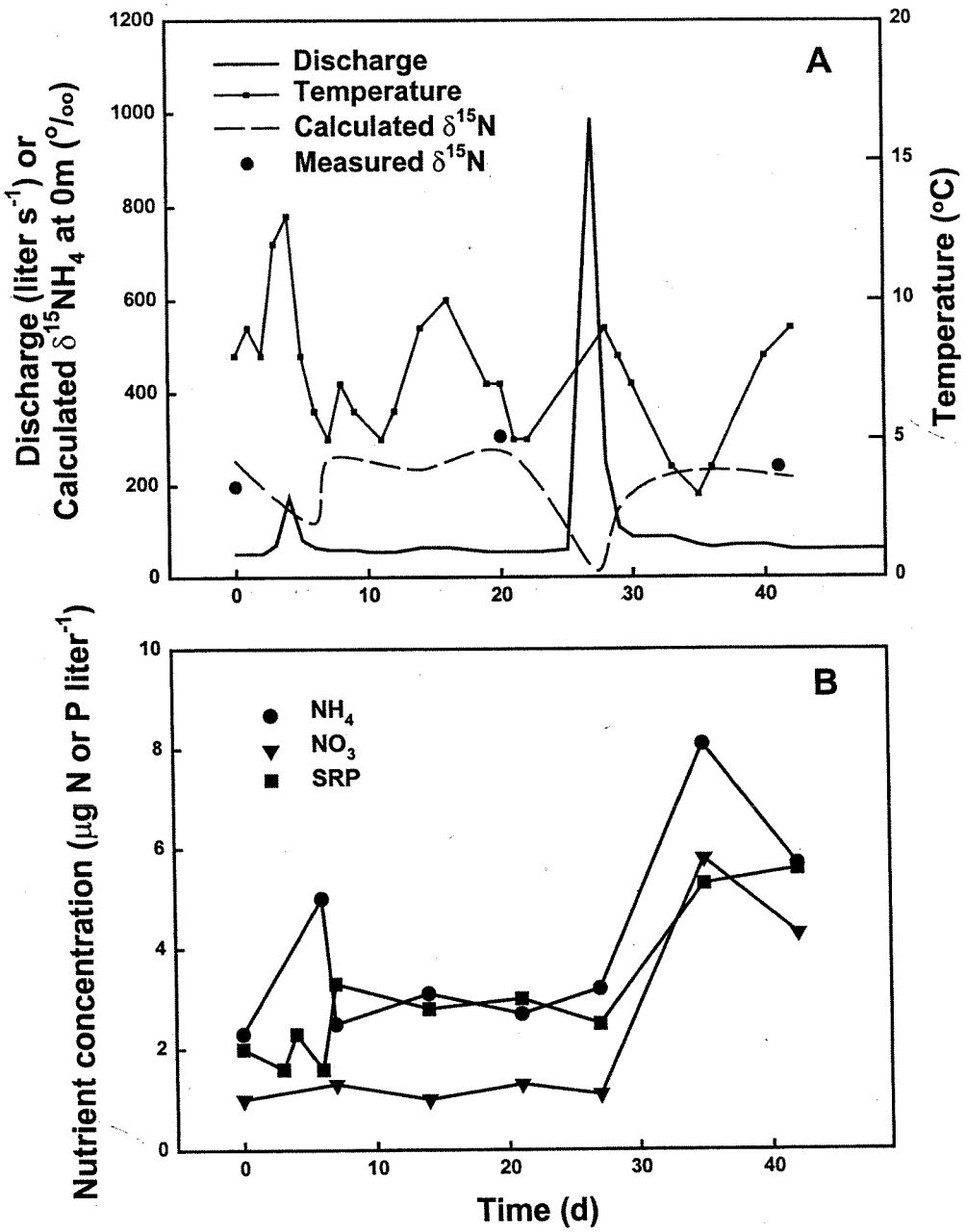

Fig. 1. (A) Discharge, stream temperature, calculated $\delta^{15}NH_4$ at 0 m, and measured $\delta^{15}NH_4$ at 27 m during the tracer addition. Calculated $\delta^{15}NH_4$ values are based on daily discharge, weekly stream ammonium concentrations, and daily drip rate of the release solution of $\delta^{15}NH_4Cl$. (B) Concentrations of ammonium, nitrate, and soluble reactive phosphorus measured approximately weekly during the tracer addition.

for calculating $^{15}NH_4$ uptake length is corrected for any dilution but assumes no significant regeneration of ^{15}N from biomass compartments, which is undoubtedly false after day 0. As a result, uptake lengths calculated using $\delta^{15}N$ values on days 20 and 41 are probably overestimates of actual uptake lengths. We corrected uptake length on these dates for ^{15}N regeneration using the $\delta^{15}NH_4$ values measured on the first day after the tracer release was terminated (day P1). We calculated regenerated $^{15}NH_4$ flux (from Eq. 4) using day P1 data at all stations and subtracted this flux (1/2 of flux used for day 20) from the original $^{15}NH_4$ flux calculated for days 20 and 41 and then calculated regeneration-corrected uptake length as described above.

Ammonium uptake rates: Two types of ammonium uptake rates were calculated using the $\delta^{15}N$ data: a whole-stream uptake rate and compartment-specific uptake rates (for biomass compartments that take up NH_4 from water only; Mulholland et al. 2000*a*). Whole-stream uptake rate (μgN m^{-2} s^{-1}) was calculated as

Table 3. Upper Ball Creek whole-stream NH_4 uptake rates (reach 27–200 m) calculated using uptake lengths (S_w), streamwater NH_4 flux (F), average stream width (w = 2.73) where F is average discharge (Q) × average NH_4 concentration, uptake rate = $F/(S_w \times w)$, and the mass transfer coefficient (v_f) = $(Q/w)/S_w$. Regeneration-corrected uptake lengths were calculated by estimating regeneration using the $\delta^{15}NH_4$ values from the stream reach on the first day after the ^{15}N tracer release was terminated (day P1). We subtracted P1 flux from the original $^{15}NH_4$ flux values calculated for days 21 and 41. NH_4 concentrations used in these calculations were measured using mass spectrometer-derived values.

Day	Average discharge (L s^{-1})	NH_4 concentration (μg L^{-1})	S_w (m)	Regeneration-corrected S_w (m)	Uptake rate (mg N m^{-2} d^{-1})	v_f ($\times 10^{-4}$) (m s^{-1})
0 (4 Nov 96)	55.6	3.0	27.7	—	190.6	7.4
20 (24 Nov 96)	60.3	3.4	86.1	82.3	78.8	2.7
40 (14 Dec 96)	70.3	3.5	102.7	93.6	83.2	2.8

$$\text{whole-stream } NH_4 \text{ uptake rate} = (NH_4 \text{ flux})/(S_w \times \hat{w}) \quad (5)$$

where NH_4 flux is the product of discharge and NH_4-N concentration, S_w = $^{15}NH_4$ uptake length calculated on day 0 (corrected for dilution), and $\hat{w}$ = average stream wetted width (m). This equation represents the rate of removal of NH_4 from the streamwater by all compartments combined.

Compartment-specific uptake rates (mg N m^{-2} d^{-1}) for the primary uptake compartments were calculated using the $\delta^{15}N$ values and nitrogen standing stocks (g N m^{-2}) of each compartment and streamwater $\delta^{15}NH_4$ values, all taken at a station close to the dripper on a date early in the release prior to substantial loss of ^{15}N via regeneration (e.g., day 7 biomass and day 0 water). Mass of ^{15}N associated with a particular compartment was calculated as

$$^{15}N_{biomass}(g\ m^{-2}) = (\delta^{15}N_{biomass}/1000) \times 0.003663 \times TN_{biomass} \quad (6)$$

where $\delta^{15}N_{biomass}$ is the $\delta^{15}N$ value of the compartment and $TN_{biomass}$ is the N standing stock in that compartment (g N m^{-2}) at the start of the tracer addition. Then compartment-specific NH_4 uptake rates (mg N m^{-2} d^{-1}) were calculated using the following equation:

$$NH_4 \text{ uptake rate} = (^{15}N_{biomass/d7}/^{15}N_{water})/7, \quad (7)$$

where $^{15}N_{biomass/d7}$ was calculated from the biomass $\delta^{15}N$ value at 27 m on day 7 from Eq. 6, and $^{15}N_{water}$ was calculated as $(\delta^{15}NH_4/1{,}000) \times 0.003663$ at 27 m on day 0, the nearest date on which $^{15}NH_4$ in water was measured. To compare whole-stream NH_4 uptake efficiencies over the 42-d experiment, we calculated a mass transfer coefficient, v_f (m s^{-1}), for NH_4 uptake using the following equation:

$$v_f = (Q/w)/S_w, \quad (8)$$

where Q is discharge (m^3 s^{-1}), w is stream width (m), and S_w is uptake length (Newbold et al. 1981).

^{15}N turnover rate: ^{15}N turnover rates for specific biomass compartments were calculated using the decline in $\delta^{15}N$ values at the 27-m station over the first 28 d after the end of the tracer addition. The slope of the relationship between ln ($\delta^{15}N_{biomass}$) and time (d) represents the compartment-specific ^{15}N turnover rates. This estimate of N turnover assumes that there is no reuptake of ^{15}N released into the water column by the biota upstream, a reasonable assumption for the upper sampling station.

Mass balance of ^{15}N retained: At the end of the 42-d release we estimated how much of the ^{15}N added to the stream was retained in each compartment. We calculated the $^{15}N_{biomass}$ (Eq. 6) retained in each compartment at each sampling site on day 42, then determined the exponential relationship between ^{15}N retained and stream distance, integrated over stream distance, and multiplied by average stream width to get mg ^{15}N $reach^{-1}$ for each compartment. This value was compared to total ^{15}N added over 42 d to obtain percentage ^{15}N retained by each compartment and summed for all compartments. We also estimated the ^{15}N lost through transport out of the stream reach by seston and dissolved forms of N (as nitrate, ammonium,

Table 4. NH_4 uptake and turnover rates for different biomass compartments. Uptake rates were calculated using $\delta^{15}N$ values from day 7 at 27 m, whereas turnover rates and times were determined from decline in $\delta^{15}N$ values at the 25 m station over 28 d after the tracer addition was completed. Postrelease data were unavailable for bryophytes.

Compartment	$TN_{biomass}$ (g m^{-2})	$\delta^{15}N_{biomass}$	NH_4 uptake rate* (mg N m^{-2} d^{-1})	Turnover rate (d^{-1})	Turnover time (d)
Epilithon	0.021	20.0	0.30	0.021	47
Bryophytes	0.003	12.6	0.03	—	—
CBOM-leaves	0.810	6.0	3.51	0.026	38
FBOM	1.028	3.1	2.31	0.019	53
Total	1.861		6.16		

* Based on $\delta^{15}N$ of each biomass compartment on day 7 at 27 m ($\delta^{15}N_{biomass/d7}$), the streamwater $\delta^{15}N$ in the NH_4 pool on day 0 at 27 m ($\delta^{15}N_{water}$), and the total N per m^2 for each biomasss compartment ($TN_{biomass}$) using the equation NH_4 uptake rate = $(^{15}N_{biomass/d7}/^{15}N_{water})/7$, where $^{15}N_{biomass/d7}$ (g m^{-2}) was calculated as $(\delta^{15}N_{biomass/d7}/1{,}000) \times 0.003663 \times TN_{biomass}$ and $^{15}N_{water}$ was calculated as $(\delta^{15}NH_4/1{,}000) \times 0.003663$.

and DON) using Eq. 4 and the $\delta^{15}N$ values of NH_4, NO_3, DON, and suspended organic nitrogen (SPON) at 200 m. To determine ^{15}N flux out of the reach over the 42-d release, we averaged the fluxes from days 0 and 20 for the first half of the release and averaged fluxes on days 20 and 41 for the second half.

^{15}N in microbes associated with organic matter: We determined the $\delta^{15}N$ value for N associated with the microbes colonizing organic matter using the chloroform fumigation technique (described above) and then applying a diffusion procedure similar to the one used to isolate and measure $^{15}NO_3$ (Brooks et al. 1989). Nitrate produced by the alkaline persulfate digestions of K_2SO_4 extracts was converted to ammonium using Devarda's Alloy; ammonia was diffused into the headspace of the bottle under basic conditions and sorbed onto an acidified GF/F sandwiched between two Teflon filters floating on the extract surface (described in detail by Mulholland et al. 2000*a*). The filters were removed, dried in a dessicator, and analyzed for $\delta^{15}N$ by mass spectrometry as described above. Microbial $\delta^{15}N$ values for microbes on wood, leaves, and FBOM were computed from the following equation:

$$\delta^{15}N_{microbes} = ((\delta^{15}N_{fumigated} \times TN_{fumigated}) - (\delta^{15}N_{unfumigated} \times TN_{unfumigated}))/TN_{microbial} \quad (9)$$

where $\delta^{15}N_{fumigated}$ and $\delta^{15}N_{unfumigated}$ are the δ values measured from the K_2SO_4 extracts, $TN_{fumigated}$ and $TN_{unfumigated}$ are the total N values (mg N g $AFDM^{-1}$) for the samples, and $TN_{microbial}$ was calculated as $TN_{fumigated} - TN_{unfumigated}$.

Results

Stream characterization—Nutrient levels (NH_4, NO_3, and SRP) are generally very low in this stream. Short-term nutrient additions indicated that ammonium was preferentially taken up over nitrate. Nitrate uptake length was three times higher than that for ammonium (Table 1). Phosphate uptake length fell between that of nitrate and ammonium (Table 1).

The ^{15}N-tracer addition was conducted in late autumn through early winter (3 November–15 December 1996), and hence daily light levels and water temperatures were low. The stream is also heavily shaded by the rhododendron understory. Rates of gross primary production (GPP) were very low compared to ecosystem respiration (*R*; Table 1) resulting in a low *P*:*R* ratio (0.002). Epilithon standing stock was also low, averaging 0.73 mg Chl *a* m^{-2} (Table 1).

Standing stocks, percentage N, C:N ratios, C:AFDM ratios, and calculated N standing stocks for all the modeled stream compartments are summarized in Table 2. FBOM represented the largest N pool (1.028 g N m^{-2}) followed by leaves, wood, epilithon, and moss. In general, allochthonous organic matter (leaves and FBOM) represented over 90% of the total N standing stocks (Table 2). However, not all of the nitrogen in organic matter was actively cycling. Results from the chloroform fumigations give an indication of the proportion of actively cycling N (microbial biomass) associated with organic matter. N in wood scrapings was 3.8% microbial N (0.32 mg microbial N g $AFDM^{-1}$), whereas FBOM was 3.6% microbial (0.84 mg microbial N g $AFDM^{-1}$) and leaf N was 2.9% microbial (0.37 mg microbial N g $AFDM^{-1}$).

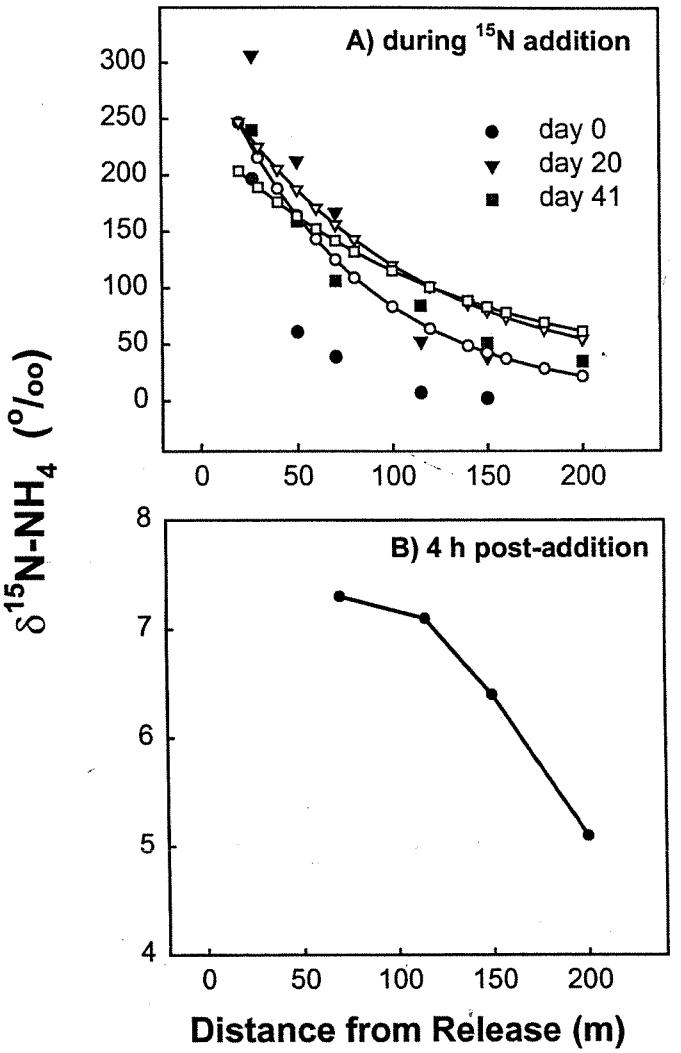

Fig. 2. (A) Longitudinal profile of observed (solid symbols) vs. predicted (open symbols) decline in $\delta^{15}NH_4$ on days 0, 20, and 41 of the tracer addition. (B) Observed longitudinal profile of $\delta^{15}NH_4$ 4 h after the tracer addition stopped on day 42.

Whole-stream estimates of N spiraling from ^{15}N-tracer data—$^{15}NH_4$ in stream water: $\delta^{15}NH_4$ in stream water changed through time as a result of varying discharge and ammonium concentration (Fig. 1A). For example, during the storm on day 27, the calculated $\delta^{15}NH_4$ in water dropped to near zero as a result of high flows. Stream ammonium concentrations remained near 3 $\mu g\ L^{-1}$ during the first 28 d of the release, but increased on days 35 and 41 (Fig. 1B).

Whole-stream NH_4 uptake: On day 0 (8 h), day 20, and day 41, $\delta^{15}NH_4$ declined with distance downstream (Fig. 2A). Uptake lengths (S_w) for $^{15}NH_4$ increased over time (Table 3). Day 0 $^{15}NH_4$ uptake length was about half of the uptake length estimated using short-term additions where stream

ammonium concentrations were elevated; hence measures of uptake length based on short-term enrichment overestimated uptake length at ambient NH_4 concentrations. In general, higher $\delta^{15}NH_4$ values predicted by the model, compared with measured values (Fig. 2A), implies that the model underestimated NH_4 uptake on all dates, probably because the model was parameterized using longer uptake lengths determined from short-term enrichment experiments.

Increasing uptake lengths over time could result from changes in biological activity or could be simply a result of significant $^{15}NH_4$ regeneration from the biota throughout the tracer addition when ^{15}N levels in the biota were high. Regeneration was apparent 4 h after stopping the addition (Fig. 2B), yet $\delta^{15}NH_4$ values of regenerated NH_4 were much lower than predicted by the model (tracer $\delta^{15}N$ = 5–7‰ vs. model $\delta^{15}N$ = 13–32‰). After recalculating uptake lengths on days 20 and 41, accounting for regeneration of $^{15}NH_4$ (Table 3), the increase in uptake length over time was still evident. Residence time of NH_4 in water, calculated as the uptake length divided by water velocity (0.106 m s^{-1}, determined using the transient storage model) increased from 4 min on day 0 to 15 min on day 42.

Whole-stream uptake rate ranged from 79 to 191 mg N m^{-2} d^{-1} and was highest on day 0 (Table 3); as ammonium uptake lengths increased through time, uptake rates decreased. Some of the variation in nitrogen uptake between dates may have been attributable to changes in physical variables through time, primarily discharge (Table 3). Temporal trends in the mass-transfer coefficients were similar to uptake rates; v_f on day 0 was more than twice as large as v_f on days 20 and 41.

Nitrification of $^{15}NH_4$: Rates of nitrification appeared to be very low in Upper Ball Creek during the 6-week tracer addition. On day 0, $\delta^{15}NO_3$ values were only slightly above background at the upper two sites (3.5 and 3.6‰ at 27 m and 50 m, respectively) and were below detection downstream. Similarly low $^{15}NO_3$ values were measured on days 20 and 41. Based on the best fit of a simple two-compartment model of nitrification and nitrate uptake, nitrification rates on day 0 were less than 3% of $^{15}NH_4$ uptake (*see* Mulholland et al. 2000*a* for detailed description of model).

Compartment-specific NH_4 uptake rates: Epilithon and bryophytes were the most highly labeled by day 7; however, NH_4 uptake rates for primary producers in the stream were generally lower than for the detrital compartments because of their low N standing stocks (Table 4). Although FBOM represents the largest pool of N (55% of the N standing stock in primary uptake compartments), leaves had the higher NH_4 uptake rate (Table 4). The sum of uptake rates for the four major compartments (representing 94% of particulate N) accounted for only 3–8% of the whole-stream uptake rate (Tables 3 and 4), which indicates a considerable underestimate of uptake by one or more compartments.

Food web N turnover: ^{15}N turnover rates for epilithon, leaves, and FBOM, determined from the decline in $\delta^{15}N$ after the tracer addition ended, were similar, ranging from 0.019 to 0.026 d^{-1} (Table 4). All three compartments had returned

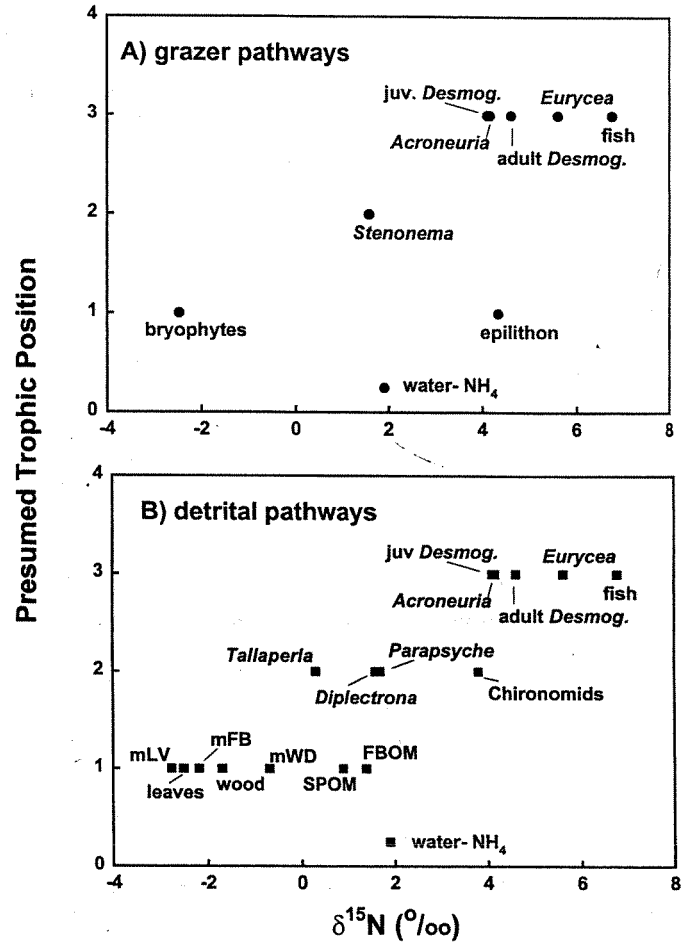

Fig. 3. Natural abundance $\delta^{15}N$ values of food web compartments and their presumed trophic levels in the (A) grazer pathway: compartments based on primary production and (B) detrital pathway: compartments based on allochthonous organic matter. The microbial portions of leaves (mLV), wood (mWD), and FBOM (mFB) are also included. The same secondary consumers are plotted on both figures as they may be eating primary consumers of both pathways. Desmog stands for *Desmognathus* salamanders and fish are YOY brook trout.

very close to background $\delta^{15}N$ values by 3 months after the ^{15}N addition stopped (Fig. 5A). Epilithon may be comprised of two pools of N—a rapid-turnover pool represented by the initial decrease in $\delta^{15}N$ (at day 50) and a slower pool that gradually decreases (by day 132; Fig. 5A). In contrast, shredders, filterers, and scrapers maintained high $\delta^{15}N$ values for up to 1 month after the ^{15}N release was terminated. This reflects the fact that consumer food resources remained enriched with ^{15}N for several weeks.

^{15}N labeling of individual food web compartments—Before examining the data from the ^{15}N-tracer addition, background $\delta^{15}N$ values for all biomass compartments in grazer and detrital pathways of the food web were used to identify food and consumer relationships in Upper Ball Creek (Fig. 3), based on the expected trophic ^{15}N fractionation of about 3 ± 1‰ per trophic level (Peterson and Fry 1987). These data are from samples collected at all stations before we

began the tracer addition and samples at the −10-m station collected throughout the tracer addition. Background $\delta^{15}N$ values in Upper Ball Creek support our prediction that the food web is composed of three trophic levels.

The mayfly *Stenonema* was predicted to be the primary epilithon consumer in the grazer pathway, but its ambient $\delta^{15}N$ value was not as high as the +6 to +8‰ expected if it was assimilating epilithon (Fig. 3A). Its ambient $\delta^{15}N$ value is closer to what would be expected if it were consuming microbes associated with detritus (Fig. 3B). On the basis of natural abundance $\delta^{15}N$, none of the invertebrates sampled appear to be feeding primarily on epilithon, although clearer relationships may have been observed for the grazer pathway if we had obtained microbial $\delta^{15}N$ values for the epilithon. Although there were no known bryophyte consumers in this stream, the caddisfly *Paraspsyche* often makes its case and net in bryophyte patches and may be feeding on some material associated with bryophytes.

Leaves, wood, FBOM, and seston represented the lowest trophic level within the detrital pathway (Fig. 3B). Ambient $\delta^{15}N$ values for the microbial fractions obtained from the chloroform fumigations of wood, leaves, and FBOM were about 4‰ lower than bulk FBOM, slightly lower than leaves, and slightly higher than wood (Fig. 3B). The stonefly *Tallaperla,* the two caddisflies (*Parapsyche* and *Diplectrona*), and chironomids were chosen as the primary consumers. *Tallaperla* appears to feed on either wood or leaves, or the microbes associated with leaves or FBOM (Fig. 3B). The filterer *Parapsyche* and the collector *Diplectrona* could feed on wood or microbes on FBOM or wood. Chironomids appear to use fine particulate organic matter (suspended or benthic; Fig. 3B). Better resolution of food resources is impossible considering the similarity of the background $\delta^{15}N$ values between FBOM and seston, leaves and wood, and the microbial fraction of FBOM and leaves (Fig. 3B). We placed the secondary consumers (invertebrate predators, salamanders, and fish) on both figures to illustrate that their $\delta^{15}N$ values are consistent with feeding on either or both the grazing and detrital pathways. Data from the ^{15}N-tracer release provided us with a much greater range of possible δ values, and therefore additional information on some of the food web relationships in Upper Ball Creek.

Overall, incorporation of the ^{15}N tracer varied among the biomass compartments that take up NH_4 from water. Primary producers were more highly labeled than allochthonous organic matter, although microbes associated with leaves were even more highly labeled than epilithon by the end of the experiment (Fig. 8). Bryophytes were the most highly labeled of all primary uptake compartments with $\delta^{15}N$ values about 2.5 times higher than epilithon (Fig. 5). Our model predictions for bryophytes were close to actual values at the four stations farthest downstream but never approached field tracer values near the ^{15}N input. Of the detrital compartments, leaves were more highly labeled than FBOM. Microbial $\delta^{15}N$ was much greater than the bulk detrital $\delta^{15}N$ for all three detritus types but especially for leaves (Fig. 8). Microbes on wood represented 69% of the ^{15}N mass in the bulk pool, whereas microbes on leaves and FBOM represented 20 and 31%, respectively.

During the ^{15}N-tracer addition, the epilithon-grazer pathway became more highly labeled than the detritus pathway. On both day 21 and day 42, $\delta^{15}N$ values for epilithon were in the range of those predicted by the model (Fig. 4A,B); however, *Stenonema* was much more highly labeled than the epilithon, in contrast to model predictions. Epilithon and grazers were labeled by day 3, and $\delta^{15}N$ increased until day 21 and changed little thereafter, which suggests that both had reached isotopic equilibrium (Fig. 5A,B).

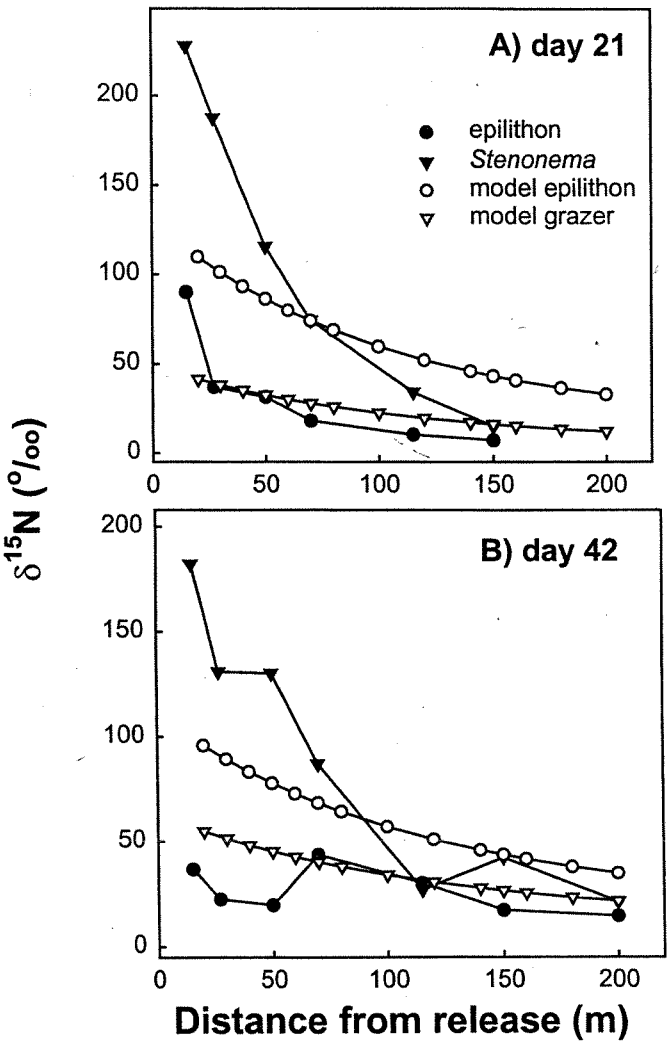

Fig. 4. Longitudinal profile of observed (solid symbols) vs. predicted (open symbols) decline in $\delta^{15}N$ values of epilithon and the grazing mayfly *Stenonema* on (A) day 21 and (B) day 42 of the tracer addition.

There was a much tighter linkage between the shredder *Tallaperla* and its presumed food resource (leaves) than observed in the epilithon-grazer pathway (Fig. 6A,B). Shredder $\delta^{15}N$ values were much lower than microbial $\delta^{15}N$ values, which suggests that the shredders were assimilating N from sources other than microbial cells alone. On day 21, the longitudinal pattern of shredder $\delta^{15}N$ paralleled that of leaf $\delta^{15}N$ (Fig. 6A). Shredder $\delta^{15}N$ values were close to those predicted by the model, although $\delta^{15}N$ of leaves never reached model predictions. Little change in $\delta^{15}N$ occurred after day 21 for

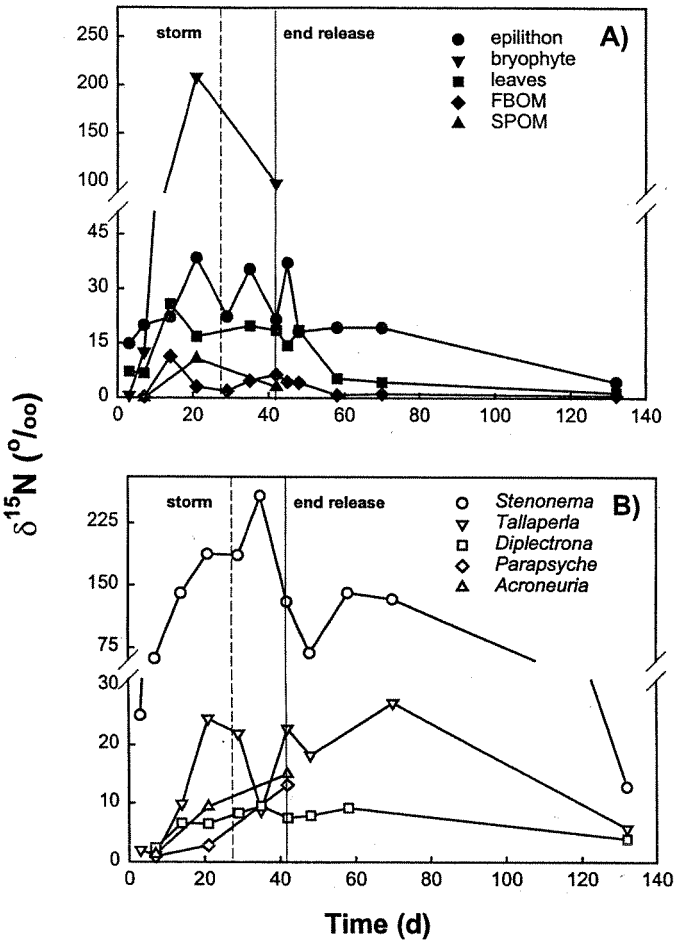

Fig. 5. Time series of observed $\delta^{15}N$ values at 27 m for the (A) primary uptake compartments, and (B) consumers. The dotted line marks the storm on day 27 and the solid line marks the end of the tracer addition on day 42.

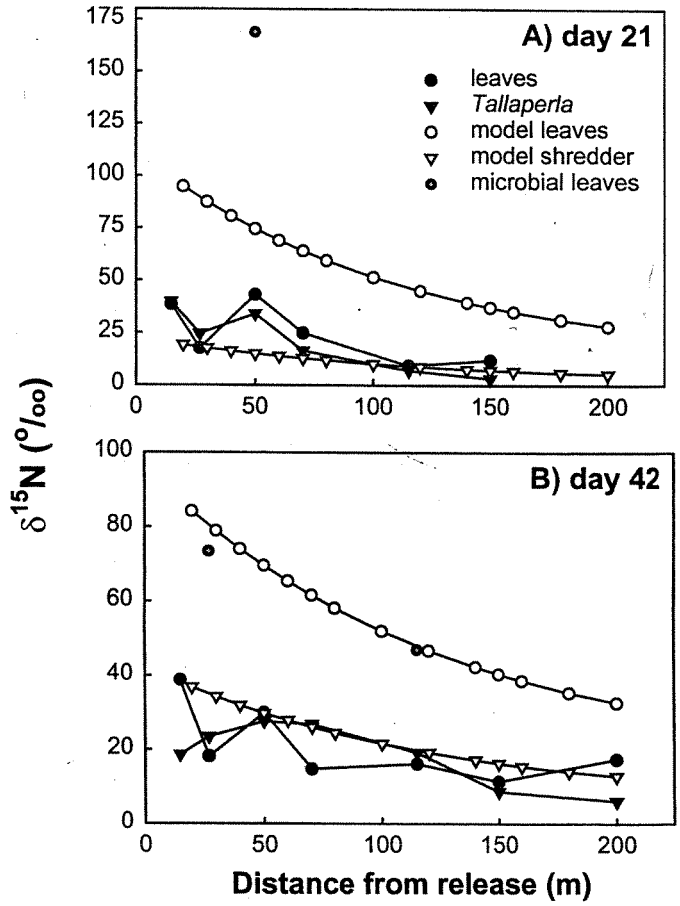

Fig. 6. Longitudinal profile of observed (solid symbols) vs. predicted (open symbols) decline in $\delta^{15}N$ values of leaves and the shredding stonefly *Tallaperla* on (A) day 21 and (B) day 42 of the tracer addition. Microbial ^{15}N value for leaves is also plotted for reference (crossed solid circle).

leaves or shredders, again suggesting they had reached isotopic equilibrium (Fig. 5A,B).

The $\delta^{15}N$ values from the tracer addition indicated that the two caddisflies may be feeding on particles from different sources. The longitudinal pattern of $\delta^{15}N$ in *Diplectrona* matched that of FBOM as expected for a collector–gatherer, whereas $\delta^{15}N$ of *Parapsyche* followed the longitudinal pattern of seston as expected for a filterer (Fig. 7A,B). Day 21 field data for *Parapsyche* and seston indicated some patchiness in the downstream labeling of both the food resource and consumer, yet $\delta^{15}N$ values remained within the range predicted by the model (Fig. 7A). In contrast, model predictions for *Diplectrona* and FBOM were higher than observed $\delta^{15}N$ values (Fig. 7B). By day 42, it appears that *Diplectrona* and *Parapsyche* may have been feeding on a combination of particulate organic matter from benthic and suspended sources (Fig. 7C,D). Both consumers show a "hump-shaped" $\delta^{15}N$ pattern over distance consistent with the effects of organic matter transport from upstream of the release site (Fig. 7C,D). Although FBOM contained very little ^{15}N tracer on day 42, *Diplectrona* was still labeled, and the *Diplectrona* del value was similar to the del value for microbes associated with FBOM (Fig. 7D). Chironomids (an additional collector–gatherer) were also sampled when available. Often, chironomids and FBOM had similar levels of ^{15}N tracer (mean $\delta^{15}N$ of 6.3‰ for chironomids), but a number of highly labeled organisms indicated very patchy ^{15}N labeling of FBOM. Also, chironomids sampled were not identified to species, and therefore species with different feeding modes likely were present.

The storm that occurred on days 27–28 (increasing discharge by an order of magnitude) did not significantly lower the $\delta^{15}N$ values in any of the consumer compartments and affected most of the biomass compartments only slightly (Fig. 5A). $\delta^{15}N$ of bryophytes decreased significantly from day 21 to day 42, but this should be viewed with caution because no data were available for the two collection periods immediately following the storm (Fig. 5A).

Juvenile *Desmognathus* was the vertebrate predator with the highest label after 42 d, which indicates that it used stream fauna as a food resource, but its peak $\delta^{15}N$ was only 5‰. In contrast, adult *Desmognathus* were not labeled. *Eurycea* and young-of-the-year brook trout had $\delta^{15}N$ values of 3 and 1‰, respectively, by day 42. Overall, the predators (the stonefly *Acroneuria* and the vertebrates) were still in-

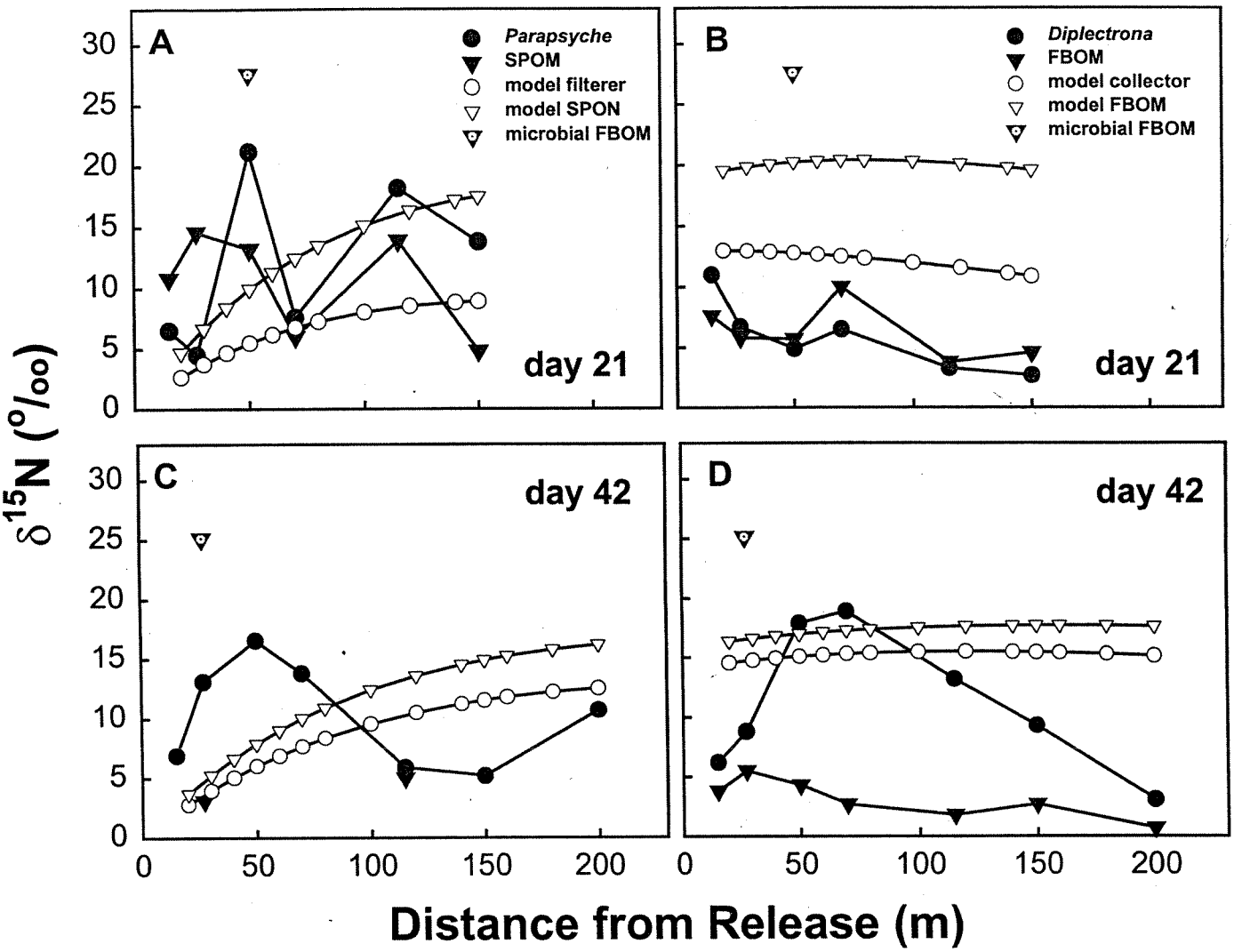

Fig. 7. Comparison of suspended vs. benthic particle pathways. Longitudinal profile of observed (solid symbols) vs. predicted (open symbols) decline in $\delta^{15}N$ values of (A) the filtering caddisfly *Parapsyche* and seston on day 21 and (C) day 42 of the tracer addition. Similar plots of the longitudinal profile of the collecting caddisfly *Diplectrona* and FBOM on (B) day 21 and (D) day 42 of the tracer addition. Microbial ^{15}N value for FBOM is plotted for reference (crossed solid triangle).

creasing in $\delta^{15}N$ from day 21 to 42, which suggests that a 6-week release was not long enough to fully label the predators. In contrast, $\delta^{15}N$ values for epilithon, leaves, scrapers, shredders, *Parapsyche,* and chironomids all showed little change between days 21 and 42, suggesting isotopic equilibrium for these compartments.

Mass balance of ^{15}N—Retention of ^{15}N by the nine major organic compartments at the end of the 42-d release only accounted for 12.3% of added ^{15}N (Table 5). Of the ^{15}N retained, most of it was in leaves and FBOM (combined 85%) with leaves representing the largest proportion at 76%. Epilithon and the grazers incorporated more ^{15}N than we would have predicted based on their relatively low N standing stocks, whereas FBOM incorporated less than predicted (Tables 2 and 5).

Additional ^{15}N can be accounted for in export by particulate and dissolved N compartments. SPO^{15}N export accounted for 11.5% of added ^{15}N, and about 29% was exported as dissolved ^{15}N, mostly as $^{15}NH_4$ (Table 5). Overall, DON and NO_3 were only slightly labeled with tracer ^{15}N. Only about 30% of the $^{15}NO_3$ samples collected on days 0, 20, and 41 had any measurable tracer, and the highest tracer $\delta^{15}N$ value obtained was 6.1‰. There were no downstream trends in the labeling of DO^{15}N, and values obtained were extremely variable due to low ambient DON concentrations (usually <20 μg N L^{-1}). The average del value for DO^{15}N was 2‰, although many samples were <1‰ and one sample had $\delta^{15}N$ of 8‰. In sum, we can account for only 53% of added ^{15}N, in the compartments we measured, over the 42-d release.

Discussion

Whole-stream N spiraling—Development of techniques to measure dissolved inorganic ^{15}N (e.g., $^{15}NH_4$) are relatively

Table 5. Mass balance of ^{15}N added over 42 days for the 200-m experimental reach.

Compartment	^{15}N retained (mg ^{15}N)	Percentage of ^{15}N added
FBOM	6.7	1.09
Leaves	57.1	9.31
Epilithon	2.0	0.33
Moss	0.5	0.08
Grazer	3.2	0.52
Collector	1.2	0.20
Filterer	0.7	0.11
Shredder	1.0	0.16
Invertebrate predator	2.7	0.44
Total biotic retention	75.1	12.25
SPO^{15}N export	70.2	11.45
$^{15}NH_4$ export	100.9	16.46
$^{15}NO_3$ export	42.0	6.85
DO^{15}N export	36.6	5.98
Total ^{15}N exported	249.8	40.75
Total ^{15}N accounted for	324.9	53.00
Total ^{15}N added	613.1	100

recent (Sigman et al. 1997; Holmes et al. 1998) and have allowed us to estimate whole-stream nitrogen uptake without the confounding effects of increasing stream N concentrations. Ammonium uptake lengths (S_w) determined from the ^{15}N experiment were similar to two other temperate forested streams, Walker Branch, Tennessee (Mulholland et al. 2000*a*) and another stream at Coweeta, Hugh White Creek (Hall et al. 1998), where ^{15}N-tracer releases were also conducted.

Ammonium uptake length in Upper Ball Creek increased almost fourfold from day 0 to day 42. The increase in ammonium uptake length was probably the result of increased discharge (56 to 70 L s^{-1}) and lower water temperature (range of 8–13°C during week 1 compared to 3–9°C during week 6). Increases in discharge have been shown to increase uptake length by increasing water depth and velocity (D'Angelo and Webster 1991; Valett et al. 1996; Webster et al. in press). In the much larger Kuparuk River, Alaska (discharge rate 20 times that of Upper Ball Creek), ammonium uptake length was over 800 m, as determined from a similar ^{15}N experiment, even though ambient ammonium concentrations were similar to Upper Ball Creek (2.8 μg L^{-1}; Wollheim et al. 1999).

The effect of NH_4 concentration on measured uptake length is demonstrated by the 2.5 times longer uptake lengths determined in the short-term nutrient releases (tenfold increase in NH_4 concentration) conducted before the ^{15}N-tracer study began. Declining water temperature as autumn progressed may have resulted in lower biological activity, which led to the nearly threefold decline in NH_4 uptake rate from day 0 to 42. Ammonium uptake length may have increased in Upper Ball Creek for a number of reasons, but it is likely that increased discharge was the dominating factor. In contrast, the decline in NH_4 uptake rates was probably more affected by decreasing stream temperatures.

$^{15}NH_4$ can be converted to $^{15}NO_3$ through nitrification, although we detected very little $^{15}NO_3$ in Upper Ball Creek during the 42-d release. Nitrification rates may have been limited by the very low ammonium concentrations (3 μg NH_4-N L^{-1}). Nitrification in Upper Ball Creek accounted for <3% of ammonium uptake on day 0, in contrast to Walker Branch, where about 20% of ammonium uptake was due to nitrification (Mulholland et al. 2000*a*). High rates of nitrification have also been shown in Bear Brook, New Hampshire, another temperate forest stream (Richey et al. 1985; Hall unpubl. data). Low quality stream sediments (FBOM with high C:N ratio) available for colonization by nitrifying bacteria may also have limited nitrification rates in Upper Ball Creek (Newbold 1992). Ambient NO_3 concentrations in Upper Ball Creek are very low (3 μg N L^{-1}) and may reflect the low nitrification rates found there.

Ammonium uptake rates: whole-stream vs. primary uptake compartments—Despite the similarity in NH_4 uptake lengths, whole-stream ammonium uptake rates were much higher in Upper Ball Creek (191 mg N m^{-2} d^{-1}) compared to Hugh White Creek (25 mg N m^{-2} d^{-1}; calculated from Hall et al. 1998) and Walker Branch (32–53 mg N m^{-2} d^{-1}; Mulholland et al. 2000*a*). Ammonium in Upper Ball Creek is removed very quickly from the water column, resulting in short uptake lengths and high mass transfer coefficients (v_f in Table 3). The pulse of allochthonous carbon during autumn leaf fall likely increased NH_4 uptake efficiency in Upper Ball Creek, which results in some of the highest mass transfer coefficients reported in the literature (v_f = 7.4 × 10^{-4} m s^{-1}, Table 3). In Hugh White Creek, located in the same basin as Upper Ball Creek, v_f was only 0.4 × 10^{-4} m s^{-1} during a summer tracer addition (calculated from Hall et al. 1998). In Walker Branch, Tennessee, during spring, v_f ranged from 1.0 × 10^{-4} m s^{-1} to 1.4 × 10^{-4} m s^{-1} (Mulholland et al. 2000*a*). In two small heterotrophic streams in Idaho, v_f ranged from 0.2 × 10^{-4} m s^{-1} to 0.8 × 10^{-4} m s^{-1} (Davis and Minshall 1999). Ammonium uptake in Upper Ball Creek had mass transfer coefficients similar to those found in an autotrophic, prairie stream where v_f ranged from 1.2 × 10^{-4} m s^{-1} to 3 × 10^{-4} m s^{-1} (Dodds et al. pers. comm.). The high mass transfer coefficients in Upper Ball Creek during autumn are likely a consequence of microbial activity on decomposing leaves.

Biotic control of nutrient uptake has been demonstrated previously in temperate forested streams. The exclusion of leaf litter from another small headwater stream at Coweeta (Catchment 55) resulted in significantly longer uptake lengths and lower uptake rates for both ammonium and phosphate, compared to the reference stream containing leaves (Webster et al. in press). Using a ^{32}P tracer addition to a forested stream, Mulholland et al. (1985) found higher phosphate uptake rates following autumn leaf fall and attributed the higher rates to heterotrophic microbes colonizing the allochthonous organic matter. Increased biological demand for nutrients in Upper Ball Creek is reflected in measurements of whole-stream respiration that were 6 times higher than in Walker Branch (Mulholland et al. 2000*a*), which emphasizes the dominance of heterotrophy in Upper Ball Creek (Table 1).

When NH_4 uptake rates were calculated for each of the four major biomass compartments on day 7 and summed,

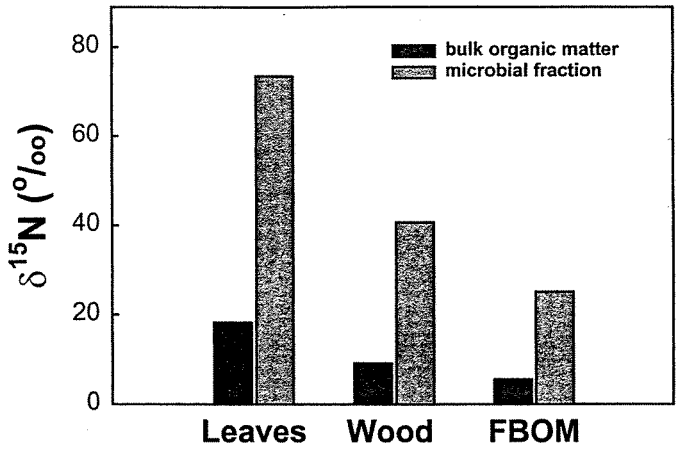

Fig. 8. Comparison of $\delta^{15}N$ values of bulk allochthonous organic matter vs. the microbial N associated with the organic matter/microbe complex on day 38. $\delta^{15}N$ values are plotted for leaves, wood, and FBOM at the 50-m site.

we could account for only about 4% of the whole-stream uptake rate measured on day 0 (Tables 3 and 4). It appears that the compartment-specific analysis greatly underestimated NH_4 uptake rate in Upper Ball Creek. There was little change in label from day 3 to day 7 for both leaves and epilithon (Fig. 5), which suggests that rapid regeneration of ^{15}N back to water may explain some of the underestimation. Our calculations of compartment-specific uptake rates assume no significant regeneration of ^{15}N back to water over the first 7 d. It is possible that standing stocks of leaves and FBOM were underestimated. Leaves, in particular, had formed leaf packs on the streambed, and our sampling method, prior to the start of the release, may have underestimated biomass. Epilithon uptake rates in Upper Ball Creek were only 17% of epilithon uptake rates in Walker Branch, Tennessee (Mulholland et al. 2000*a*), probably because of lower autotrophic biomass (as indicated by low estimates of chlorophyll *a* and GPP, Table 1).

N turnover rates and estimates of actively cycling N—N turnover rates and times for epilithon, leaves, and FBOM were calculated using the decline in $\delta^{15}N$ values over the first 28 d after termination of the ^{15}N addition (Table 4). Thus, these turnover rates should reflect release of actively cycling N only. By dividing uptake rate by turnover rate, we can estimate the biomass of N in a compartment that is actively cycling. For epilithon, the biomass of actively cycling N (0.014 g N m^{-2}) represented about 67% of total epilithon N. This estimate of actively cycling N assumes that all N uptake is as NH_4. If there were large amounts of NO_3 uptake by epilithon as well, the total N uptake rate would be underestimated, as would the proportion of actively cycling N. Actively cycling N in leaves (0.135 g N m^{-2}) was about 17% of total N, close to our estimate of 20% microbial ^{15}N in leaves calculated from the chloroform fumigations (Fig. 8). In contrast, actively cycling N estimated for FBOM (0.122 g N m^{-2}) was a smaller fraction of total N in FBOM (11%) than the 31% microbial ^{15}N estimated using the fumigation technique (Fig. 8). Thus, unlike epilithon, most of the N in the detrital compartments is not actively cycling.

Food web relationships—Relationships within the Upper Ball Creek food web were more complex than predicted. The grazer *Stenonema* had a consistently higher $\delta^{15}N$ than epilithon, its proposed food resource, on days 21 and 42, in contrast to model predictions. This apparent contradiction is a likely consequence of our epilithon sampling methods. Epilithon was sampled by scraping entire rock surfaces, and all material adhering to that surface was considered epilithon. The $\delta^{15}N$ results indicate that *Stenonema* is either selectively ingesting and/or assimilating a more highly labeled component of the epilithon (e.g., diatoms and bacteria in the outer layer) rather than the bulk epilithon, which likely includes nonliving, less nutritious organic matter (Lock et al. 1984). Epilithon in Upper Ball Creek has a very low Chl *a*: AFDM ratio (Table 1), which implies that little of the organic portion is algae. Using a standard AFDM : Chl *a* ratio of 100 : 1 for algae, we calculate that only about 6% of the Upper Ball Creek epilithon was algal biomass. Additionally, gut analysis data for *Stenonema* from a different stream in the Coweeta basin showed that they were consuming 30% FPOM and 70% algal material (Hall et al. 1998). During a ^{15}N-tracer addition conducted in Hugh White Creek, at Coweeta, during summer, *Stenonema* had $\delta^{15}N$ values less than its epilithon food resource (Hall et al. 1998), which suggests that seasonal differences in availability of algal N may be occurring. In a similar ^{15}N-tracer addition in the Kuparuk River, Alaska, Wollheim et al. (1999) also found that the dominant grazer, *Baetis,* had a higher $\delta^{15}N$ value than epilithon and that the bulk epilithon had a lower $\delta^{15}N$ than their model predicted. They resolved these discrepancies in the model by dividing the epilithon into highly labeled autotrophic and less-labeled detrital portions, which resulted in *Baetis* tracking the $\delta^{15}N$ of its food resource (autotrophic epilithon). During a spring ^{15}N addition in Walker Branch, where the measured epilithon Chl *a*: AFDM ratio was 10 times higher than in Upper Ball Creek, *Stenonema* also tracked the bulk epilithon $\delta^{15}N$ closely (Mulholland et al. 2000*b*).

In contrast to the grazer-epilithon patterns, the $\delta^{15}N$ values for the dominant shredder *Tallaperla* closely tracked those of leaves on both day 21 and 42 and were consistently lower than $\delta^{15}N$ values for microbes colonizing leaves (Fig. 6). Leaves collected in the field were almost completely skeletonized; both the biofilm and the leaf were consumed. The $\delta^{15}N$ signal in *Tallaperla* tracked the $\delta^{15}N$ signal of the leaves, not the microbes. This is consistent with previous observations on *Tallaperla* feeding. *Tallaperla* was somewhat less labeled than leaves during a summer ^{15}N addition (Hall et al. 1998); incorporation of fungal and bacterial biomass could account for only 25% of *Tallaperla* respiratory demands in a laboratory feeding experiment (Findlay et al. 1986); and from 19 to 43% of carbon in *Tallaperla* was derived from bacteria in two headwater streams at Coweeta (Hall and Meyer 1998). These results all support the same conclusion, namely, that *Tallaperla* growth is based on assimilation of more than microbial biomass.

The feeding mode of two caddisflies, *Diplectrona* and *Parapsyche,* differed considerably based on the day 21 $\delta^{15}N$

data. *Diplectrona,* typically found on the underside of rocks, followed the $\delta^{15}N$ pattern of FBOM, whereas *Parapsyche,* found in moss of fast-flowing riffle areas, followed the $\delta^{15}N$ signal of seston (Fig. 7A,B). Focusing on day 21 data, collector–gatherers and filterers had only slightly higher $\delta^{15}N$ values than their presumed food resource, which suggests that consumer N is derived from more than just the microbial portion of the FBOM-microbe complex. In contrast, on day 42, the $\delta^{15}N$ results suggest little difference in feeding behavior of the two caddisflies, although we had only two seston $\delta^{15}N$ values to compare. The consistently low label in both bulk FBOM and seston in Upper Ball Creek implies that at least part of the seston may be entrained FBOM particles transported from upstream and containing little actively cycling N (Fig. 7C,D). In contrast, in the more autotrophic Walker Branch, the seston label was almost double that of FBOM, and seston in that stream may be a combination of entrained FBOM and sloughed algae (Mulholland et al. 2000*b*).

The $\delta^{15}N$ values for both invertebrate and vertebrate predators demonstrated that the tracer addition was not long enough to achieve isotopic equilibrium in larger bodied organisms with N pools that turn over slowly. On day 42, $\delta^{15}N$ values in predators were always considerably higher than on day 21, and because of this we were able to make few conclusions about food web linkages. In general, $\delta^{15}N$ values for the invertebrate predator *Acroneuria* were approaching those of smaller invertebrate consumers by day 42. Both small brook trout and the immatures of two species of salamander were labeled only slightly above background $\delta^{15}N$ levels, but the presence of measurable tracer indicated that at least part of their N came from the stream food web. In contrast, there was no evidence for stream food resources in the del values of adult salamanders (*Desmognathus*).

Mass balance of tracer ^{15}N—Although whole-stream uptake of NH_4 in Upper Ball Creek was high (79–190 mg N m^{-2} d^{-1}, Table 3), we could only partially account for the ^{15}N added over the 42-d tracer study in Upper Ball Creek. ^{15}N found in the food web compartments in the 200-m reach accounted for about 12% of the total ^{15}N added (Table 5). The large standing stock of leaves that entered during autumn leafall and was subsequently colonized by microbes accounted for most of the ^{15}N retention. When we included the amount of ^{15}N exported from the reach through transport of $SPO^{15}N$, $^{15}NH_4$, $^{15}NO_3$, and $DO^{15}N$ in the water column, we were able to account for 53% of ^{15}N added. Most of the export of dissolved ^{15}N was as $^{15}NH_4$, whereas the smallest export was as $DO^{15}N$.

Overall, DON and NO_3 were only marginally labeled with tracer ^{15}N. Marine studies have shown autotrophically derived DON to be an important sink for inorganic nitrogen (Bronk et al. 1994), as well as a source of N for both phytoplankton and bacteria (Bronk and Glibert 1993). In contrast, most DON in headwater streams is thought to be of terrestrial origin, particularly in autumn when leaching of leaf litter elevates DON concentrations in forest soils (McDowell et al. 1998). Although our study could not clarify the role of DON in nitrogen cycling in streams, the lack of ^{15}N tracer in the DON pool suggests that in-stream processes are not a significant source of DON in this forest stream during autumn.

There remains a paradox in Upper Ball Creek: nitrogen uptake was rapid, yet there is considerable tracer ^{15}N uptake for which we cannot account. An underestimate of the role played by subsurface processes in Upper Ball Creek could influence our overall ^{15}N mass balance. Some of the missing tracer uptake could be accounted for through uptake, retention, and loss of ^{15}N through microbial processes occurring in the hyporheic zone (Hendricks and White 1991; Mulholland et al. 1997), which would not be included in our compartment-specific uptake rates. Using conservative tracer injections conducted 4 times during the study, we estimated an $A_s:A$ of 0.37 in Upper Ball Creek, which indicates that the area of the transient storage zone may be modest in comparison to the surface area of the stream bottom. Additionally, the possible loss of ^{15}N from Upper Ball Creek through denitrification remains unknown, but could be important (Duff and Triska 1990). Although the sediments in Upper Ball Creek, a high-gradient headwater stream, likely remain well oxygenated, there could be anaerobic microsites where denitrification occurs, coupled with mineralization and nitrification in adjacent aerobic zones. Loss of N through denitrification was measured in two smaller streams at Coweeta, despite high gradient and presumably well-aerated sediments (Caskey and Swank 1983). Nonetheless, at this time we know little about the contribution of subsurface processes to overall nutrient retention in Upper Ball Creek, and future studies are planned to address this possible sink.

It is also likely that we have underestimated standing stocks of organic matter at the end of the tracer release, which leads to an underestimate of ^{15}N retention in those compartments. Standing stock of organic matter was measured 4 d prior to the start of the tracer release (the first week of November). Although the majority of litterfall had occurred by then, it is likely that fresh litter continued to fall or blow into the stream during the release. In a nearby watershed within the Coweeta basin, approximately 25% of total litter input occurred after November 5 (Webster and Benfield unpubl. data). Input and accumulation of unlabeled leaves in the reach would have diluted the ^{15}N signal for leaves and resulted in an underestimate of ^{15}N retention in that very large compartment. Our "missing isotope" problem is not surprising given the difficulties of sampling large detrital pools (leaves and FBOM) combined with large through-system fluxes of organic nitrogen. It is similar in magnitude to those found in terrestrial ^{15}N studies, where accounting for only half of ^{15}N tracer is not uncommon (e.g., Jordan et al. 1997; Seely and Lajtha 1997).

Because of high standing stocks, leaves and FBOM represent much of the ^{15}N retained in Upper Ball Creek; hence any losses from these pools downstream should significantly affect the mass balance (Table 5). After the storm that occurred on day 27, the $\delta^{15}N$ values of leaves and FBOM decreased (Fig. 5), which indicates a possible dilution by the addition of "new" unlabeled material from upstream or the riparian zone and presumably export of labeled material out of the study reach. When we calculate a mass balance using ^{15}N data from before the storm (day 21), leaves and FBOM accounted for the retention of 12 and 7% of the ^{15}N added.

Yet at the end of the release (day 42), 9% of all ^{15}N was in leaves and only 1% was in FBOM (Table 5). The FBOM pool appears to have been more impacted by the storm than was leaf litter. Contrary to our expectations, total retention was not changed by the storm, and transport during the storm accounted for only a small amount of the ^{15}N added to the stream.

Summary—Our study, combined with several other ^{15}N-tracer studies, demonstrates that nitrogen cycling strongly reflects the overall energy dynamics of streams. Primary producers dominated N uptake in two unshaded streams; the Kuparuk River, Alaska (Wollheim et al. 1999) and S. Kings Creek, Kansas (Dodds et al. pers. comm.). In Walker Branch, Tennessee both heterotrophic and autotrophic pathways of N cycling were important during the transition period associated with spring leafout (Mulholland et al. 2000*a*). Results from the Upper Ball Creek study demonstrate the tremendous ammonium demand (and high v_f) associated with microbes colonizing leaf detritus and the resultant linkage to invertebrate shredders. In Upper Ball Creek in autumn, spiraling of NH_4 is very tight, NH_4 residence time in water is short, and uptake rates are very high, even among heterotrophic streams (*see* Hall et al. 1998). Although epilithon and grazers became highly labeled with ^{15}N, due to their fast turnover rates, they represented only a small portion of overall nitrogen uptake because of their low standing stock. Additionally, nitrification rates were low, as were ambient NH_4 and NO_3 concentrations, and much of the detrital N does not appear to be actively cycled through in-stream processes. Minimal labeling of stream DON is consistent with allochthonous DON sources at this site, in contrast to DON dynamics in marine systems (Bronk et al. 1994; Bronk and Ward 1999) and possibly more autotrophic streams (Kaplan and Bott 1982).

Although our understanding of N dynamics in streams has been advanced by whole-stream ^{15}N-tracer studies, most of these have been conducted in relatively low nutrient streams that are limited by either N, P, or both. In many streams anthropogenic inputs of N have greatly increased N transport (Vitousek et al. 1997), and the resulting overenrichment of N is a widespread problem ultimately affecting estuaries and oceans (Carpenter et al. 1998). Analyses of N spiraling in unimpacted streams provide an ecological foundation for assessment of spiraling in high-N streams. A challenge for the future lies in applying these models and experimental techniques to further our understanding of the processes controlling N dynamics in N-enriched streams.

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