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Characterizing nitrogen dynamics, retention and transport in a tropical rainforest stream using an *in situ* ^{15}N addition

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SUMMARY

1. This study was part of the Lotic Intersite Nitrogen eXperiment (LINX); a series of identical $^{15}NH_4$ tracer additions to streams throughout North America. $^{15}NH_4Cl$ was added at tracer levels to a Puerto Rican stream for 42 days. Throughout the addition, and for several weeks afterwards, samples were collected to determine the uptake, retention and transformation pathways of nitrogen in the stream.
2. Ammonium uptake was very rapid. Nitrification was immediate, and was a very significant transformation pathway, accounting for over 50% of total NH_4 uptake. The large fraction of NH_4 uptake accounted for by nitrification (a process that provides energy to the microbes involved) suggests that energy limitation of net primary production, rather than N limitation, drives N dynamics in this stream.
3. There was a slightly increased ^{15}N label in dissolved organic nitrogen (DON) the day after the $^{15}NH_4$ addition was stopped. This $DO^{15}N$ was < 0.02% of DON concentration in the stream water at the time, suggesting that nearly all of the DON found in-stream is allochthonous, or that in-stream DON production is very slow.
4. *Leptophlebiidae* and *Atya* appear to be selectively feeding or selectively assimilating a very highly labelled fraction of the epilithon, as the label found in the consumers became much higher than the label found in the food source.
5. A large spate (>20-fold increase in discharge) surprisingly removed only 37% of in-stream fine benthic organic matter (FBOM), leaves and epilithon. The fraction that was washed out travelled downstream a long distance (>220 m) or was washed onto the stream banks.
6. While uptake of $^{15}NH_4$ was very rapid, retention was low. Quebrada Bisley retained only 17.9% of the added ^{15}N after 42 days of ^{15}N addition. Most of this was in FBOM and epilithon. Turnover rates for these pools were about 3 weeks. The short turnover times of the primary retention pools suggest that long-term retention (>1 month) is minimal, and is probably the result of N incorporation into shrimp biomass, which accounted for < 1% of the added ^{15}N.

Keywords: ammonium uptake, LINX, ^{15}N tracer, nitrification, nitrogen retention

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Introduction

Nitrogen availability can limit primary production in many terrestrial and aquatic habitats (e.g. Grimm & Fisher, 1986; Vitousek & Howarth, 1991). Humans affect the N cycle through activities such as burning fossil fuels, fertilization and waste disposal. Whether through N deposition (via combustion of fossil fuels), or direct input to streams and rivers (e.g. fertilizer run off, input of treated and untreated waste), N inputs to the world's landscapes have increased dramatically over the past several decades (Vitousek *et al.*, 1997). Changes in N inputs are occurring most rapidly in tropical regions (Matson *et al.*, 1999) and could have dramatic impacts on stream chemistry and ecosystem processes. Matson *et al.* (1999) contend that retention of anthropogenic N will be much less in tropical ecosystems than temperate ecosystems, resulting in increased N flux at the soil–water and soil–air interfaces, with little or no lag in response time. Because streams and rivers link terrestrial ecosystems to lentic, estuarine and marine ecosystems, the processes and transformations that occur in lotic systems can have impacts on downstream ecosystems. These linkages can be even tighter in many tropical islands, such as Puerto Rico, because of the proximity of headwaters to the ocean and because of the many biotic linkages, such as amphidromous shrimp that migrate from the estuaries to the headwaters (e.g. March *et al.*, 1998).

Nitrogen concentration in streams is influenced by inputs from the landscape, but also by processes occurring in the channel and hyporheic zone of the stream. These processes include nitrification, denitrification, uptake, retention and mineralization, and they are poorly described for tropical streams. There have been many studies using enrichment techniques to measure N dynamics such as nitrification (e.g. Richey, McDowell & Likens, 1985; Triska *et al.*, 1993; Kopacek & Blazka, 1994), uptake length (e.g. Richey *et al.*, 1985; Munn & Meyer, 1990; Marti & Sabater, 1996) and retention (e.g. Newbold *et al.*, 1983; Marti & Sabater, 1996). Because these studies raise stream N concentrations, however, they probably overestimate nitrification and uptake (Mulholland, Steinman & Elwood, 1990). Potential nitrification rates in stream benthic substrata (sediment, bryophytes or detrital pools) have also been measured (e.g. Cooper, 1983; Richey *et al.*, 1985; Triska *et al.*, 1993; Kopacek & Blazka, 1994; Holmes *et al.*, 1996). While measuring the potential rate of nitrification in the substratum is valuable for comparisons among streams, these measurements do not necessarily represent the processes occurring in the stream at ambient concentrations. The recent development of tracer additions of stable N isotopes (Fry *et al.*, 1995) allows for the measurement of the in-stream rate of N spiralling, while maintaining an ambient concentration of N in the stream and avoiding the limitations of enrichment experiments and incubations.

In this study we used $^{15}NH_4$ as a tracer to follow the uptake, transformation, retention and export of N through Quebrada Bisley, the main headwater stream of the Rio Mameyes in the Luquillo Experimental Forest (LEF), Puerto Rico. This study is one component of the multisite Lotic Intersite Nitrogen eXperiment (LINX), a series of identical 6-week ^{15}N additions to streams throughout North America. Currently there are only a handful of ^{15}N tracer studies in streams published (Peterson, Bahr & Kling, 1997; Hall, Peterson & Meyer, 1998; Mulholland *et al.*, 2000; Tank *et al.*, 2000). This experiment is the first such study performed in the tropics, allowing for the first time direct measurement of whole stream N uptake, nitrification and retention at ambient nutrient concentrations in a tropical stream.

Methods

Site description

Quebrada Bisley is a second order stream at approximately 240 m above sea level located in the LEF, in the north-east corner of Puerto Rico. Altitude in the forest ranges from 200 to 1000 m, with vegetation dominated by tabonuco (*Dacryodes excelsa* Vahl.) forest type at the lower altitudes and colorado (*Cyrilla racemiflora* L.) forest type higher up. Average monthly air temperature ranges from 21 °C in January to 25 °C in August, decreases with altitude, and the daily fluctuation is 6–7 °C. Annual rainfall ranges from 250 cm at lower altitude to more than 450 cm at higher altitude. Precipitation is greatest from June to December. Leaf fall occurs all year, with a peak in April–June and a minimum in December–March (Weigert, 1970; Lodge *et al.*, 1991; Zou *et al.*, 1995), averaging 470.9 g dry mass m^{-2} $year^{-1}$. The LEF is

characterized by steep slopes. More detailed descriptions of the LEF can be found in Brown *et al.* (1983) and Scatena (1989).

Soils in the Bisley basin are dense clays and groundwater flow paths are shallow (McDowell, Bowden & Asbury, 1992). Precipitation in the area of our study is approximately 310 cm $year^{-1}$ (García-Martinó *et al.*, 1996). Floods are frequent and 20-fold increases in stream discharge are common over a few hours. Discharge closely follows precipitation and quickly returns to baseflow.

A 225-m reach of the main branch of the Quebrada Bisley was chosen, starting approximately 150 m below an access road. The reach was selected because it lacked significant tributaries. The stream reach was marked at 10-m intervals, and sampling stations were selected at −10, 25, 50, 67, 110, 140, 180 and 225 m, relative to the location of the ^{15}N-drip point (0 m). The average slope of the reach was approximately 13%. Using a point-transect approach the stream reach was 67% riffle and 33% pool, with the substratum containing approximately 45% boulder/bedrock, 40% cobble, 14% gravel and 1% sand. Average stream width was 4.7 m (Table 1).

Table 1 Stream characteristics measured prior to, or during the ^{15}N addition

Parameter	Value
Physical (reach average):	
Discharge (6 week average)	20.2 L s^{-1}
Width (day 0)	4.7 m
Depth (day 0)	12.8 cm
Slope	13.3%
Temperature (6-week average)	22 °C
Light (PAR) (6-week average)	0.3 mol m^{-2} day^{-1}
Water chemistry (experimental average)	
NH_4	2–3 μg N L^{-1}
NO_3	129 μg N L^{-1}
DON	120 μg N L^{-1}
SRP	14 μg P L^{-1}
pH	7.4
Metabolism (from P.J. Mulholland *et al.*, unpublished data)	
Gross primary productivity (GPP)	0.1 g O_2 m^{-2} day^{-1}
Respiration (*R*)	9.0 g O_2 m^{-2} day^{-1}
P : *R* ratio	0.01

Stream biotic community

Freshwater shrimp are the dominant organisms in small streams of the LEF (Covich & McDowell, 1996; Buzby, 1998), where three genera are found: *Xiphocaris* (*X. elongata* Guerin-Meneville) *Atya* (three species; *A. lanipes* Holthius, *A. innocous* Herbst and *A. scrabra* Leach) and *Macrobrachium* (five species; *M. carcinus* L., *M. heterochirus* Wiegmann, *M. crenulatum* Holthius, *M. faustinum* De Saussure and *M. acanthurus* Wiegmann) and are most abundant in pools. Large adult *Atya* were primarily found in only one pool at approximately 80 m, and were not routinely sampled. Small *Atya* (< 1 cm) and small freshwater crabs (*Epilobocera sinuatifrons* A. Milne-Edwards) were found in shallow riffles, buried in gravel. Insect densities and species diversity were very low, and were dominated by relatively immobile taxa (Buzby, 1998). Macroinvertebrate shredders are uncommon in Bisley, accounting for only 1.3% of the aquatic insects, as is typical of tropical streams (Buzby, 1998). The predaceous mountain mullet, *Agonostomus monticola* Bancroft, is common in Bisley (Buzby, 1998), and the goby, *Sicydium plumieri* Bloch, is present. The eel, *Anguilla rostrata* Leseur, was seen at the lower end of our study reach on one occasion. A detailed listing of the species present in Bisley can be found in Buzby (1998) and a review of the stream food web of the LEF can be found in Covich & McDowell (1996).

Methods

^{15}N addition

A solution of ^{15}N enriched NH_4Cl (10% enrichment) was continuously added to the stream using a battery operated fluid metering pump at a constant rate (2 mL min^{-1}) for 6 weeks (15 January 1998 to 27 February 1998). Our goal was to raise the $\delta^{15}N$ of stream NH_4 to 500‰ (based on an expected discharge of 0.025 m^3 s^{-1} and NH_4 concentration of 10 μg NH_4-N L^{-1}). Actual stream discharge was lower for much of the experiment and NH_4 concentrations were lower than anticipated. Therefore, our enrichment was higher than we planned. The drip rate to the stream was checked periodically (approximately every other day) and corrected to maintain a constant addition rate. The addition rate was not adjusted for changing discharge or fluctuations in NH_4 concentration, so the overall enrichment to the stream varied day to day.

During the 6-week addition, 1495.9 mg of ^{15}N was added to the stream. This resulted in a negligible increase in stream NH_4 concentration (approximately

0.2 μg NH_4-N L^{-1}). Therefore the added $^{15}NH_4$ was an ammonium tracer and followed pathways identical to ambient NH_4.

Physical parameters

Discharge, light and water temperature were recorded throughout the ^{15}N-tracer addition. Discharge in our study reach was calculated using the relationship between upstream discharge (from a USFS gauging station) and stream reach discharge calculated from conservative tracer additions (Br^-, $n = 9$). Photosynthetically active radiation (PAR) (mol m^{-2} day^{-1}) was measured approximately 2 m above the stream surface at the reference station (−10 m). Stream water temperature was recorded hourly at −10 m.

Water chemistry: sampling and analysis

Water samples for routine nutrient analysis [NO_3^-, NH_4^+, PO_4^{3-} and total dissolved nitrogen (TDN)] were collected twice weekly during the tracer addition, as well as each day when water samples for ^{15}N analysis were collected. Nitrate samples were filtered in the field using 0.2 μm sterile Acrodisc filters (Pall, Gelman, Ann Arbor, MI, U.S.A.) and refrigerated. All other samples were filtered in the field using precombusted glass fibre filters (Whatman, GF/F, Maidstone, U.K.), and frozen within 3 h of collection. Nitrate was analysed using ion chromatography (micromembrane suppression, Dionex, Sunnyvale, CA, U.S.A.), and ammonium and ortho-phosphate were measured using colorimetric flow injection analysis (Lachat, Milwaukee, WI, U.S.A.). The TDN was measured by high-temperature catalytic oxidation (HTCO), using a Shimadzu TOC-5000 coupled to an Antek chemiluminescent N detector (Houston, TX, U.S.A.) (Merriam, McDowell & Currie, 1996). Water chemistry analyses were performed at the University of New Hampshire.

Sampling of detritus pools and epilithon

Standing stocks of coarse benthic organic matter (CBOM > 1 mm) and fine benthic organic matter (FBOM < 1 mm) were estimated in pools and riffles. Samples were collected using an open-ended cylinder (0.07 m^2) placed into the sediments as deep as possible (approximately 5–10 cm; $n = 6$ per habitat). Large CBOM was picked out by hand. The sediment was agitated, and the contents of the cylinder were filtered through a 1-mm sieve to collect the remaining CBOM. A subsample (60 mL) of the water remaining in the cylinder was collected and filtered onto precombusted GF/F filters (Whatman) to measure FBOM. Samples were dried at 60 °C, weighed, combusted at 500 °C for 4 h, re-wetted, dried and re-weighed for calculation of dry mass and ash-free dry mass (AFDM). Standing stocks for the entire stream reach was calculated from weighted habitat averages (pools and riffles).

For routine ^{15}N analysis, CBOM was divided into small wood and leaves. Samples were hand picked from several locations at each station, dried at 60 °C for several days until dry, and ground. The FBOM was collected from the stream bottom using light suction, and approximately 100 mL of FBOM slurry was returned to the laboratory, filtered onto precombusted GF/F filters, dried at 60 °C for several days and stored in sealed glass scintillation vials until ^{15}N analysis.

The concentration of suspended particulate organic matter (SPOM) was measured by filtering known quantities of stream water through precombusted GF/F filters. Dry weight and AFDM were calculated as described above. Mass per m^2 was calculated by multiplying mass per volume by average stream depth. For ^{15}N analysis, stream water was filtered in the field through precombusted GF/F filters until the filter clogged. Filters were dried at 60 °C for several days and sealed in glass scintillation vials.

Biomass and chlorophyll *a* content in epilithon was scraped from randomly selected rocks collected from riffle areas of the stream. A known area of the rock was scrubbed with a wire brush and rinsed with stream water. The epilithic slurry was collected in a container and returned to the laboratory. Samples for chlorophyll *a* were filtered onto precombusted GF/F filters and frozen until they could be analysed by extraction in the dark for 24 h at 4 °C, using 90% acetone. The extracts were analysed at 664 and 750 nm with a spectrophotometer before and after acidification to determine chlorophyll *a* (APHA, 1992). Samples for dry weight and AFDM were processed as FBOM samples. Samples for ^{15}N analysis were collected by pooling the epilithon scrubbed from five rocks at each station. The scrubbed material was washed into a container using stream water. Samples were filtered and prepared for analysis in the manner of FBOM.

Sampling of consumer biomass

Representative organisms from different trophic levels and feeding guilds were selected for ^{15}N analysis based on abundance (Table 2). Samples were collected from each station prior to starting the ^{15}N addition to estimate standing stocks. During the addition, samples were collected weekly from each station. The predatory mountain mullet (*Ag. monticola*), and other fish (e.g. *S. plumieri*) were not sampled because of technical difficulties with fish shocking equipment.

Adult shrimp (*M. carcinus* and/or *M. crenulatum* and *Xiphocaris*) were collected from large pools in baited minnow traps on three successive nights prior to the ^{15}N addition to estimate population biomass. Biomass estimates were calculated using known relationships between carapace length and mass, and habitat-corrected based on the percentage of habitat found in the stream. Small *Atya* (*A. lanipes*) and *Epilobocera*, were collected by agitating a known area of riffle and collecting the specimens in a D-net (i.e. 'kick sample'). Samples were dried at 60 °C for several days and weighed. A subsample was combusted at 500 °C to calculate AFDM and the remainder was analysed for C : N ratio. All values were habitat corrected to provide units per m^2 of stream bottom. We used estimates of insect standing stock biomass from Buzby (unpublished data).

We sampled the smaller invertebrates for ^{15}N analysis during the tracer addition using a D-net, without regard to area sampled. *Veliidae* were collected off the water's surface with a net. Shrimp were captured in minnow traps containing leaves as bait. Traps were put in the stream the night prior to sampling. At least five individuals were pooled from each site, with the exception of *Macrobrachium* and *Xiphocaris*, where typically only one individual or a claw from a single individual was used. Organisms were separated by family or genus, dried at 60 °C for several days and then ground. Larger shrimp were dried more thoroughly (for a week or more). Table 2 summarizes standing stocks and C : N ratios of compartments sampled during the experiment.

^{15}N analysis

All ^{15}N analyses were conducted at the Ecosystems Center Laboratory, Marine Biological Laboratory, Woods Hole, MA, USA. The $^{15}N : {}^{14}N$ ratio was determined by automated high temperature combustion with cryogenic separation followed by measurement of $^{15}N : {}^{14}N$ using a Finnigan Delta S mass spectrometer (Thermo Finnigan, San Jose, CA, U.S.A.). All $^{15}N : {}^{14}N$ ratios are expressed as $\delta\ ^{15}N$ (units of ‰) according to the following equation:

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

Table 2 Habitat-corrected estimates of ash-free dry mass (AFDM) and nitrogen for compartments sampled during and after the ^{15}N addition. The percent of total measured stream AFDM and nitrogen are in parentheses

Compartment	Representative sampled	AFDM ($g\ m^{-2}$)	C : N	N ($mg\ m^{-2}$)
Epilithon		3.54 (6.7)	5.56	217.0 (12.4)
Fine benthic organic matter (FBOM)	<1 mm	32.23 (61.3)	9.90	1110.0 (63.5)
Coarse benthic organic matter (CBOM) (leaves and wood combined)	>1 mm	11.98 (22.8)	32.2	277.0 (15.8)
Suspended particulate organic matter (SPOM)		4.17 (7.9)	4.47	57.6 (3.3)
Insect shredder	Not sampled			
Insect filterer	*Hydropsychidae*	2×10^{-4} (0.0)		0.02 (0.0)
Insect collector/gatherer	*Leptophlebiidae* *Neohagenulus julio*	3×10^{-3} (0.05)		0.28 (0.0)
Insect predator	*Veliidae*	2×10^{-4} (0.0)		0.02 (0.0)
Shredder	*Xiphocaris*	0.087 (0.16)	4.09	10.5 (0.6)
Filterer/grazer	*Atya* (>1 cm)	0.052 (0.10)	4.38	7.2 (0.4)
Omnivore	*Epilobocera sinuatifrons*	0.081 (0.2)	4.71	10.9 (0.6)
Grazer	*Atya* (<1 cm)	0.138 (0.3)	4.57	16.6 (0.9)
Predator	*Macrobrachium*	0.315 (0.6)	4.31	40.0 (2.3)
Stream total		52.60		1747.1

where $R = {}^{15}N : {}^{14}N$, and the standard is air ($R_{standard} = 0.003663$). Reported $\delta^{15}N$ values were corrected for background levels of ^{15}N by subtracting the value of the −10 m station from the downstream value, and do not represent any naturally occurring ^{15}N. To avoid carryover contamination, field sampling for ^{15}N analysis was conducted from the bottom of the reach (low ^{15}N) to the site closest to the dripper (high ^{15}N). Upon completion of field sampling on each date, equipment was rinsed off in the stream above the drip site, but downstream of the reference station. Samples from each compartment were collected from several locations within 5 m of the specified station (e.g. 110 m = 105–115 m) and pooled to ensure a representative sample, as well as decreasing the risk of sample depletion during the experiment. The SPOM was collected at only one location at the upstream limit of each station.

Full sampling (all compartments, all stations) or abbreviated sampling (selected compartments, all stations) was conducted weekly during the ^{15}N addition and for 4 weeks after the addition ended. In addition, epilithon at 25 m was sampled approximately every 3 days during the release, and weekly thereafter, to track short-term changes in ^{15}N in a fast-turnover compartment.

^{15}N water chemistry

Water samples for ^{15}N analysis were collected from all stations, plus two stations further downstream (260 and 290 m) on days 0, 20, 41 and postday 1. A conservative tracer addition was conducted during ^{15}N sampling of dissolved compartments to quantify changes in discharge along the study reach. Samples were collected and analysed as described in Mulholland *et al.* (2000).

There are two potential sources of error in the stream water $\delta^{15}NH_4$ and $\delta^{15}NO_3$ values measured using the diffusion procedures. First, nitrogen contamination in the reagents acts to dilute the sample $\delta^{15}N$ values because the reagents are unenriched in ^{15}N relative to the samples. The reagent blanks were estimated for the $^{15}NH_4$ method and the $^{15}NO_3$ method, and sample values were corrected. The second source of error is because of the breakdown of DON to either NH_4 or NO_3 during sample processing. At our site, this is a potentially significant problem for $^{15}NH_4$ analysis, but not for $^{15}NO_3$ analysis, given the ambient concentrations of each. To minimize the potential error of the DON blank associated with the $^{15}NH_4$ analysis, we calculated $^{15}NH_4$ flux using the mass spectrometer value for stream water NH_4 concentration, rather than the NH_4 value from the wet chemistry method.

DO^{15}N analysis method

Samples for DO^{15}N analysis were oxidized using hydrogen peroxide and UV radiation for 20–24 h to convert all ^{15}N to $^{15}NO_3$. The resulting sample was analysed for $^{15}NO_3$ following the procedure described by Mulholland *et al.* (2000), with DO^{15}N being calculated by subtraction of the dissolved inorganic ^{15}N.

^{15}N calculations from water samples

Calculations of NH_4 uptake length and rate, nitrification rate, NO_3 uptake length and rate using ^{15}N label were performed as described by Mulholland *et al.* (2000), with few exceptions. In this study, $^{15}NH_4$ and $^{15}NO_3$ flux were estimated by multiplying the average flux (μg $^{15}NH_4$-N day^{-1} and μg $^{15}NO_3$-N day^{-1}) on three dates at the most downstream station by 42 days.

Because uptake length is strongly influenced by discharge, uptake lengths can only be directly compared when discharge is similar. We eliminated the influence of discharge by calculating a mass transfer coefficient (V_f), which equals stream depth times stream velocity divided by uptake length (Stream Solute Workshop, 1990; Davis & Minshall, 1999). Average stream depth and velocity were measured directly on day 0 but, for days 20 and 41, depth and velocity were estimated from Q based on the relationship of Leopold & Maddock (1953). The V_f represents the vertical velocity at which a solute moves through the sediment/water interface (Stream Solute Workshop, 1990) or the uptake efficiency (Davis & Minshall, 1999). Uptake rate can be calculated as the product of V_f and the concentration of an ion in the stream.

^{15}N calculations from biomass samples

The mass of ^{15}N associated with each food web compartment was calculated using standing stock estimates and the ^{15}N signal in each compartment.

Compartment-specific NH_4 uptake rates were calculated using the ^{15}N label in each primary uptake compartment on day 7 and the estimated ^{15}N label in stream water. Nitrogen turnover was estimated from the decay rates in each compartment over the first 28 days following the addition. These calculations are described by Mulholland *et al.* (2000).

Results

Physical and chemical parameters

Table 1 summarizes stream characteristics and conditions. Discharge declined slightly from days 0 to 20 (Fig. 1). Heavy rains on day 20 caused discharge to peak at over 1400 L s^{-1}, and sampling on day 21 was postponed for 24 h to allow discharge to return to near baseflow. Discharge remained near baseflow for the remainder of the addition with the exception of a few small storms (Fig. 1). Periods of high flow were brief, with the stream returning to baseflow within a few days at most.

Bisley is heavily shaded and light readings were low, averaging 0.27 mol quanta m^{-2} day^{-1}. Daily average light intensity ranged from 0.09 to 0.91 mol quanta m^{-2} day^{-1} during the ^{15}N addition. Water temperature was nearly constant, ranging from 21 to 23 °C. Nutrient concentrations were relatively constant during the experiment (Fig. 2). Average concentrations of NH_4, NO_3, DON and soluble reactive phosphorous (SRP) were 2–3, 129, 120 μg N L^{-1} and 14 μg P L^{-1}, respectively. Stream pH averaged 7.4.

Biomass and N standing stocks

Total standing stock of the major food web compartments was 52.6 g AFDM m^{-2} (Table 2). Detrital pools accounted for 92% of all organic material. Most of this was FBOM (32.2 g AFDM m^{-2}) and CBOM (12 g AFDM m^{-2}), followed by SPOM (4.2 g AFDM m^{-2}) and epilithon (3.5 g AFDM m^{-2}). Consumer standing stock was only slightly greater than 1% of the total organic material (0.68 g AFDM m^{-2}) and was dominated by the three genera of shrimps and the freshwater crab. Insect biomass was negligible, accounting for less than 0.01% of the measured organic standing stock (Table 2).

Nitrogen standing stock totalled 1.75 g N m^{-2} and, again, detrital pools were dominant, making up 83% of the measured N in food web compartments. Epilithon contained 12.5% of the total N standing stock. Consumers accounted for less than 5% of the total N standing stock, with insects

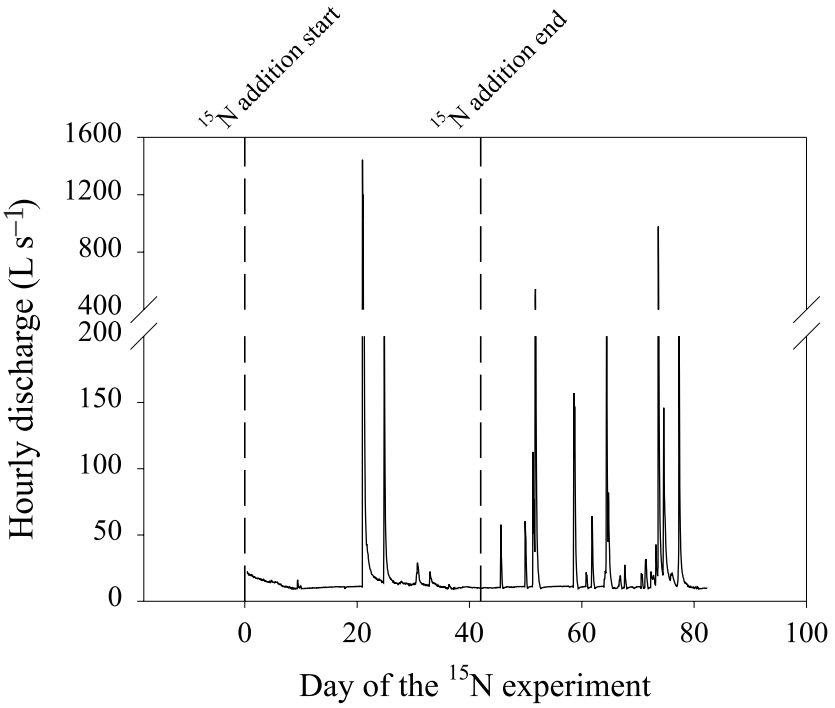

Fig. 1 Hourly discharge for Quebrada Bisley from January to April 1998. Data taken from a USGS gauging station approximately 150 m upstream of the start of the study reach.

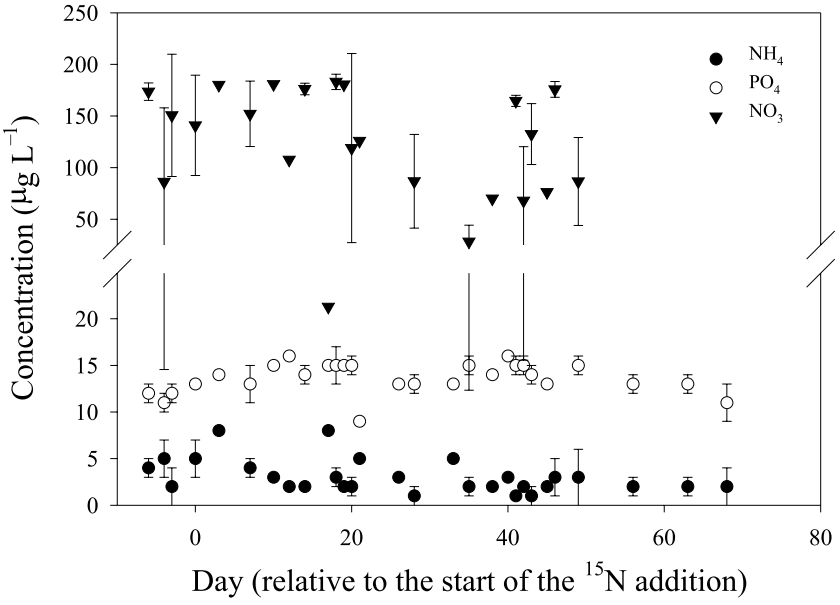

Fig. 2 Average measured concentration of NO_3-N, NH_4-N and PO_4-P throughout the experiment. Samples were collected from one to eight stations for each sample point. Error bars are 1 standard deviation. Experimental average concentrations were 2–3 μg NH_4-N L^{-1}, 129 μg NO_3-N L^{-1}, and 14 μg PO_3-P L^{-1}.

making up less than 1% of the consumer nitrogen fraction (Table 2).

Nitrogen dynamics

Ammonium uptake in Quebrada Bisley was very rapid, with uptake lengths of 26.4 and 15.5 m and uptake rates of 0.611 and 0.559 μg m^{-2} s^{-1} (days 0 and 41, respectively) (Table 3). However, the discharge on day 41 was almost half of the day 0 discharge, and NH_4 concentration had decreased from 4.2 and 3.3 μg N L^{-1}. By comparing uptake to efficiency (V_f), we see that uptake was similar on the two dates, although slightly higher on day 41 (Table 3), indicating more efficient uptake of available NH_4 on day 41.

The ^{15}N-labelled NO_3, from direct nitrification of the added $^{15}NH_4$, was evident in the stream water on day 0, only 6 h after the $^{15}NH_4$ addition began (Fig. 3a). The increase in $^{15}NO_3$ flux closely follows the decrease in stream water $^{15}NH_4$ flux (Fig. 3). Direct nitrification rates were 0.351 and 0.320 μg N m^{-2} s^{-1} on days 0 and 41, respectively. Direct nitrification averaged 57.4% of total NH_4 uptake. Nitrification rate and nitrification uptake velocity (V_f) were nearly constant during the tracer release.

Nitrate uptake length was calculated to be 1192 m on day 0, increased to 1689 m on day 20, and continued to increase to greater than 3000 m on day 41. Nitrate uptake rates declined dramatically during the 6-week addition, from 0.454 μg N m^{-2} s^{-1} on day 0 (nearly equal to NH_4 uptake rate) to 0.066 μg N m^{-2} day^{-1} on day 41 (Table 3). Nitrate uptake efficiency (V_f) followed the same pattern.

The importance of NO_3 and NH_4 as an assimilatory source of N shifted during the experiment. Initially, 36% of assimilated N came from NH_4, with the remaining fraction assumed to be coming from NO_3. By day 41, ammonium supplied 78% of the total N assimilated by the stream.

Samples were collected and analysed for DO^{15}N analysis 1 day after the release ended. There was a detectable ^{15}N signal in the DON collected from downstream stations (Fig. 3d), and we calculated a DO^{15}N flux of 0.0318 μg ^{15}N s^{-1}.

Uptake rates in primary uptake compartments

The FBOM had the most rapid NH_4 uptake rate (9.0 mg N m^{-2} day^{-1}). Epilithon was highly labelled after only 3 days of ^{15}N addition and had an N uptake rate of 8.5 mg N m^{-2} day^{-1}. Leaves and small wood had low NH_4 uptake (0.78 and 0.23 mg N m^{-2} day^{-1}, respectively) (Table 4), and δ^{15}N values were generally low throughout the 42-day release. Although FBOM had a higher uptake rate than epilithon on a per m^2 basis, this was largely a result of its 10-fold higher standing stock. In contrast, when uptake rates are expressed as per gram AFDM, epilithon has a much higher uptake rate than any other primary uptake compartment (Table 4).

Table 3 Nitrogen dynamics calculated from DI^{15}N data collected on days 0, 20 and 41

	Units	Day 0	Day 20	Day 41
Physical and chemical discharge	$m^3\ s^{-1}$	0.0184	0.0118	0.0113
Parameters (whole stream averages)				
Depth	m	0.128	0.107	0.105
Velocity	$m\ s^{-1}$	0.030	0.026	0.025
NH_4	$\mu g\ N\ L^{-1}$	4.2	3.9	3.3
NO_3	$\mu g\ N\ L^{-1}$	141	139	164.7
NH_4 dynamics				
S_w	m	26.4	15.5*	15.5
Uptake rate	$\mu g\ m^{-2}\ s^{-1}$	0.611	0.700*	0.559
V_f ($\times 10^4$)	$m\ s^{-1}$	1.455	1.795*	1.694
Direct nitrification dynamics				
S_w	m	45.9	35.9	27.1
Uptake rate	$\mu g\ m^{-2}\ s^{-1}$	0.351	0.302	0.320
V_f ($\times 10^4$)	$m\ s^{-1}$	0.836	0.775	0.970
Assimilatory NH_4 dynamics				
Uptake rate	$\mu g\ m^{-2}\ s^{-1}$	0.260	0.398*	0.239
V_f ($\times 10^4$)	$m\ s^{-1}$	0.618	1.020*	0.723
NO_3 dynamics				
S_w	m	1192	1689	>3000
Uptake rate	$\mu g\ m^{-2}\ s^{-1}$	0.454	0.229	0.066
V_f ($\times 10^4$)	$m\ s^{-1}$	0.032	0.016	0.004
Nitrification/total NH_4 uptake	%	57.5	43.2*	57.3
Assimilatory N uptake from NH_4	%	36.4	63.5*	78.2
Assimilatory N uptake from NO_3	%	63.6	36.5*	21.8

*Several day 20 values are estimated by assuming NH_4 S_w and uptake rate are the same as on day 41.

The sum total N uptake rate for all primary uptake compartments is 18.5 mg N m^{-2} day^{-1}, which is less than the whole stream uptake rate calculated on day 0. If nitrification is included, however, total uptake is 48.9 mg N m^{-2} day^{-1}, much closer to the whole stream uptake rate of 52.8 mg N m^{-2} day^{-1} (Table 4).

Uptake by consumers

All of the consumers sampled were labelled with ^{15}N at the end of the 6-week tracer addition, indicating rapid incorporation of N at all trophic levels. The $\delta^{15}N$ values for *Leptophlebiidae* and small *Atya* (< 1 cm) closely followed the ^{15}N signal of epilithon throughout the experiment, indicating that epilithon is the major food source for these taxa. By the end of the tracer addition, the $\delta^{15}N$ signal in *Leptophlebiidae* and *Atya* surpassed the ^{15}N label in epilithon (Fig. 4b, c) which suggests that there is selective feeding or assimilation of a more highly labelled portion of the epilithon. A similar trend was observed for *Hydropsychidae* and its presumed food source, FBOM (Fig. 4), although *Hydropsychidae* may also be grazing epilithon directly. The larger of these consumers (i.e. the shrimp) did not reach a steady state ^{15}N concentration, and therefore we are limited in the conclusions we can make from these data.

Mass balance

On the last day of the ^{15}N addition, 17.9% of the total ^{15}N added during the experiment could be accounted for in the sampled food web compartments. Epilithon and FBOM accounted for 6.3 and 10.2% of the added ^{15}N, respectively. None of the other food web compartments individually contained more than 1% of the added ^{15}N (Table 5). The $^{15}NO_3$ flux out of the study reach over 42 days represented 50% of the tracer ^{15}N, while 7.7% of the tracer ^{15}N left the reach as DO^{15}N (based on postday 1 flux), and $^{15}NH_4$ flux accounted for only 1% of the added ^{15}N. The SPOM transport totalled < 1% of the added ^{15}N.

Major floods similar to the one that occurred on day 20 are common in streams of the LEF, and we expected

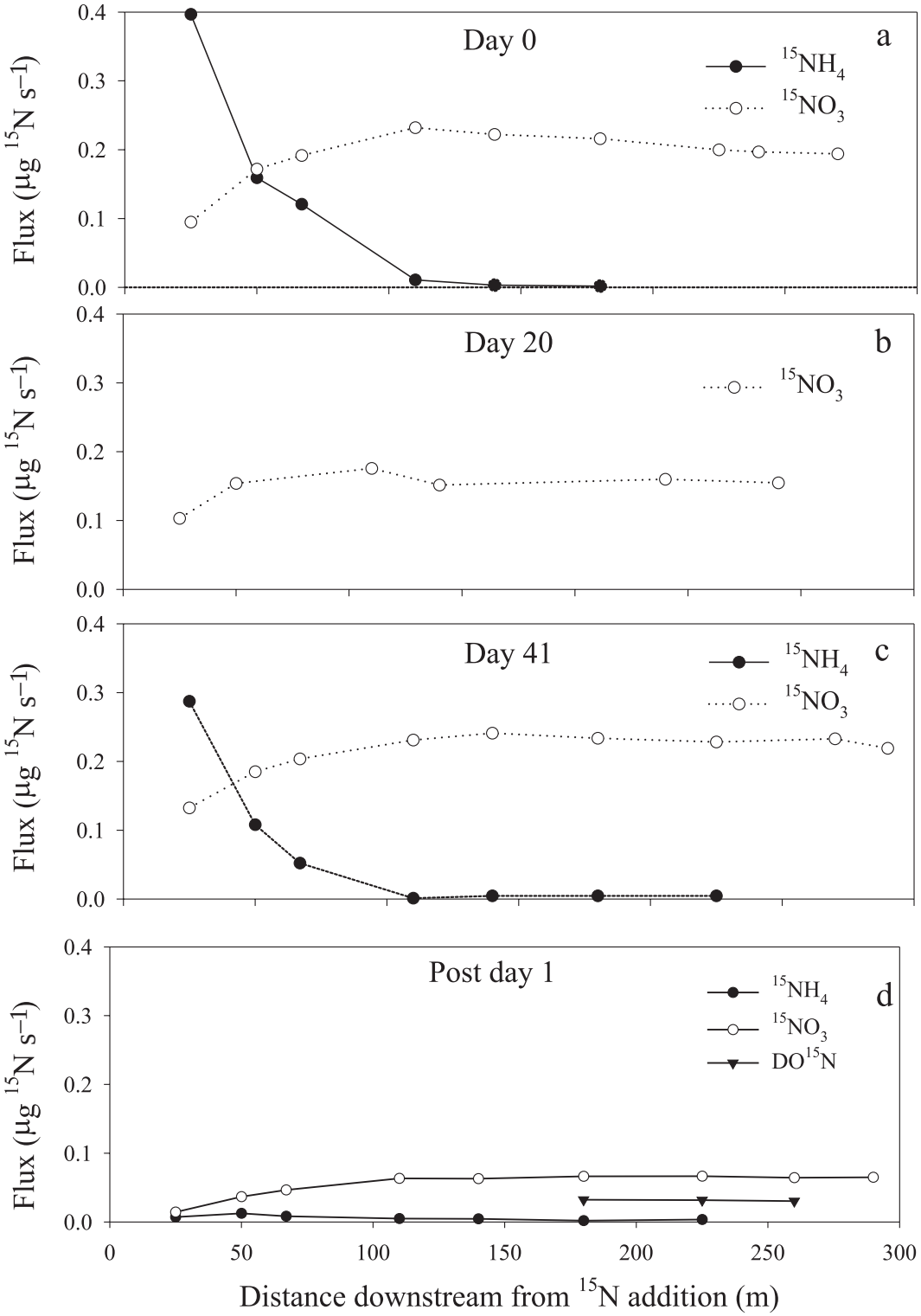

Fig. 3 Dissolved ^{15}N flux on 4 days during the experiment. Panels (a–c) are during the ^{15}N addition, and (d) is 1 day after ceasing the addition. ^{15}N labelled NH_4Cl was added at 0 m. Ammonium data for day 20 was lost. Days 20 and 41 flux values have been corrected for regeneration of $^{15}NH_4$ and $^{15}NO_3$, and Panel (d) shows regeneration of NH_4 from the stream bottom, and subsequent nitrification of the regenerated NH_4 (indirect nitrification) 1 day after the addition ended.

Table 4 Uptake rates for in-stream compartments calculated on day 7. All rates are corrected for turnover

Compartment	NH_4 uptake rate (mg N m^{-2} day^{-1})	NH_4 uptake rate (mg N g^{-1} AFDM day^{-1})
Epilithon	8.5	2.40
Leaves	0.78	0.13
Small wood	0.23	0.04
FBOM	9.0	0.28
Nitrification (day 0)	30.36	
Total of all compartments measured	48.9	
Whole stream uptake (day 0)	52.8 (38.8–66.8)*	

*95% Confidence limits.

that ^{15}N labelled detritus would be washed from our study reach and replaced by upstream and/or terrestrial sources. A simple mass balance model was applied to epilithon and detrital δ ^{15}N data from before and after the flood to estimate changes in ^{15}N retention resulting from such events using eqns 2 and 3.

$$\delta^{15}N_{\text{prestorm}} \times F_{\text{prestorm}} + \delta^{15}N_{\text{natural abundance}} \times F_{\text{natural abundance}} = \delta^{15}N_{\text{poststorm}} \quad (2)$$

$$F_{\text{natural abundance}} + F_{\text{prestorm}} = 1 \quad (3)$$

where $\delta^{15}N_{\text{prestorm}}$ is the ^{15}N label of the compartment prior to the storm, F_{prestorm} is the fraction of the poststorm ^{15}N signal supplied by the prestorm material, $\delta^{15}N_{\text{poststorm}}$ is the ^{15}N signal in the compartment after the storm and $F_{\text{natural abundance}}$ is the fraction of the poststorm ^{15}N signal supplied by unenriched material from upstream or terrestrial sources.

The model assumes that the standing stock within a given compartment remains constant before and after the storm. Buzby (1998) found storms had minimal effects on leaf and wood mass in Bisley. Although it seems likely that there would be some scouring of the epilithon, we assumed that this reduction was minimal, because there was very little epilithic material or filamentous algae prior to day 20. Results from the mass balance showed that the storm had a small effect on the ^{15}N retained in the primary uptake compartments, despite the dramatic rise in discharge (Table 6). There was some replacement of ^{15}N labelled leaves by unlabelled leaves and replacement was higher at 25 m than at 50 m (66 and 13%, respectively). Percent replacement of labelled FBOM was similar at both sites, with about one-third of the material being replaced. Epilithon showed similar replacement as FBOM, at about 33%. Using these calculations, we estimated that 5.2% of the tracer ^{15}N was washed out of the study reach during the day 20 storm. In sum, we could account for approximately 83% of the tracer ^{15}N added during the 6-week release, and more than 65% of total added was lost from the reach via downstream transport.

Nitrogen turnover

Decreasing $\delta^{15}N$ values in the food web samples collected after the ^{15}N addition stopped were used to calculate N turnover rates. Samples were collected weekly for the first 4 weeks after the addition, on postdays 7, 14, 21 and 28. We used the data from the 50-m station for turnover rate calculations for all compartments except epilithon, where we used data from the 25-m station (a more extensive data set). Our calculation assumes there is no re-uptake of ^{15}N released into the water column. Because re-uptake of ^{15}N is likely, this calculation is probably an underestimate of turnover time.

Because of the rapid decrease in $\delta^{15}N$ values after the release stopped, FBOM had the shortest turnover time of 7.5 days, followed by SPOM and leaves with turnover times of 10.5 and 11.9 days, respectively. Epilithon had the longest turnover time of 22.7 days (Table 7). We did not have enough postaddition wood samples to calculate a turnover time.

Consumers were sampled less frequently postaddition, because they were easily depleted. Samples were collected on postdays 7 and 28, and post 3 months. We cannot calculate turnover time, because ^{15}N label in consumers continued to increase after the addition ended (Fig. 5), and $\delta^{15}N$ values had not reached steady-state in any of the consumer compartments. This is probably the result of continued feeding on

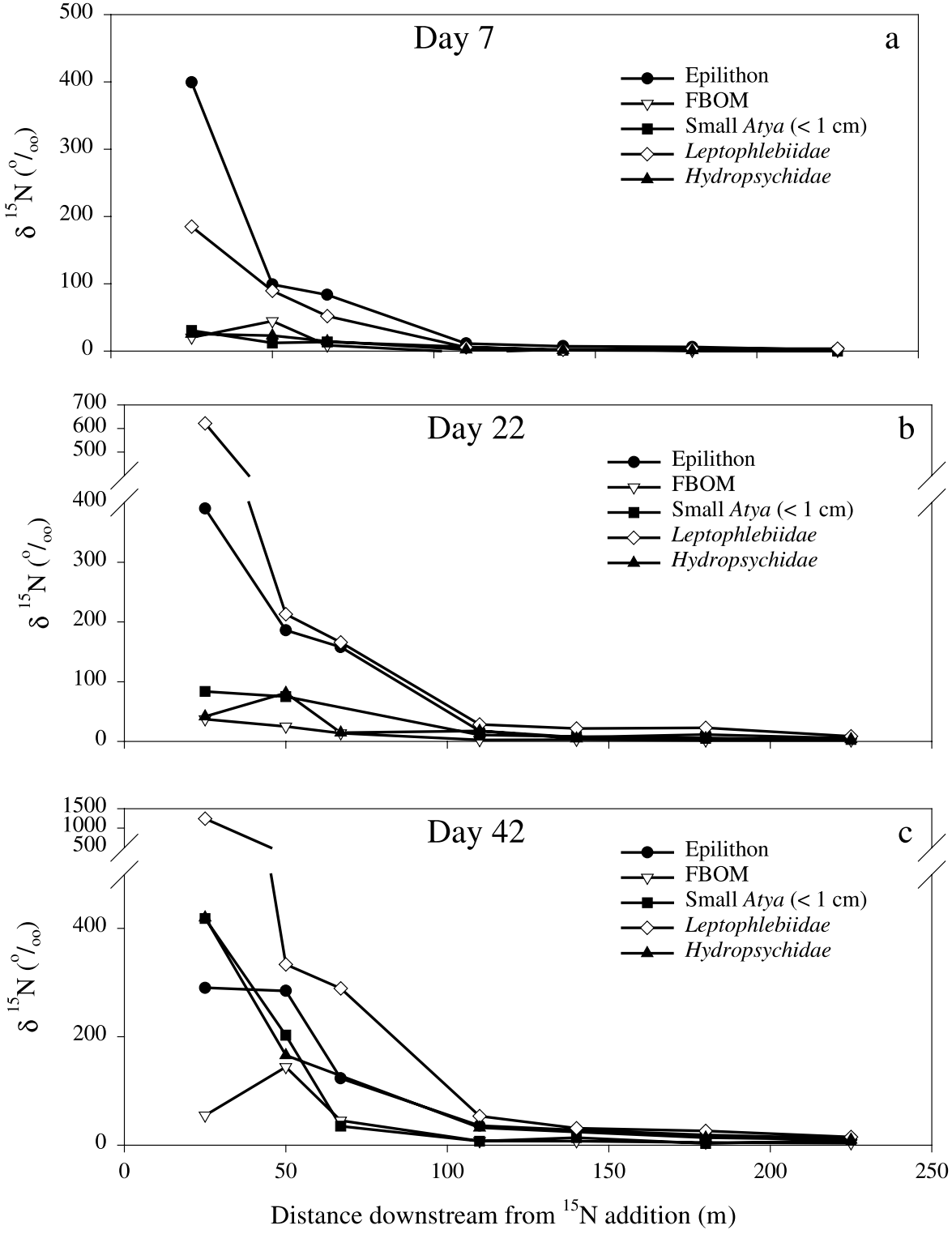

Fig. 4 The $\delta^{15}N$ signal in consumers and their probable food sources on 3 days during the experiment.

Table 5 Mass balance of added ^{15}N, with the amount of ^{15}N found in each compartment after 42 days, and estimated losses from the reach caused by transport

Compartment	Mg ^{15}N accounted for	Percentage of ^{15}N added
Epilithon	93.6	6.26
Leaves	5.4	0.36
Wood	2.8	0.19
FBOM	153.1	10.24
Leptophlebiidae	0.31	0.02
Hydropsychidae	0.01	0.00
Veliidae	0.00	0.00
Macrobrachium	2.84	0.19
Atya (<1 cm)	5.71	0.38
Xiphocaris	0.59	0.04
Epilobocera	1.27	0.09
NH_4 flux	17.78	1.19
NO_3 flux	751.2	50.2
DON flux	115.2	7.7
SPOM flux	12.23	0.82
Day 20 storm – detrital flux	77.99	5.21
Total	1260	82.89

labeled food resources. Postday 28, ^{15}N label in all consumers, except the large predatory shrimp *Macrobrachium*, had begun to decrease, although $\delta^{15}N$ values were often still higher than the respective day 42 value. Even after 3 months, larger consumers sampled from the upstream sites (most highly enriched) still had significant ^{15}N label. This would be expected, as shrimps can live long (>5 years, S. Johnson, unpublished data).

Discussion

Nitrogen dynamics and flux

Ammonium uptake rates in Bisley were similar to those reported in other ^{15}N tracer studies (Table 8), which ranged from 81.6 μg m^{-2} min^{-1} in Upper Ball Creek, TN (Mulholland *et al.* 2000) to 17.4 μg m^{-2} min^{-1} in Hugh White Creek, NC (Hall *et al.*, 1998). Nitrification rate in Bisley is higher than those published for the other LINX sites to date (Table 8). Mulholland *et al.* (2000) showed a nitrification rate of 4.1 μg N m^{-2} min^{-1} in Walker Branch, TN, with direct nitrification accounting for 20% of total NH_4 uptake on day 0. In contrast, Tank *et al.* (2000) found almost no nitrification in Upper Ball Creek, NC, with an average rate of 0.8 μg N m^{-2} min^{-1}. A higher rate of nitrification in Bisley is consistent with a higher water temperature and low light; factors that have been shown to increase nitrification (Warwick, 1986; Kopacek & Blazka, 1994). Direct nitrification averaged 57.4% of total NH_4 uptake, similar to the 50% estimate of export as $^{15}NO_3$ from the study reach. The $DO^{15}N$ was a significant export pathway for the added ^{15}N, but accounted for only 0.02% of the total DON concentration in stream water, indicating that DON in streamwater is allochthonous (presumably from terrestrial sources), or that the in-stream pathways by which $^{15}NH_4$ is converted to $DO^{15}N$ are very slow.

Nitrate uptake rate was high in Bisley but because of the high NO_3 concentration (129 μg N L^{-1}), uptake length was very long (Table 3). Once N was transformed to NO_3^-, it was essentially exported from the study reach. Overall, nitrate uptake rate in Bisley was higher than found in Walker Branch, TN or Upper Ball Creek, NC, although all were of the same order of magnitude (Table 8).

Ammonium uptake efficiency (V_f) increased on day 41, as did the assimilatory NH_4 uptake velocity (V_f). These increases could be the result of an increase in filamentous algae in the last few weeks of the tracer addition, possibly brought about by an extended period of low flow. Filamentous algae became visible in the stream and data from nutrient releasing substrata showed a twofold increase in chlorophyll *a* on GF/F filters (Tank *et al.*, unpublished data). The shift in the source of assimilated N during the

Table 6 Effect of day 20 storm on epilithon and detrital pools at the two upper sampling stations. Values are calculated from a mass balance model described in the text

	Percentage replacement from upstream or terrestrial sources			Percentage of ^{15}N retained			Mass ^{15}N lost (mg)
	25 m	50 m	Average	25 m	50 m	Average	
Leaves	66	13	40	34	87	60	1.82
FBOM	41	34	37	59	66	63	29.5
Epilithon	33		33	67		67	46.66

Table 7 Turnover times based on postaddition ^{15}N data, assuming no re-uptake of regenerated N. This assumption is probably not met, therefore these values are probably underestimates

Compartment	Turnover time (days)
Epilithon	22.7
Leaves	11.9
Small wood	Not calculated
FBOM	7.5
SPOM	10.5

experiment is driven by the order of magnitude decrease in NO_3 uptake.

Biomass uptake

Ammonium uptake rates expressed per unit area of stream bottom for primary uptake compartments were very high compared with other published ^{15}N tracer studies (Table 8), with the exception of leaves. Uptake by epilithon was approximately seven times higher and FBOM uptake was twofold greater than that found in Walker Branch, TN (Mulholland *et al.* 2000). Nitrogen uptake by leaves expressed per unit area of stream bottom was very low compared with previous ^{15}N tracer studies (Table 8). This is primarily because of the scarcity of leaves in Bisley. Nitrogen uptake rates per g AFDM of leaf material were much higher in Bisley compared with other published studies (Table 8) suggesting that the microbial N uptake associated with leaf material in Bisley is quite high. Overall, leaves never became highly labelled with ^{15}N, and this is possibly the result of rapid breakdown and transport of leaf material. Leaf breakdown and/or transport must occur on a continuous basis, as detrital pools remain constant over time (Buzby, 1998) and build up of leaf packs was not observed between storms during this experiment. Above-ground leaf litter fall in the Bisley watershed averages 470.9 g dry mass m^{-2} $year^{-1}$ (Lodge *et al.*, 1991). Decay rates of in-stream leaves (Vogt *et al.*, 1996; Buzby, 1998) are much slower than input rates. Without whole leaf transport, or breakdown and transport of subsequent CBOM and FBOM, in-stream leaf standing stocks would increase over time. We measured the in-stream standing stock of CBOM to be 14.8 g dry mass m^{-2} prior to the ^{15}N addition, which is approximately 11.5 days input of leaf litter. Leaf turnover time (calculated from postaddition ^{15}N data) was 11.9 days.

^{15}N mass balance

Approximately 18% of the ^{15}N added over the 42-day tracer addition was retained within the Bisley study reach, which was similar to retention in the other published LINX sites. Walker Branch, TN, and Upper Ball Creek, NC, had retention of 48 and 12% of added

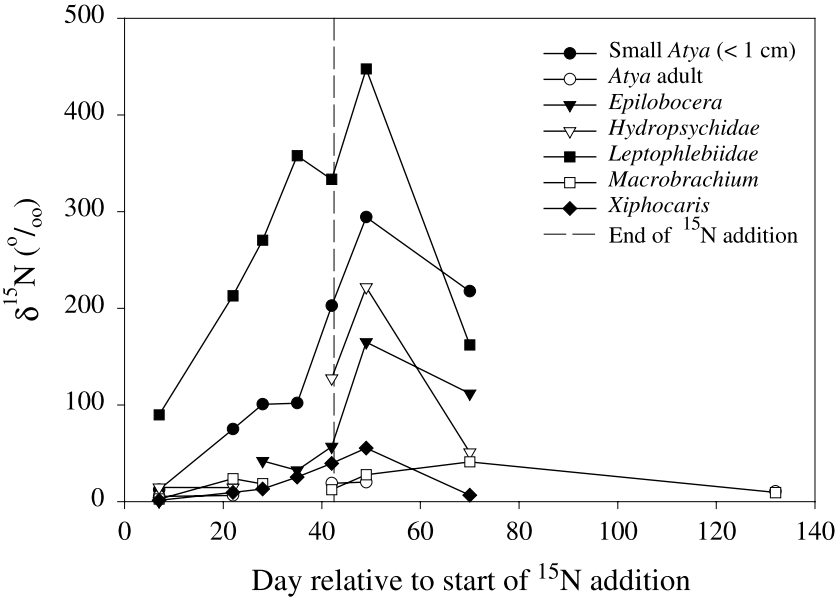

Fig. 5 The $\delta^{15}N$ signal in consumers at 50 m below the ^{15}N addition point. Note the increase in signal even after day 42 (the end of the ^{15}N addition).

Table 8 A comparison of retention and export from two other published ^{15}N tracer studies

	Units	Quebrada Bisley, PR	Walker Branch, TN*	Upper Ball Creek, NC†
Retained in biota	%	17.8	48	12.2
Lost via NO_3 export	%	50.2	23	6.7
Lost via NH_4 export	%	1.19	4	17.97
Lost via SPOM export	%	0.82	4	11.09
Sum of export losses (not including DON)	%	57.4‡	31	35.5
Lost via DON export	%	7.7		
Total ^{15}N accounted for	%	82.9	79	48.0
NH_4 uptake rate	$\mu g\ m^{-2}\ min^{-1}$	35.1§	27.3¶	81.6¶
Direct nitrification rate	$\mu g\ m^{-2}\ min^{-1}$	20.1§	4.10**	0.8¶
NO_3 uptake rate	$\mu g\ m^{-2}\ min^{-1}$	16.0¶	12.7¶	11.5††
Epilithon	$\mu g\ m^{-2}\ min^{-1}$	5.90‡‡	0.90‡‡	0.21‡‡
CBOM (leaves)	$\mu g\ m^{-2}\ min^{-1}$	0.54‡‡	2.92‡‡	2.44‡‡
FBOM	$\mu g\ m^{-2}\ min^{-1}$	6.25‡‡	2.85‡‡	1.60‡‡
Epilithon	$\mu g\ g^{-1}$ AFDM min^{-1}	1.67‡‡	0.24‡‡	0.16‡‡
CBOM (leaves)	$\mu g\ g^{-1}$ AFDM min^{-1}	0.09‡‡	0.04‡‡	0.04‡‡
FBOM	$\mu g\ g^{-1}$ AFDM min^{-1}	0.19‡‡	0.01‡‡	0.04‡‡

*From Mulholland *et al.* (2000).
†From Tank *et al.* (2000).
‡Includes export of detritus during the day 20 storm.
§An average of two dates.
¶An average of three dates.
**From day 0 only.
††An estimate from limited data, Tank *et al.* (unpublished data).
‡‡From day 7 only.

^{15}N (Mulholland *et al.*, 2000; Tank *et al.*, 2000, respectively). Export of ^{15}N from the study reach in Bisley was much higher than either of these streams (Table 8). The SPOM and dissolved inorganic nitrogen (DIN) export from Bisley accounted for 52.2% of the added ^{15}N, with the majority being exported as NO_3. In contrast, Walker Branch, TN, exported 31% of ^{15}N, with about three quarters leaving as NO_3, and Upper Ball Creek, NC, exported about 36% of the added ^{15}N with only one-fifth lost as NO_3.

Because 50% of NH_4 uptake is the result of nitrification (a process that provides energy to the microbes involved), energy limitation, rather than nitrogen limitation, of net primary production, appears to drive N dynamics in this stream. These data also support the contention of Matson *et al.* (1999) that anthropogenic N additions to tropical sites will result in rapid and large losses of N, largely because of the lack of N limitation to primary productivity (Vitousek & Sanford, 1986). These losses will be rapidly exported to the ocean, as in-stream retention is minimal.

We were able to account for about 83% of the added ^{15}N but, despite our high recovery, we recognize that several omitted food web compartments may represent the missing ^{15}N. As noted previously, filamentous algae were not sampled, although it did certainly take up ^{15}N. In addition, fish were not collected because sampling equipment was unavailable. Admittedly, the biomass of these compartments is small compared with the shrimp (F. Scatena, personal communication), but undoubtedly some of the missing ^{15}N would be found there. Additional ^{15}N may have been lost to downstream transport of ^{15}N labelled material during three smaller storms that occurred over our 6-week addition (Fig. 1), but we cannot accurately estimate the amount. Finally, a small amount of ^{15}N may have moved laterally out of the stream, into the riparian zone, through insect emergence, and/or feeding by terrestrial predators.

N retention and transport

Storms were frequent, yet they transported surprisingly small quantities of CBOM, FBOM and epilithon from upstream to downstream sites in our study reach. A careful examination of the detrital

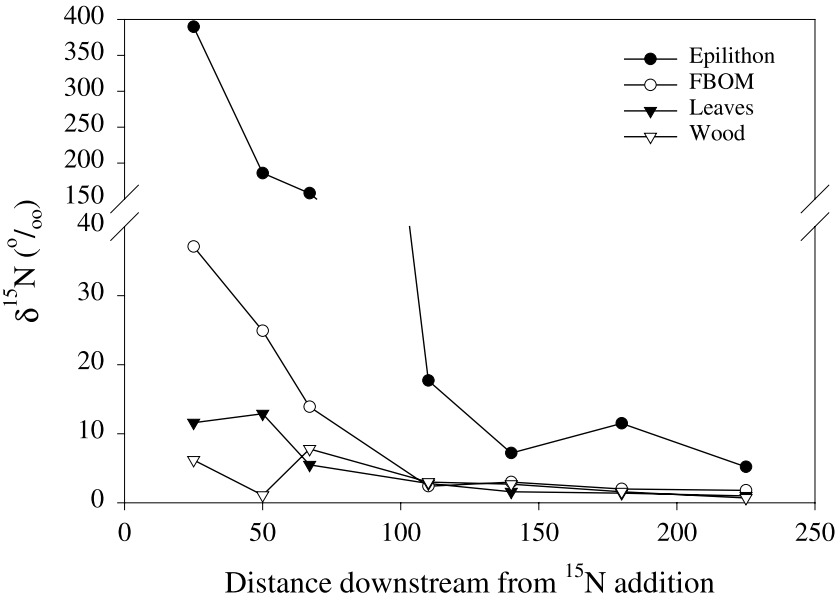

Fig. 6 The $\delta^{15}N$ signal in primary uptake pools on day 22, 1 day following a very intense flood.

and epilithic $\delta^{15}N$ values along the entire study reach before and after the day 20 storm indicates that material that was moved by a storm is transported a long distance (>220 m). There was no increase in $\delta^{15}N$ values of any compartment in the lower part of the study reach (Fig. 6), which would be expected if, for example, highly labelled FBOM from the 25-m station was deposited at the 180-m sampling site. Long travel distances of particulate detritus may be facilitated by the lack of large woody debris in Bisley, which probably minimizes debris dams and leaf packs typical of temperate forested streams.

In summary, ammonium uptake in Bisley was rapid, as demonstrated by the very short uptake lengths, and nitrification accounted for one-half of the ammonium uptake. Nitrification occurred immediately after the $^{15}NH_4$ was introduced to the stream, and very quickly following regeneration of $^{15}NH_4$ from stream biota. Despite the rapid uptake of $^{15}NH_4$ from the water column, retention of ^{15}N was low in our study reach. Fully 50% of the $^{15}NH_4$ loss is a result of nitrification and subsequent loss of ^{15}N from the stream reach as $^{15}NO_3$ flowing downstream. Although $^{15}NO_3$ was an important source of assimilatory N at the beginning of the tracer addition, high ambient nitrate concentrations result in very long uptake lengths, in the order of kilometres. There is clearly short-term retention (on the order of weeks) of ^{15}N in detrital pools and epilithon, but N cycles rapidly and turnover times are typically 3 weeks or less.

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