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Food resources of stream macroinvertebrates determined by natural-abundance stable C and N isotopes and a ^{15}N tracer addition

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Abstract. Trophic relationships were examined using natural-abundance ^{13}C and ^{15}N analyses and a ^{15}N-tracer addition experiment in Walker Branch, a 1st-order forested stream in eastern Tennessee. In the ^{15}N-tracer addition experiment, we added $^{15}NH_4$ to stream water over a 6-wk period in early spring, and measured ^{15}N:^{14}N ratios in different taxa and biomass compartments over distance and time. Samples collected from a station upstream from the ^{15}N addition provided data on natural-abundance ^{13}C:^{12}C and ^{15}N:^{14}N ratios. The natural-abundance ^{15}N analysis proved to be of limited value in identifying food resources of macroinvertebrates because ^{15}N values were not greatly different among food resources. In general, the natural-abundance stable isotope approach was most useful for determining whether epilithon or detritus were important food resources for organisms that may use both (e.g., the snail *Elimia clavaeformis*), and to provide corroborative evidence of food resources of taxa for which the ^{15}N tracer results were not definitive. The ^{15}N tracer results showed that the mayflies *Stenonema* spp. and *Baetis* spp. assimilated primarily epilithon, although *Baetis* appeared to assimilate a portion of the epilithon (e.g., algal cells) with more rapid N turnover than the bulk pool sampled. Although *Elimia* did not reach isotopic equilibrium during the tracer experiment, application of a N-turnover model to the field data suggested that it assimilated a combination of epilithon and detritus. The amphipod *Gammarus minus* appeared to depend mostly on fine benthic organic matter (FBOM), and the coleopteran *Anchytarsus bicolor* on epixylon. The caddisfly *Diplectrona modesta* appeared to assimilate primarily a fast N-turnover portion of the FBOM pool, and Simuliidae a fast N-turnover component of the suspended particulate organic matter pool rather than the bulk pool sampled. Together, the natural-abundance stable C and N isotope analyses and the experimental ^{15}N tracer approach proved to be very useful tools for identifying food resources in this stream ecosystem.

Key words: stream macroinvertebrates, food resources, N cycling, tracer addition, stable isotope, ^{15}N, ^{13}C.

Over the past 2 decades, stable isotope ratios have been used increasingly to identify food web linkages and organic matter sources in ecosystems. In particular, ^{13}C:^{12}C and ^{15}N:^{14}N ratios appear to be most useful for identifying trophic relationships (Peterson and Fry 1987, Kling 1994).

Stable isotope food web investigations are generally of 2 types. Natural-abundance stable isotope studies rely on variation in the isotope ratios of elements in plants from different environments or different photosynthetic pathways and relatively constant fractionation against the heavier isotope during assimilation at each higher trophic level. Isotope tracer addition experiments involve addition of elements highly enriched in the heavy isotope (e.g., ^{15}N) and detection of changes in isotope ratios of organic matter in response to tracer addition once isotopic equilibrium is achieved.

Both stable isotope approaches have been used to determine trophic relationships in streams. Rounick et al. (1982) used variations in natural abundance of stable C isotopes to show that allochthonous sources of organic C supported invertebrate production in forested

streams, whereas autochthonous C sources were more important food resources for invertebrates in streams in clearcut catchments. Similarly, Rosenfeld and Roff (1992) used natural-abundance ^{13}C measurements to show a dependence of invertebrates on autochthonous sources of organic matter in unforested and forested streams in spring in southern Ontario. Hamilton et al. (1992) used natural-abundance ^{13}C and ^{15}N measurements to determine that the predominant food resource for many invertebrates and fishes in the Orinoco River floodplain was microalgae rather than the more abundant and conspicuous aquatic vascular plants. Angradi (1994) used natural-abundance ^{13}C and ^{15}N measurements to show that there were 3 trophic levels in the lower Colorado River, with fish being supported by autochthonous production in 1 tributary but riparian or upland organic matter inputs in other tributaries. In a variation of the natural-abundance approach, Peterson et al. (1993) used shifts in the fractionation of N stable isotopes resulting from N fertilization to alter the stable isotope ratios of algae and, in turn, document the increased trophic importance of epilithic algae in the Kuparuk River, Alaska, food web after fertilization.

The use of stable isotope tracer addition experiments in food web studies is a more recent development. Hall (1995) added ^{13}C-labeled acetate to a forested headwater stream to identify the role of bacteria as a food resource for invertebrates. Peterson et al. (1997) used ^{15}N contents of insects during a $^{15}NH_4$ tracer addition experiment in the Kuparuk River to show the differences in food utilization between grazers and filter-feeders. Hall et al. (1998) used a similar $^{15}NH_4$ addition approach in Hugh White Creek, North Carolina, and found evidence for selective assimilation of microbial versus detrital N by several invertebrate detritivores. Hughes et al. (2000) added $^{15}NO_3$ to an oligohaline reach of the Parker River estuary in Massachusetts to determine the relative importance of planktonic and benthic primary producers and detritus derived from the surrounding marsh in fueling the food web. Last, Wollheim et al. (1999) showed how a mass balance model of N flow could be used with field ^{15}N tracer results to more clearly identify food web relationships.

In this study, we used both natural-abundance stable C and N isotope and experimental ^{15}N tracer addition approaches to identify food web relationships in Walker Branch, a 1st-order forested stream in eastern Tennessee. We show how this combination of stable isotope approaches provides additional and more definitive information on trophic relationships than either approach alone. This experiment was part of the Lotic Intersite Nitrogen Experiment (LINX), a large multi-site project on N cycling involving identical ^{15}N tracer additions to streams throughout the United States.

Study Site

The study was conducted during early spring 1997 in a 125-m reach of the West Fork of Walker Branch (hereafter referred to as Walker Branch), a 1st-order, forested stream in the Ridge and Valley geophysical province of eastern Tennessee (lat 35°58′N, long 84°17′W). The climate is typical of the humid Appalachian region of the southeastern USA, with a mean annual temperature of 14.5°C and mean annual precipitation of 140 cm. Although precipitation (mostly as rain) is distributed relatively evenly throughout the year, stream baseflow discharge is highly seasonal because of high rates of evapotranspiration during the growing season. The West Fork of Walker Branch drains a 38.4 ha catchment at the Oak Ridge National Environmental Research Park. The vegetation is dominated by oak (*Quercus* spp.), hickory (*Carya* spp.), red maple (*Acer rubrum*), and yellow-poplar (*Liriodendron tulipifera*). The catchment is underlain by dolomite, and the stream arises from several springs discharging water from the bedrock aquifer (Mulholland 1992). The stream gradient is ~0.035 m/m. Stream water is moderately alkaline (alkalinity of 2–3 meq/L) and the pH is slightly basic (usually 8.0–8.3).

At the time of the year when this study was conducted, primary production is at the annual maximum in Walker Branch, although total metabolism is dominated by heterotrophy with P:R ratios <0.5 (Marzolf et al. 1994, Mulholland et al. 2000). Invertebrate biomass is dominated by the pleurocerid snail *Elimia clavaeformis* (~95% of invertebrate biomass), although several insect and other macroinvertebrate taxa can be numerically abundant in some seasons (Newbold et al. 1983, J. G. Smith, ORNL, unpublished data).

Methods

Beginning on 1 April 1997 and continuing for 6 wk, a solution of ^{15}N-enriched NH_4Cl (10% ^{15}N) was pumped from a stream-side carboy into a constricted section of the stream to achieve rapid mixing. The ^{15}N addition was designed to achieve a 50% increase in the ^{15}N:^{14}N ratio in the dissolved NH_4 pool (based on an assumed discharge rate of 10 L/s and a NH_4 concentration of 3 μg N/L). The ^{15}N addition resulted in a very small increase in the concentration of NH_4 in stream water (~0.05 μg N/L). The ^{15}N input was maintained at a rate of ~0.20 mg ^{15}N/h throughout the 6-wk period, except for a 12 to 16 h period on 28 to 29 April when the pump malfunctioned and the supply of ^{15}N to the stream was interrupted. The actual ^{15}N addition rate was determined based on the volume remaining in the carboy when the $^{15}NH_4$ solution was replenished each week.

Samples of water, organisms, and different types of benthic organic matter were collected for ^{15}N measurements at a station ~10 m upstream from the ^{15}N input and used for natural-abundance analysis of ^{15}N and ^{13}C. A small waterfall (~0.5 m in height) 2 m upstream from the ^{15}N input prevented upstream movement of most organisms from the ^{15}N-enriched reach to the station used for natural-abundance analysis. Samples for ^{15}N were also collected at stations located at distances of 10 m, 25 m, 50 m, 75 m, and either 100 m or 125 m downstream from the ^{15}N addition at approximately weekly intervals during the addition (at about mid-morning on each sampling date). Because of the large number of samples collected and the high analytical costs, the samples at all stations were analyzed for ^{15}N only on the last sampling date (day 42) of the experiment. Samples collected at 25 m on each sampling date were analyzed for ^{15}N to provide a time course of ^{15}N content for each sample type. Some samples from other stations and dates were analyzed for ^{15}N to provide additional data for spatial and temporal analyses.

We collected epilithon, bryophytes, leaves, epixylon, fine benthic organic matter (FBOM, <1 mm), suspended particulate organic matter (SPOM), and macroinvertebrates at each station. Epilithon was scraped from the surface of 4 to 6 randomly chosen rocks at each station using a stiff-bristled brush and washed into a small volume of water. Epilithon slurries were filtered through precombusted glass fiber filters (Whatman GF/F) within 2 h of collection. Bryophytes were removed from rock surfaces at 4 to 6 locations at each station. The distal ends of each frond (~1 cm) were removed and only this material was used for ^{15}N analysis. Leaves and pieces of wood were randomly collected from 4 to 6 locations at each station. The outer surface of wood was removed by scraping, collected, and the remainder discarded. Thus, our ^{15}N values of wood represent epixylon (wood biofilm only). FBOM was collected by suctioning surface material from 4 to 6 areas of fine sediment accumulation at each station. FBOM slurries were filtered through precombusted glass fiber filters (Whatman GF/F) within 2 h of collection. One sample of SPOM was collected at each station by filtering 2 to 4 L of streamwater through precombusted glass fiber filters (Whatman GF/F). Samples of epilithon, bryophytes, leaves, epixylon, and FBOM collected from all areas at each station were combined to form 1 composite sample of each type for isotope analysis.

Approximately 20 to 30 *E. clavaeformis* (3–5 mm shell width) were collected randomly at each station, allowed to clear their guts overnight in the laboratory, and the soft tissue was removed after 30 s in a microwave oven. We collected other common macroinvertebrates (generally 5–20 individuals at each station) by kick sampling with a D-frame net positioned just downstream and by hand-picking organisms from rocks and detritus. Some macroinvertebrate taxa varied greatly in abundance over the experiment and we were able to collect individuals of these taxa only on some dates and at some stations. Organisms were held overnight in the laboratory to clear guts prior to further processing. Individuals of each taxon collected at each station were combined to form 1 composite sample.

All samples were dried at 60°C for at least 2 d and, with the exception of the samples on filters, were ground using a Wiley mill. Subsamples were analyzed for ^{13}C:^{12}C and ^{15}N:^{14}N ratios using a Europa Model 20/20 isotope ratio mass spectrometer at the Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts. All isotope ratios are expressed as either $\delta^{13}C$ or $\delta^{15}N$ values (units of ‰) according to the following equation:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R_{sample} is the ^{13}C:^{12}C or ^{15}N:^{14}N ratio of the sample and $R_{standard}$ is the ^{13}C:^{12}C or ^{15}N:^{14}N ratio of a standard (PeeDee belemnite carbonate for $\delta^{13}C$, atmospheric N for $\delta^{15}N$). Usually only 1 subsample of each homogenized sample per station per date was analyzed. Where replicate subsamples were analyzed, the mean of the replicate values was used. Analysis of replicates for 40 samples indicated a mean difference in $\delta^{15}N$ of 0.50‰, although 2/3 of the replicates differed by <0.5‰.

The $\delta^{13}C$ and $\delta^{15}N$ values of samples collected from the station upstream from the ^{15}N addition point were considered natural-abundance values and used in the natural-abundance analyses. In the experimental ^{15}N tracer analyses, all $\delta^{15}N$ values for samples collected at stations downstream from the ^{15}N addition point were corrected for natural-abundance levels of ^{15}N by subtracting the $\delta^{15}N$ value of similar samples collected upstream from the ^{15}N addition. Thus, all $\delta^{15}N$ values determined for the ^{15}N tracer experiment at stations downstream from the ^{15}N input represent only the tracer ^{15}N content of the sample (i.e., they do not include naturally occurring ^{15}N).

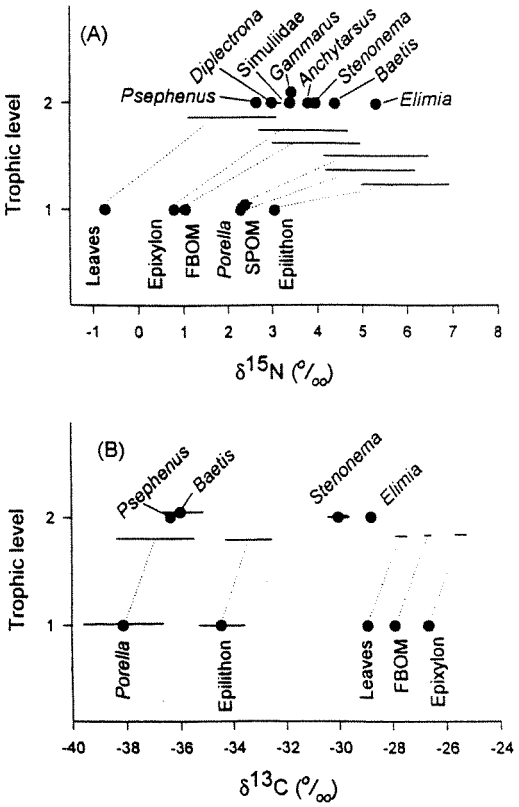

FIG. 1. Natural-abundance $\delta^{15}N$ (A) and $\delta^{13}C$ values (B) of primary consumer taxa and their potential food resources in Walker Branch. Expected ranges in consumer $\delta^{15}N$ and $\delta^{13}C$ values based on consumption of each individual food resource are indicated by the horizontal lines below the consumers. These lines reflect the mean and SD of the isotope ratios for that food resource, and trophic isotopic enrichments of +2 to +4‰ for ^{15}N and +1‰ for ^{13}C (for organisms relative to their food indicated by the dotted lines). Horizontal lines through data points indicate 1 SD (where large enough to be seen, except for epixylon, *Porella pinnata*, *Baetis* spp., and *Anchytarsus bicolor*, for which only 1 sample was analyzed). FBOM = fine benthic organic matter, SPOM = suspended particulate organic matter.

Results

Natural-abundance ^{15}N and ^{13}C analysis

The natural-abundance ^{15}N analysis, based on an expected trophic ^{15}N enrichment (i.e., increase in $\delta^{15}N$ values of an organism compared with its food) of +2 to +4‰ (Ehleringer et al. 1986, Peterson and Fry 1987, Keough et al. 1996), provided only a limited amount of definitive information (Fig. 1A). Only epilithon and leaves had $\delta^{15}N$ values that differed by more than 3‰, so it was difficult to discriminate among other potential food resources. Our results suggested that epilithon may be the primary food resource for *E. clavaeformis* but not for other taxa. Among the other presumed scrapers, *Baetis* spp. and *Stenonema* spp. appeared to be assimilating at least some detritus (probably FBOM) because their natural-abundance $\delta^{15}N$ values were lower than would be expected from a diet solely of epilithon. The natural-abundance $\delta^{15}N$ values for the liverwort *Porella pinnata* and epilithon were very close, and thus *Porella* could not be ruled out as a possible food resource for *Elimia* and the mayflies as well.

Leaves had the lowest natural-abundance $\delta^{15}N$ values of all potential food resources and did not appear to be a primary resource for any consumer taxa. The amphipod shredder *Gammarus minus* and the coleopteran shredder *Anchytarsus bicolor* had natural-abundance $\delta^{15}N$ val-

ues consistent with a diet primarily of FBOM or epixylon rather than leaves.

The natural-abundance ^{15}N analysis showed several surprises. Natural-abundance $\delta^{15}N$ values for the coleopteran scraper *Psephenus herricki* were considerably lower than those expected from a diet of either epilithon or FBOM, as constituted in bulk samples of these resources. Simuliidae had considerably lower natural-abundance $\delta^{15}N$ values than expected from a diet primarily of the bulk SPOM pool, the presumed food resource for these filterers. The natural-abundance $\delta^{15}N$ values for SPOM and FBOM differed by 1.4‰, suggesting that SPOM was not simply the result of entrainment of the FBOM pool that we sampled (surface material that could be readily suctioned off the bottom). These data suggested that SPOM included substantial amounts of a more highly ^{15}N-enriched pool, such as the epilithon.

The natural-abundance ^{13}C analysis was primarily focused on determining whether the dominant scraper taxa were dependent on epilithon or detritus. Based on this analysis, *Elimia* and *Stenonema* appeared to use a combination of detritus and epilithon, although the natural-abundance $\delta^{13}C$ values of *Baetis* and *Psephenus* were lower than expected from a diet of epilithon, detritus, or a combination of both (Fig. 1B). The data are consistent with a diet consisting primarily of *Porella* for these taxa, although *Psephenus* was usually found only on the undersides of rocks.

^{15}N tracer addition experiment

Data from the ^{15}N tracer addition experiment provided more definitive information on food web relationships than did the natural-abundance stable C and N isotope analyses. If organisms of a specific taxon were feeding primarily on a particular food resource and if their body N turned over at relatively rapid rates (on the order of several days), then we expect their tracer $\delta^{15}N$ values to generally follow the tracer $\delta^{15}N$ values of their food over time and space. Early in the experiment, organisms would likely have tracer $\delta^{15}N$ values considerably lower than their food if their N pools had not reached isotopic equilibrium. Later in the experiment, organisms should have tracer $\delta^{15}N$ values closer to their food resources, unless their N turnover rates are quite slow (weeks or greater). Thus, tracer $\delta^{15}N$ values of small taxa with rapid growth rates (e.g., mayflies, simuliids) would be expected to correspond closely with the tracer $\delta^{15}N$ values of their food resources, but tracer $\delta^{15}N$ values of larger taxa or those with low growth rates (e.g., *Elimia*) may not.

The time course tracer $\delta^{15}N$ profiles at 25 m (Fig. 2A) and the longitudinal $\delta^{15}N$ profiles on day 42 (Fig. 2B) suggested that *Stenonema* spp. and *Baetis* spp. depended on epilithon as their primary food resource, although similarity in the $\delta^{15}N$ profiles for epilithon and *Porella* did not rule out the latter as a food resource for these organisms. The generally higher tracer $\delta^{15}N$ values for *Baetis* than epilithon over time and space also suggested that this taxon assimilated a portion of epilithon that acquires NH_4 from water more rapidly than the bulk epilithon sampled (e.g., actively growing algal or bacterial cells). The generally lower tracer $\delta^{15}N$ values for *Stenonema* than epilithon over time at 25 m (Fig. 2A) suggested that this organism may also consume some detritus, although this pattern was not observed in the longitudinal profile (Fig. 2B). Surprisingly, the temporal and longitudinal tracer $\delta^{15}N$ profiles for *Elimia* and *Psephenus* followed those of leaves and FBOM more closely than of epilithon, the presumed food resource for these scrapers (Fig. 2A,B). However, *Elimia* is a relatively large organism (average mass of nearly 1 mg ash-free dry mass [AFDM] per individual for the samples collected) with a relatively long life cycle. Thus, tracer $\delta^{15}N$ levels for *Elimia* may have lagged well behind those of its food because of its slow N turnover rate.

The ratio of tracer $\delta^{15}N$ in an organism to tracer $\delta^{15}N$ in its food resource at the same time and station should be equal to 1.0 if the organism N is in isotopic equilibrium with its food (the effect of trophic fractionation is eliminated because we consider only the tracer ^{15}N by subtracting background values as described in the methods). Organism:epilithon tracer $\delta^{15}N$ ratios for scrapers over time suggested that only *Stenonema* was at isotopic equilibrium with respect to epilithon by the end of the 42-d experiment (Fig. 3A). *Stenonema*:epilithon tracer $\delta^{15}N$ ratios appear to converge on ~1.0 over time, suggesting that bulk epilithon was the primary food resource for this taxon. *Baetis*:epilithon tracer $\delta^{15}N$ ratios >1 later in the experiment suggested that *Baetis* assimilated a more highly ^{15}N-labeled portion of the epilithon (e.g., actively growing

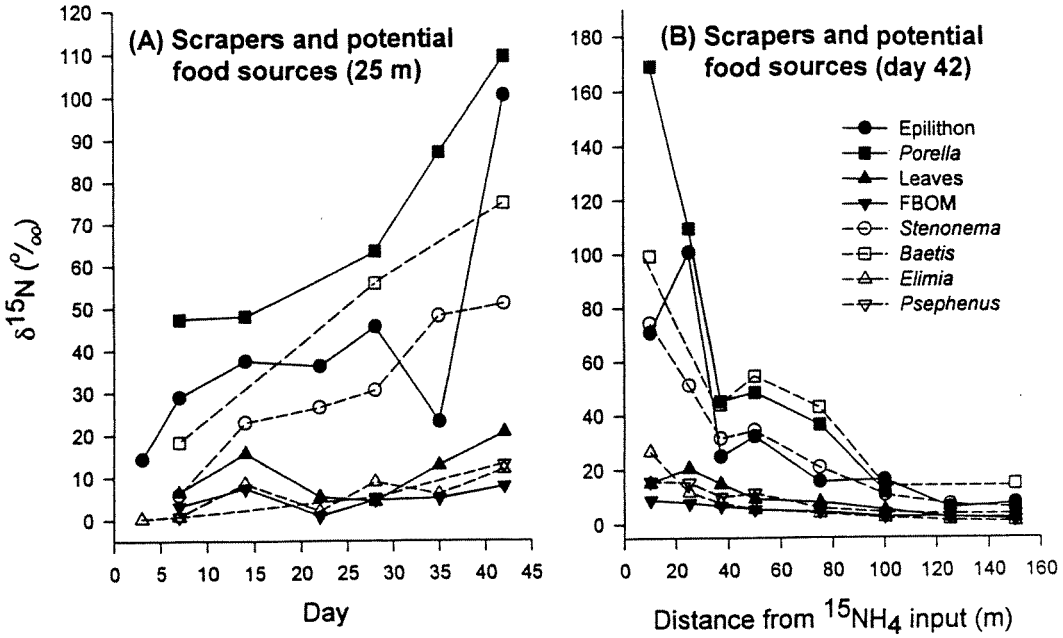

FIG. 2. Time course profiles at the 25 m station (A) and longitudinal profiles on day 42 (B) of tracer $\delta^{15}N$ values for scrapers and their potential food resources. Time course data were available for *Baetis* spp. and *Psephenus herricki* for days 7, 28, and 42 only. The day 42 value for *Baetis* is the average between the stations at 10 m and 50 m because the sample from 25 m was lost. Each data point represents only 1 subsample of composited material and has an uncertainty of approximately ±0.5‰ (see methods).

algal cells on the epilithon surface) than the bulk pool sampled (Fig. 3B). It was not clear whether the time-course trend in *Elimia*:epilithon tracer $\delta^{15}N$ ratio was approaching an asymptote by the end of the experiment because the values remained relatively low throughout the study (Fig. 3C). If so, this would suggest that *Elimia* fed primarily on a food resource (e.g., detritus) that was much less highly ^{15}N-labeled than the epilithon. Time course *Psephenus*:epilithon tracer $\delta^{15}N$ ratios suggested that this organism had not reached isotopic equilibrium with respect to epilithon by the end of the experiment (Fig. 3D).

A model of tracer $\delta^{15}N$ in *Elimia* was developed based on a N turnover rate for this taxon (0.003/d) determined from previous measurements of NH_4 excretion and N content (Mulholland et al. 1991, P. J. Mulholland, unpublished data) and the tracer $\delta^{15}N$ values of potential food resources measured during this study (Fig. 4). The model predicted the tracer $\delta^{15}N$ of *Elimia* over time at the 25 m station assuming assimilation of epilithon, leaves, and FBOM separately. Comparison of model predictions with actual measurements of *Elimia* tracer $\delta^{15}N$ suggested that this organism relied on a combination of epilithon and detritus, particularly later in the experiment. Because leaf detritus was relatively scarce at this time of year (spring), our results probably reflected assimilation of epilithon and FBOM, roughly in equal proportions.

Among presumed detritivore taxa, the temporal tracer $\delta^{15}N$ profile for *G. minus* indicated that this taxon was at or near isotopic equilibrium by the end of the experiment and that its primary food resource was more likely FBOM than leaves (Fig. 5A). The sharp declines in $\delta^{15}N$ of FBOM and leaves on day 22 at 25 m was the result of the storm 2 d earlier, which transported more highly enriched materials downstream and deposited unlabeled materials from upstream and the channel margins in the experimental reach. The $\delta^{15}N$ of leaves and FBOM increased again in the period following the storm as the newly deposited material became labeled. The longitudinal tracer $\delta^{15}N$ profiles for *Gammarus* were in better agreement with those of epixylon than FBOM or leaves (Fig. 5B). *Gammarus*:detritus tracer $\delta^{15}N$ ratios suggested that

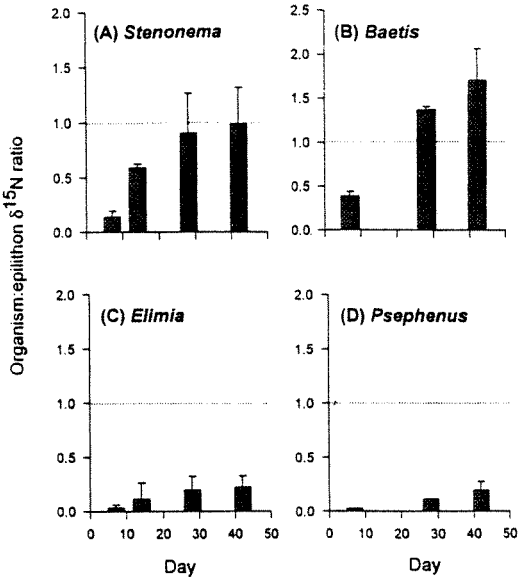

FIG. 3. Organism:epilithon $\delta^{15}N$ ratios for scrapers over time during the ^{15}N tracer experiment. Histogram heights represent means of values from all sampling stations on a particular date for which epilithon tracer $\delta^{15}N$ values were >5‰ (n = 2–5 per date). Vertical error bars represent 1 SD.

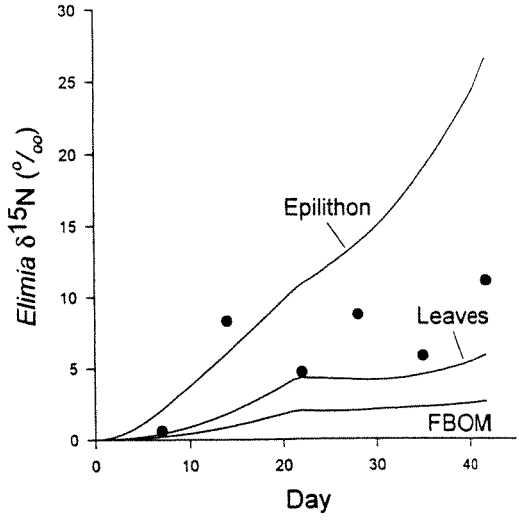

FIG. 4. Values of $\delta^{15}N$ for *Elimia clavaeformis* at the 25 m station over time. Solid lines are model simulations based on consumption of single food resources (epilithon, leaves, fine benthic organic matter [FBOM]) and points are measured values.

FBOM was the most likely primary food resource for this taxon and that it appeared to be at approximate isotopic equilibrium with FBOM by the end of the experiment, although variation was large reducing the certainty of this assessment (Fig. 6A,B).

The longitudinal $\delta^{15}N$ profile for *A. bicolor* was more similar to that for epixylon than for other detritus pools (Fig. 5B) and the *Anchytarsus*:food $\delta^{15}N$ ratio at the end of the experiment was closer to 1 for the case of epixylon than leaves (Fig. 6C,D). Merritt and Cummins (1996) indicated that larvae in this family are long-lived (up to 3 y), however, and it is likely that the tracer $\delta^{15}N$ values for this organism would lag well behind those of its food (organism food ratios <1 throughout the experiment). Unfortunately, we have no information on N turnover rate in *Anchytarsus* and cannot evaluate its food resources using a model similar to that used for *Elimia*.

The longitudinal tracer $\delta^{15}N$ profile for the Simuliidae was distinctly hump-shaped and similar to that for SPOM with maxima observed at 25 to 40 m (Fig. 7). The hump-shaped longitudinal profile in SPOM $\delta^{15}N$ is the result of gradual deposition of unlabeled SPOM from upstream, generation of highly labeled SPOM in the upper part of the experimental reach via resuspension of FBOM and sloughing of epilithon or direct labeling of SPOM, and gradual replacement of highly labeled SPOM by entrained FBOM and epilithon farther downstream. Among the potential food resources, only SPOM clearly had this longitudinal profile shape. Further, Simuliidae tracer $\delta^{15}N$ values were considerably more enriched than SPOM throughout the study reach, suggesting assimilation of a portion of the SPOM that takes up NH_4 from water more rapidly than the bulk material (e.g., attached microbes, algal cells).

The values and shape of the longitudinal tracer $\delta^{15}N$ profile for the caddisfly *Diplectrona modesta* were intermediate between those for SPOM and FBOM (Fig. 7). Our results suggested that either *Diplectrona* used a combination of FBOM and SPOM, or that it assimilated a portion of the FBOM pool that cycled N more rapidly than the bulk material as constituted in our samples.

Discussion

Comparison of approaches

Each of the 2 types of analysis used to infer food web relationships (natural-abundance sta-

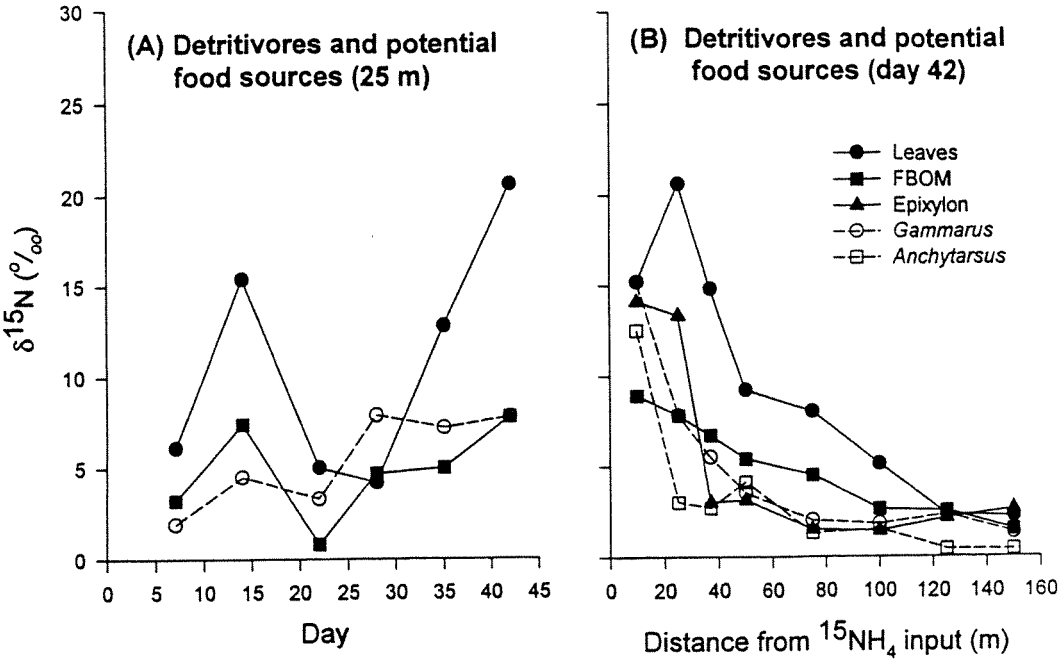

FIG. 5. Time course profiles at the 25 m station (A) and longitudinal profiles on day 42 (B) of tracer $\delta^{15}N$ values for the detritivores *Gammarus minus* and *Anchytarsus bicolor* (longitudinal profile only) and their potential food resources. Each data point represents only 1 subsample of composited material and has an uncertainty of approximately ±0.5‰ (see methods).

ble C and N analysis and ^{15}N tracer experiments) has limitations, and the combination of approaches provided a more complete picture than either alone. Natural-abundance stable isotope analysis relies on substantial separation between potential food resources (generally at least 3–4‰) and this was true only for epilithon and leaves for $\delta^{15}N$, and epilithon and all detritus compartments for $\delta^{13}C$. Further, trophic enrichment in natural-abundance $\delta^{15}N$ values is variable, depending on an organism's age, size, and nutritional status; values as low as +2 and as high as +4‰ have been reported in different studies (Minagawa and Wada 1984, Ehleringer et al. 1986, Peterson and Fry 1987, Keough et al. 1996). Last, an organism may assimilate a portion of the food resource that has a different stable isotope ratio than the bulk material sampled (e.g., bacterial or algal cells that have different $\delta^{15}N$ or $\delta^{13}C$ values than the material as a whole). The strengths of the natural-abundance stable isotope approach are that it integrates over time and that organisms are likely to be at or near isotopic equilibrium with respect to their food resources, assuming there have been no recent shifts in physical or chemical conditions that alter the isotopic content or relative abundance of food resources.

The natural-abundance ^{15}N and ^{13}C analyses taken together appeared to show that *E. claviformis* and *Stenonema* spp. assimilated a mixture of epilithon and detritus (more likely FBOM than leaves). These analyses also showed that *Baetis* spp. assimilated either a more highly ^{15}N-enriched portion of the epilithon or a mixture of epilithon and *Porella*. There was disagreement in the natural-abundance analyses as to food resources for *P. herricki*, with the ^{15}N analysis suggesting detritus and the ^{13}C analysis a mixture of epilithon and *Porella*. The natural-abundance ^{15}N analysis also suggested that *G. minus* and *A. bicolor* fed mostly on FBOM and/or epixylon rather than on leaves. *Gammarus* is known to shred leaves and was commonly found in leaf accumulations in Walker Branch; however, our analysis suggested that leaves were not its primary food resource. Our results for *Anchytarsus* were in agreement with Merritt and Cummins (1996), who indicated that rotting wood is its primary food resource.

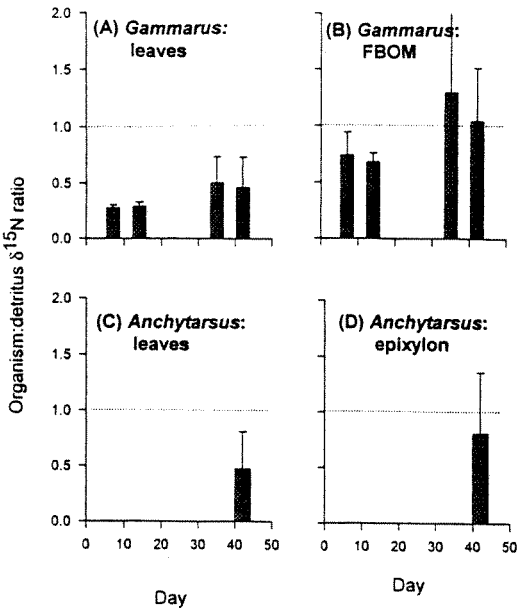

FIG. 6. Organism:detritus $\delta^{15}N$ ratios for detritivores over time during the ^{15}N tracer experiment. Histogram heights represent means of values from all sampling stations on a particular date for which detritus tracer $\delta^{15}N$ values were >5‰ (n = 2–4 per date). Vertical error bars represent 1 SD. Only day 42 values were determined for *Anchytarsus*.

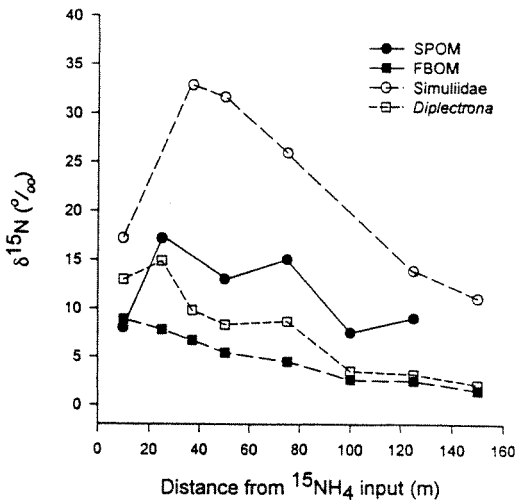

FIG. 7. Longitudinal profiles (day 42) of tracer $\delta^{15}N$ values for the collector/filterers Simuliidae and *Diplectrona modesta* and their potential food resources (suspended particulate organic matter [SPOM], fine benthic organic matter [FBOM]). Time series samples were not collected for these taxa. Each data point represents only 1 subsample of composited material and has an uncertainty of approximately ±0.5‰ (see methods).

The strength of the experimental ^{15}N tracer approach for determining food web relationships is that there is likely to be greater separation in isotope ratios between potential food resources. Food resources of primary consumers can acquire different amounts of tracer ^{15}N as a result of differential use of the various forms of dissolved N in water (NH_4, NO_3, dissolved organic N), only 1 of which (NH_4) was labeled with tracer ^{15}N. Different food resources also may have different levels of tracer ^{15}N because the proportion of actively cycling N may differ. For example, the much lower tracer $\delta^{15}N$ levels of detritus compared with epilithon and *Porella* during the latter portions of our tracer experiment likely reflected the large pool of N of terrestrial origin within detritus. Another strength of the tracer ^{15}N approach is that it can sometimes identify when a food resource is exploited by a consumer in a manner different from the way in which it was sampled. For example, epilithon usually consists of many layers of living and dead algal cells as well as excreted mucilage and adsorbed organic matter (Lock et al. 1984). Tracer ^{15}N is primarily incorporated into the living components of the epilithon (and recently excreted organic compounds), which are likely to be concentrated at or near the upper surface. Thus, tracer ^{15}N is not likely to be distributed homogeneously within the epilithon matrix, and scrapers that remove and assimilate primarily living cells in the upper layers would become more highly labeled than the bulk material sampled.

Herbivores

Our experimental ^{15}N tracer analyses suggested that *Stenonema* spp. and *Baetis* spp. depend primarily on epilithon as their primary resource, although as with the natural-abundance analysis, our tracer results do not rule out the possibility that *Baetis* may also consume *Porella*. Results from a ^{13}C tracer experiment in Cold Spring, North Carolina (Hall 1995), and ^{15}N tracer experiments in Hugh White Creek, North Carolina (Hall et al. 1998), Upper Ball Creek, North Carolina (J. L. Tank and co-workers, unpublished data), and the Kuparuk River, Alaska (Peterson et al. 1997, Wollheim et al. 1999) have

also shown that these taxa feed primarily on epilithon. *Stenonema* in Cold Spring and Upper Ball Creek and *Baetis* in the Kuparuk River reached tracer $\delta^{15}N$ levels that were substantially greater than those for the bulk epilithon by the end of the respective experiments, suggesting assimilation of a more metabolically active, fast N-turnover pool (e.g., living algal or bacterial cells) within the bulk epilithon. In the Kuparuk River study, Wollheim et al. (1999) showed that when the epilithon were separated into fast N-turnover (diatoms) and slow N-turnover (detritus) pools, the longitudinal $\delta^{15}N$ profile for *Baetis* agreed more closely with that of the fast N-turnover epilithon pool than the bulk epilithon. In Walker Branch, there was evidence for assimilation of fast-turnover N in the epilithon by *Baetis* but not by *Stenonema* (Fig. 2). Our results suggested that *Baetis* may feed primarily on the surface of the epilithon whereas *Stenonema* appears to consume the bulk epilithon material in Walker Branch.

Differences in the way *Stenonema* exploits the epilithon as a food resource in different streams may be related to the quality of the epilithon. In heavily shaded streams, such as Cold Spring and Upper Ball Creek, much of the epilithon may be detrital material and *Stenonema* may preferentially consume the more nutritious component of the epilithon (e.g., algal or bacterial cells). In Walker Branch where light levels during the period of this experiment (early spring) were relatively high (12–15 mol quanta m^{-2} d^{-1}, Mulholland et al. 2000), the bulk epilithon appeared to be of higher quality, as indicated by considerably higher chlorophyll:AFDM ratios (3.2×10^{-3}, Mulholland et al. 2000), compared with Upper Ball Creek (0.47×10^{-3}, J. L. Tank, unpublished data). Thus, the epilithon may have been used more completely as a food resource by *Stenonema* in Walker Branch.

Detritivores

The tracer ^{15}N results for *G. minus* and *A. bicolor* were largely consistent with the natural-abundance ^{15}N results, suggesting that *Gammarus* feeds primarily on FBOM and *Anchytarsus* on epixylon, at least in early spring. *Gammarus* has been described as a collector-gatherer in Walker Branch (Newbold et al. 1983), but we found it mostly in leaf packs and thought, a priori, that it might be primarily a shredder. *Gammarus* sometimes may function as a shredder in Walker Branch, but our results suggested that it relied on FBOM as its primary food resource in spring.

For *D. modesta*, the natural-abundance and experimental ^{15}N tracer analyses together suggested that FBOM was its primary food, although neither analysis alone was very clear. Merritt and Cummins (1996) classified this taxon as both a collector-gatherer and filterer. Assuming that FBOM was the primary food of *Diplectrona*, the experimental ^{15}N tracer analysis suggested that this taxon may digest and assimilate disproportionately the microbes associated with FBOM (or selectively consume portions of the FBOM that are higher in microbial cells) because its tracer $\delta^{15}N$ values during the experiment were consistently higher than those for bulk FBOM.

The experimental ^{15}N tracer analysis also suggested that the presumed scraper *P. herricki* (Merritt and Cummins 1996) is actually a detritivore, in agreement with the natural-abundance ^{15}N analysis (but not the ^{13}C analysis). In a stream in middle Tennessee, Smith (1978) found that detritus was a considerable proportion of the diet of *Psephenus*, based on analysis of stomach contents. Alternatively, *Psephenus* may feed on epilithon, and our ^{15}N tracer results failed to show this relationship because this organism may have a low N turnover rate, despite its small size. *Psephenus* has a relatively long life cycle in Walker Branch (~2 y, J. G. Smith, personal communication), and the time course tracer $\delta^{15}N$ values for *Psephenus* also suggested that it may not have been in isotopic equilibrium with its food resource by the end of the experiment.

Filterers

The experimental ^{15}N tracer analysis proved considerably more informative than the natural-abundance ^{15}N analysis for the food resources of Simuliidae. The natural-abundance ^{15}N analysis suggested that FBOM might be a food resource for black flies, but not SPOM. The ^{15}N tracer experiment, however, clearly showed that SPOM was the primary food resource for these organisms, and that they assimilated a portion of the SPOM pool that was more highly labeled with tracer ^{15}N (e.g., free-living or particle surface microbes, algal cells sloughed from the epi-

lithon) than the bulk pool. Black fly larvae consume a disproportionate share of bacteria in the seston load of several streams (Edwards and Meyer 1987, Hershey et al. 1996). Mulholland et al. (2000) focused on N cycling and showed that microbes associated with benthic detritus had tracer $\delta^{15}N$ values 2–4 times higher than the bulk detrital material, and it is likely that this was true of SPOM as well.

Use of modeling with the tracer ^{15}N approach

A weakness of the tracer ^{15}N approach is that the $\delta^{15}N$ values of consumers will lag behind those of their food, with the lag period being a function of the rate of N turnover within the consumer. Thus, the experimental ^{15}N tracer approach is most useful for determining food resources of consumers that have high N turnover rates (on the order of several days). We believe that this was the case at least for *Stenonema* and *Baetis*, based on time course plots of organism: food tracer $\delta^{15}N$ ratios (Fig. 3A,B), and for *G. minus*, based on its time course tracer $\delta^{15}N$ values (Fig. 5A). The tracer ^{15}N approach can also be very useful for organisms with slower N turnover rates if N turnover rate is known or can be estimated, and a model of expected $\delta^{15}N$ over time developed for assimilation of each potential food resource (Wollheim et al. 1999). We were able to show that *Elimia* assimilated a mixture of epilithon and detritus, probably mostly FBOM, using this approach (Fig. 4). This result was consistent with the natural-abundance ^{13}C analysis, but conflicted with the natural-abundance ^{15}N analysis, which suggested that *Elimia* assimilated primarily epilithon. Previous studies have shown that *Elimia* feeds on epilithon, FBOM, and leaves (Mulholland et al. 1985a, Rosemond et al. 1993), and its primary diet may depend on which of these resources is most plentiful at a particular time. The importance of epilithon as a food resource for *Elimia* may be greater in the spring when light levels are highest and algal productivity is greatest (Marzolf et al. 1994), whereas the importance of leaves is likely greater in late autumn when they are most abundant (Mulholland et al. 1985b).

Bryophytes as a food resource

Our ^{15}N results did not rule out the possibility that *P. pinnata* may be a food resource for some macroinvertebrates in Walker Branch because the natural-abundance and tracer $\delta^{15}N$ values for *Porella* and epilithon were similar. The similarity in the tracer $\delta^{15}N$ values did not appear to be the result of epiphytes because the $\delta^{15}N$ values for *Porella* were determined on entire fronds and the large amount of N in *Porella* tissues (3.7% of dry mass, Mulholland et al. 2000) would preclude high tracer $\delta^{15}N$ values for the bulk material if only epiphytes were labeled. We have observed no physical evidence of feeding on *Porella* and suspect that, despite its high abundance (Steinman and Boston 1993) and importance in uptake of NH_4 from water (Mulholland et al. 2000), it is not a major food resource for macroinvertebrates in Walker Branch. However, it is intriguing that the natural-abundance ^{13}C analysis suggested that *Psephenus* and *Baetis* may consume *Porella*. It is possible that *Baetis* may consume a component of the epilithon that is more similar in ^{13}C content to *Porella* than to the bulk epilithon pool, as suggested by the ^{15}N tracer results. Nonetheless, the possible role of *Porella* as a food resource in Walker Branch and elsewhere deserves further study.

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Literature Cited

ANGRADI, T. R. 1994. Trophic linkages in the lower Colorado River: multiple stable isotope evidence.

Journal of the North American Benthological Society 13:479–498.

EDWARDS, R. T., AND J. L. MEYER. 1987. Bacteria as a food source for black fly larvae in a blackwater river. Journal of the North American Benthological Society 6:241–250.

EHLERINGER, J. R., P. W. RUNDEL, AND K. A. NAGY. 1986. Stable isotopes in physiological ecology and food web research. Trends in Ecology and Evolution 1:42–45.

HALL, R. O. 1995. Use of a stable carbon isotope addition to trace bacterial carbon through a stream food web. Journal of the North American Benthological Society 14:269–277.

HALL, R. O., B. J. PETERSON, AND J. L. MEYER. 1998. Testing a nitrogen-cycling model of a forest stream by using a nitrogen-15 tracer addition. Ecosystems 1:283–298.

HAMILTON, S. K., W. M. LEWIS, AND S. J. SIPPEL. 1992. Energy sources for aquatic animals in the Orinoco River floodplain: evidence from stable isotopes. Oecologia 89:324–330.

HERSHEY, A. E., R. W. MERRITT, M. C. MILLER, AND J. S. MCCREA. 1996. Organic matter processing by larval black flies in a temperate woodland stream. Oikos 75:524–532.

HUGHES, J. E., L. A. DEEGAN, B. J. PETERSON, R. M. HOLMES, AND B. FRY. 2000. Nitrogen flow through the food web in the oligohaline zone of a New England estuary. Ecology 81:433–452.

KEOUGH, J. R., M. E. SIERSZEN, AND C. A. HAGLEY. 1996. Analysis of a Lake Superior coastal food web with stable isotope techniques. Limnology and Oceanography 41:136–146.

KLING, G. W. 1994. Ecosystem-scale experiments: the use of stable isotopes in freshwaters. Pages 91–120 *in* L. A. Baker (editor). Environmental chemistry of lakes and reservoirs. Advances in Chemistry. American Chemical Society, Washington, DC.

LOCK, M. A., R. R. WALLACE, J. W. COSTERTON, R. M. VENTULLO, AND S. E. CHARLTON. 1984. River epilithon: toward a structural-functional model. Oikos 42:10–22.

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

MERRITT, R. W., AND K. W. CUMMINS. 1996. An introduction to the aquatic insects of North America. 3rd edition. Kendall/Hunt, Dubuque, Iowa.

MINAGAWA, M., AND E. WADA. 1984. Stepwise enrichment of 15N along food chains: further evidence of the relation between d15N and animal age. Geochimica et Cosmochimica Acta 48:1135–1140.

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

MULHOLLAND, P. J., J. W. ELWOOD, J. D. NEWBOLD, AND L. A. FERREN. 1985a. Effect of a leaf-shredding invertebrate on organic matter dynamics and phosphorus spiralling in heterotrophic laboratory streams. Oecologia 66:199–206.

MULHOLLAND, P. J., J. W. ELWOOD, J. D. NEWBOLD, L. A. FERREN, AND J. R. WEBSTER. 1985b. Phosphorus spiralling in a woodland stream: seasonal variations. Ecology 66:1012–1023.

MULHOLLAND, P. J., A. D. STEINMAN, A. V. PALUMBO, J. W. ELWOOD, AND D. B. KIRSCHTEL. 1991. Role of nutrient cycling and herbivory in regulating periphyton communities in laboratory streams. Ecology 72:966–982.

MULHOLLAND, P. J., J. L. TANK, D. M. SANZONE, W. M. WOLLHEIM, B. J. PETERSON, J. R. WEBSTER, AND J. L. MEYER. 2000. Nitrogen cycling in a forest stream determined by a ^{15}N tracer addition. Ecological Monographs (in press).

NEWBOLD, J. D., J. W. ELWOOD, R. V. O'NEILL, AND A. L. SHELDON. 1983. Phosphorus dynamics in a woodland stream ecosystem: a study of nutrient spiralling. Ecology 64:1249–1265.

PETERSON, B. J., M. BAHR, AND G. W. KLING. 1997. A tracer investigation of nitrogen cycling in a pristine tundra river. Canadian Journal of Fisheries and Aquatic Sciences 54:2361–2367.

PETERSON, B. J., AND B. FRY. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293–320.

PETERSON, B. J., B. FRY, L. DEEGAN, AND A. HERSHEY. 1993. The trophic significance of epilithic algal production in a fertilized tundra river ecosystem. Limnology and Oceanography 38:872–878.

ROSEMOND, A. D., P. J. MULHOLLAND, AND J. W. ELWOOD. 1993. Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. Ecology 74:1264–1280.

ROSENFELD, J. S., AND J. C. ROFF. 1992. Examination of the carbon base in southern Ontario streams using stable isotopes. Journal of the North American Benthological Society 11:1–10.

ROUNICK, J. S., M. J. WINTERBOURN, AND G. L. LYON. 1982. Differential utilization of allochthonous and autochthonous inputs by aquatic invertebrates in some New Zealand streams: a stable carbon isotope study. Oikos 39:191–198.

SMITH, J. G. 1978. The life histories, production rates and feeding habits of selected benthic macroinvertebrates of Spring Creek, Overton County, Tennessee. MS Thesis, Tennessee Technological University, Cookeville, Tennessee.

STEINMAN, A. D., AND H. L. BOSTON. 1993. The ecological role of aquatic bryophytes in a woodland

stream. Journal of the North American Benthological Society 12:17–26.

WOLLHEIM, W. M., B. J. PETERSON, L. A. DEEGAN, M. BAHR, J. E. HOBBIE, D. JONES, W. B. BOWDEN, A. E. HERSHEY, G. K. KLING, AND M. C. MILLER. 1999. A coupled field and modeling approach for the analysis of nitrogen cycling in streams. Journal of the North American Benthological Society 18:199–219.

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