The role of instream vs allochthonous N in stream food webs: modeling the results of an isotope addition experiment

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Abstract. Stable isotope enrichment experiments offer a potentially powerful way to examine the base of aquatic food webs, but interpretation of the data from these experiments may be confounded by problems such as selective ingestion/assimilation of bulk food sources by consumers, variable tracer enrichment over time, and the failure of consumers to approach isotopic equilibrium with the tracer in their diets over the course of the experiment. Our study examined data from a stable isotope addition experiment in which ^{15}N-labeled NH_4^+ was added to a midwestern US stream. A compartment model was used to provide insights into the kinetics of ^{15}N uptake and release from algae, heterotrophic microbes colonizing detritus, and invertebrate consumers. The model accounted for temporal variation in the degree of isotopic enrichment and did not require the assumption of isotopic equilibrium between consumers and their diets. The importance of instream production (i.e., growth of algae and microbes within the study reach during the experiment) relative to allochthonous and upstream inputs was ~38 to 50% for heptageniids and *Psephenus*, 10 to 20% for *Orconectes propinquus*, *Gammarus*, hydropsychids, and larval *Stenelmis*, and <10% for the filter-feeding *Similium* and the unionid mussel *Pleurobema sintoxia*. The alternative choices of algae or heterotrophic microbes as the basis of consumer diets made little difference in these estimates, even though the microbes became more ^{15}N-enriched than the algae because microbes had higher turnover rates. These results were subject to a number of caveats, and guidelines for experimental design are suggested for future studies to help address some of these problems.

Key words: nitrogen, ^{15}N, streams, stable isotopes, tracer experiments, food webs.

The relative roles of various sources of organic matter as the bases of stream food webs are

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difficult to ascertain. Streams commonly receive substantial allochthonous inputs of detrital organic matter, but they vary greatly in their potential 1° production, often because shade from riparian vegetation limits light (Allan 1995). Most streams are heterotrophic in their overall metabolism; i.e., the ratio of photosynthesis to respiration (P:R) is <1 (Mulholland et al. 2001). However, invertebrate consumers frequently are reliant on algal production, suggesting that much heterotrophic microbial production is not consumed by the invertebrates that support higher trophic levels, including fishes. Many common invertebrate consumers in woodland streams are specialized for processing leaf litter. However, the relative nutritional importance of litter vs subsequent heterotrophic microbial production on that litter is not clear (Cummins and

Klug 1979). Riparian land use and nutrient pollution frequently alter the rates of instream production and allochthonous inputs of organic matter in streams. Thus, investigation of the roles that these basal sources of organic matter play in supporting food webs has important implications for management, as well as theoretical interest.

Stable isotope addition experiments are widely used as tools for studying the sources of organic matter that support food webs, particularly in flowing waters (e.g., Peterson et al. 1997, Hall and Meyer 1998, Dodds et al. 2000, Hughes et al. 2000, Mulholland et al. 2000a, b, Tank et al. 2000). In these experiments, a compound enriched in the heavy isotope of an element (^{15}N or ^{13}C) is added continuously to the stream for several weeks to provide a tracer of elemental flow through the food web without significantly altering nutrient availability or rates of nutrient uptake and biological production. Algae, detritus colonized by bacteria and fungi, invertebrates, and other organisms are sampled downstream of the addition point during and after the isotope tracer addition. The accumulation and eventual disappearance (depuration) of the isotope tracer in the biotic compartments provides information on assimilation pathways, elemental turnover rates, and foodweb structure. Stable isotope addition experiments offer a powerful way to study aquatic food webs, particularly in ecosystems where natural abundances of stable isotopes do not permit separation of autochthonous sources of biological production from allochthonous inputs of organic matter.

Several problems confound interpretation of the results of stable isotope tracer addition studies, and some of these problems also are important in studies of natural isotope abundance (Gannes et al. 1997). One problem is that the actual food sources for organisms that collect, scrape, or filter fine or coarse particulate organic matter usually cannot be sampled directly; instead, samples typically consist of bulk material that is likely to include a mixture of edible and inedible components in unknown proportions (Hamilton and Lewis 1992, Hall et al. 1998). Consumers often become more isotopically enriched than their presumed food sources during tracer experiments (e.g., Dodds et al. 2000, Mulholland et al. 2000a, Tank et al. 2000), revealing that these animals are capable of selectively in-

gesting or assimilating the actively cycling fraction of the bulk organic matter. This fraction presumably is the living component (e.g., algae and microbes) rather than the refractory detritus. Another problem is that the consumer must be close to isotopic steady state (equilibrium) with respect to its diet before the isotopic enrichment of a consumer can be compared with that of its potential food at a particular point in time. Isotopic equilibrium between consumers and their diets generally is assumed to exist in studies of natural abundances of stable isotopes, even though changing ecological conditions may change the isotopic composition of the diets of consumers (e.g., O'Reilly et al. 2002). However, it is difficult to determine whether isotopic equilibrium has been reached in isotope addition experiments, especially when the degree of isotopic enrichment varies over time. Variable enrichment often occurs in stream tracer experiments because changing discharge and ambient nutrient concentrations cause the isotopic enrichment to vary even if the rate of isotope addition is kept constant. Variable enrichment also may result from spiking the system periodically with isotopically enriched material, as sometimes is done in mesocosm experiments.

Wollheim et al. (1999) described a steady state box model that predicted the spatial and temporal distribution of tracer ^{15}N in organisms and detritus in streams during isotope addition studies. The model has been used to generate predictions that could be compared with observed patterns of ^{15}N enrichment (Hall et al. 1998, Dodds et al. 2000). The model has successfully predicted some aspects of ^{15}N distributions, but significant disagreements between model predictions and observations have been observed, presumably because of the problems mentioned above.

Our experiment was part of a larger study called the Lotic Intersite Nitrogen eXperiment (LINX). This project entailed similar experiments at 9 sites in diverse biomes across North America. In our experiment, $^{15}NH_4^+$ was added to a low-gradient midwestern US stream to study the food web and to trace biogeochemical transformations. Hamilton et al. (2001) presented a complete description of the experiment and study site and an analysis of stream N biogeochemistry, and Raikow and Hamilton (2001) analyzed the diets of an assemblage of mussels in the stream. In our present study, ^{15}N was used

as a tracer of energy and nutrient flow from potential basal food sources into 1° consumers. The objective was to determine the relative importance of allochthonous inputs of organic matter, instream production by algae, and instream production by heterotrophic microbes as basal sources that support the stream food web. A compartment model was used to provide insights into the kinetics of ^{15}N uptake and release by biotic compartments at the base of the food web and at the level of the 1° consumer. This approach accounted for temporal variation in the degree of isotopic enrichment and addressed problems regarding consumer diets and isotopic equilibrium. The results indicated the relative importance of instream production compared to allochthonous and upstream inputs for a variety of invertebrate consumers, but they also pointed to new questions about the interpretation of data from experiments using isotopic tracer additions.

Study Site

Site description

Eagle Creek, a 2nd-order tributary of the Kalamazoo River, is located in southwestern Michigan between the cities of Kalamazoo and Battle Creek. Eagle Creek exits a small reservoir (Eagle Lake) ~1200 m above the study reach and passes through small beaver ponds and wetlands filled with emergent marsh vegetation before entering the study reach, where the channel has no riparian wetland and is lined by deciduous trees and shrubs. The stream channel in the study reach averages 5 m wide and 0.2 m deep and has a longitudinal gradient of ~0.25%. The stream bottom is largely covered by coarse sand, gravel, and some cobbles (54%, 35%, and 10%, respectively, of the total area in the study reach). Fine particulate organic matter and leaf and wood fragments accumulate in depositional areas along the edges, and organic matter is mixed with the sand in variable proportions. Eagle Creek water is alkaline with relatively low concentrations of available nutrients.

Conditions during the experiment

The experiment was done during June and July 1998 when the discharge of Eagle Creek was stable at ~200 L/s. Discharge increased about 2-fold after the largest storm (Hamilton et al. 2001). The mean concentrations of NH_4^+-N, NO_3^--N, and soluble reactive P during the experiment were 16 μg/L, 17 μg/L, and 3 μg/L, respectively. Hamilton et al. (2001) presented several lines of evidence showing that algae and microbes took up NH_4^+ in preference to NO_3^-. Assays using nutrient-diffusing substrata indicated that algal growth probably was limited by light rather than by nutrients because of shading by the forest canopy during the experiment (Hamilton et al. 2001). Whole-stream, 24-h respiration exceeded gross 1° production by ~8-fold, and epilithic microalgae were the only significant aquatic autotrophs in the stream; the reach-weighted mean chlorophyll a density in epilithon was 7.2 mg/m². Chlorophyll a concentrations in the stream water were measured on several occasions and ranged from 2.2 to 3.3 μg/L; presumably, this chlorophyll originated largely as algae exported from upstream wetlands or the reservoir, or from algae sloughed off the stream bottom. The ratio of organic C to chlorophyll a in suspended material was 571, indicating that algae made up a minority of the total suspended C.

Methods

The crux of the LINX experiments was a continuous 6-wk tracer $^{15}NH_4^+$ addition to the stream water, with intensive sampling during and after the addition to track the fluxes of tracer ^{15}N through foodweb compartments. Methods used in the Eagle Creek experiment were standardized across the LINX sites and were fully described in Mulholland et al. (2000b) and Hamilton et al. (2001). Seven sampling stations were distributed throughout the study reach (116, 176, 251, 301, 351, 396, and 461 m downstream of the ^{15}N addition point). A reference site ~10 m upstream of the addition point was sampled to provide information on background δ15N in all foodweb compartments. Background δ15N values did not increase over the course of the experiment, and no evidence was found for the appearance of tracer ^{15}N at the reference site.

N standing stocks in detrital and living organic matter

Biomass of detrital material and living organisms involved in N uptake and cycling was es-

timated during the 2 wk prior to the start of the ^{15}N addition. Biomass as ash-free dry mass (AFDM) was determined by combustion of subsamples at 500°C for 4 h. Biomass for unionids did not include shells. Standing stocks of organic matter were sampled using a stratified random approach to yield area-weighted mean densities for the entire study reach. Sampling of potential basal food sources for aquatic consumers (epilithon on cobble, fine particulate organic matter, leaves, and small woody debris) was described in Hamilton et al. (2001). Benthic invertebrates were collected using a Surber sampler (0.1 m²) at 35 points across the study reach. Invertebrates were collected from coarse woody debris by picking the organisms by hand from known areas of wood surfaces. Unionid bivalves (Raikow and Hamilton 2001) and crayfish were sampled by collecting individuals within several m² of stream bottom at several points across the reach. Crayfish density was estimated by sampling juveniles in 1998 and 1999 and from information on the relative abundance of adults and juveniles at nearby Augusta Creek during the summer (Creed 1994). Standing stocks of fine benthic organic matter (FBOM) were estimated by collection of core samples; this sampling was repeated using improved methods 1 y later (July 1999), and those data are used here (Hamilton et al. 2001).

Addition of tracer ^{15}N

The tracer ^{15}N addition was designed to enrich the δ^{15}N of the dissolved NH_4^+ in the stream by ~500‰, based on the stream discharge and NH_4^+ concentration at the start of the addition. A peristaltic pump dripped a solution of $^{15}NH_4Cl$ (10 atom %) into the stream continuously at 2 mL/min to achieve the target rate of 506 mg ^{15}N/d. Several wooden planks were staked in the channel immediately downstream of the dripper to deflect the flow towards the center of the stream and to enhance cross-channel mixing. The efficacy of mixing was verified during a preliminary Br^- addition by measuring Br^- concentrations along cross-channel transects at several points downstream of the addition site. The $^{15}NH_4Cl$ addition began on 16 June 1998 (day 0) and ended on 28 July (day 42). The pump flow rate was monitored daily throughout the addition, and the rate of addition was adjusted if necessary. The mean

δ^{15}N in NH_4^+ (δ^{15}N-NH_4^+) across the reach over the course of the 42-d isotope addition was calculated from the daily discharge, ambient NH_4^+ concentration, rate of ^{15}N addition, and the downstream rate of decrease in δ^{15}N-NH_4^+ that was measured on days 0, 20, and 41 (Hamilton et al. 2001).

Sample collection for δ^{15}N in organic matter

Consumers and their potential food sources were sampled weekly for δ^{15}N during the tracer ^{15}N addition and for 1 mo after the addition ended. The consumers were selected to represent the most abundant organisms in the stream and to span a range of functional feeding groups. Multiple individuals of small macroinvertebrate species were collected from each sampling station on each sampling date, and individuals were combined by taxon into composite samples, which were processed and analyzed. Several mussels and crayfish were sampled at only 1 or 2 points along the reach on most dates, so replication across sites was low for these taxa. Mussels and crayfish were processed and analyzed individually.

Whole-body samples were analyzed for δ^{15}N for all taxa except mussels and adult crayfish, which were large enough to allow dissection of muscle and digestive gland tissue for analysis, providing a comparison between tissues with different turnover rates. Animals were allowed to clear their guts overnight when whole-body samples were to be analyzed. Samples of organic matter, organisms, and tissues were dried at 50°C and ground to a fine powder before subsampling for isotopic analysis.

Stable isotope measurements

Stable N isotope analyses were done at the Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts, or the Center for Environmental Science and Technology at the University of Notre Dame, South Bend, Indiana. Both laboratories used automated analytical systems in which the isotope ratio mass spectrometers were coupled to high-temperature combustion columns and cryogenic separation units. Samples analyzed at both labs showed good agreement (i.e., within 1–2‰). Stable isotope measurements were expressed as δ^{15}N (in units of ‰) according to the equation:

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

where $R = {}^{15}N:{}^{14}N$ ratio, and the N isotope standard was air. All $\delta^{15}N$ values reported hereafter reflect tracer ^{15}N because background $\delta^{15}N$ measurements made at the upstream reference site were subtracted from $\delta^{15}N$ measurements made in the enriched reach (Peterson et al. 1997).

Data analysis and modeling

N turnover in potential basal food sources.— Hamilton et al. (2001) analyzed the observed ^{15}N accumulation in primary uptake compartments during the experiment. Measures of algal and microbial biomass (C, chlorophyll *a*, ergosterol, and direct bacterial cell counts) showed that only a small proportion of the N in the samples of epilithon, leaves, and FBOM was actively cycling. The actively cycling fractions of total N were 23% for epilithon, 1% for FBOM, and 7% for leaves, and these proportions could be accounted for by algal, fungal, and bacterial biomass. The algae, fungi, and bacteria that derived their N by uptake of NH_4^+ from the stream water were regarded as instream producers for the purpose of modeling ^{15}N uptake and transfer into the food web, even though heterotrophic microbes may have derived energy (C) from allochthonous organic C while assimilating all or part of their inorganic N from the water.

Turnover rate constants of tracer ^{15}N in specific biomass compartments were determined by Hamilton et al. (2001) from the decline in biomass $\delta^{15}N$ (^{15}N depuration) at the 116 m station over the first 28 d after the end of the ^{15}N addition by assuming 1st-order dynamics (i.e., from the slope of the linear relationship between $\ln(\delta^{15}N_{biomass})$ and time). This slope reflected the turnover rate constant of the actively cycling N; the turnover rate constant of N in the whole detritus/organism aggregate was much slower. Turnover rate constants can be determined in this way only for 1° uptake compartments (e.g., algae and microbes that assimilate $^{15}NH_4^+$ directly from the stream water) because consumers continue to assimilate tracer ^{15}N in their food after the isotope addition ceases as long as their food sources remain enriched. Hamilton et al. (2001) estimated that tracer ^{15}N turnover rate constants were $0.075 \pm 0.007/d$ (mean $\pm$ SE) for algae in epilithon, $0.22 \pm 0.01/$ d for heterotrophic microbes in leaves, and $0.14 \pm 0.02/d$ for heterotrophic microbes in FBOM. Based on measures of the microbial biomass, fungi were likely to be the primary cause of uptake and release of tracer ^{15}N from leaves, while bacteria were likely to be the primary cause in the FBOM (Findlay et al. 2002); nonliving detrital organic matter was assumed to play a negligible role in $^{15}NH_4^+$ uptake.

Model formulation.—A model was formulated to simulate the flow of tracer ^{15}N between potential food sources and consumers. The model was designed to interpret measured changes in tracer $\delta^{15}N$ in 1° consumers over time to reveal the relative importance of aquatic and terrestrial production to food webs. The theoretical trajectory of tracer ^{15}N uptake and depuration from an actively cycling 1° uptake compartment ($\delta^{15}N_t$) was calculated from the empirically determined depuration rate and the $\delta^{15}N-NH_4^+$ using an inverse exponential model (Doucet and Sloep 1992):

$$\delta^{15}N_t = (\delta^{15}N\text{-}NH_4^+) \times [1 - e^{(-kt)}] \qquad [2]$$

where t = time in days and k = the ^{15}N turnover rate constant in units of $/t$. This equation is based on the assumption that the value of $\delta^{15}N\text{-}NH_4^+$ is constant. In Eagle Creek, where $\delta^{15}N\text{-}NH_4^+$ varied over time, equation 2 was approximated over daily time intervals by a linear difference equation:

$$\delta^{15}N_t = [\delta^{15}N_{t-1} \times (1 - k)] + [(\delta^{15}N\text{-}NH_4^+)_t \times k]$$
$$[3]$$

Equations 2 and 3 were used in the linear, open-system compartment model (Doucet and Sloep 1992) depicted in Fig. 1. The rate constants k (2, 1) and k (3, 2) were for assimilation and release, respectively, of tracer ^{15}N (compartment 1) from the 1° uptake compartment represented by heterotrophic microbes or algae (compartment 2).

The compartment model also was used to simulate the isotope accumulation and depuration in 1° consumers, the organisms that consumed the algae or heterotrophic microbes containing tracer ^{15}N (compartment 3). The 1° consumers potentially assimilated N from the ^{15}N-enriched microbes and algae (compartment 2) and from unlabelled sources. The rate of assimilation of algae or microbes containing tracer ^{15}N was k (3, 2), while the rate of assimilation of unlabelled N from sources such as allochthon-

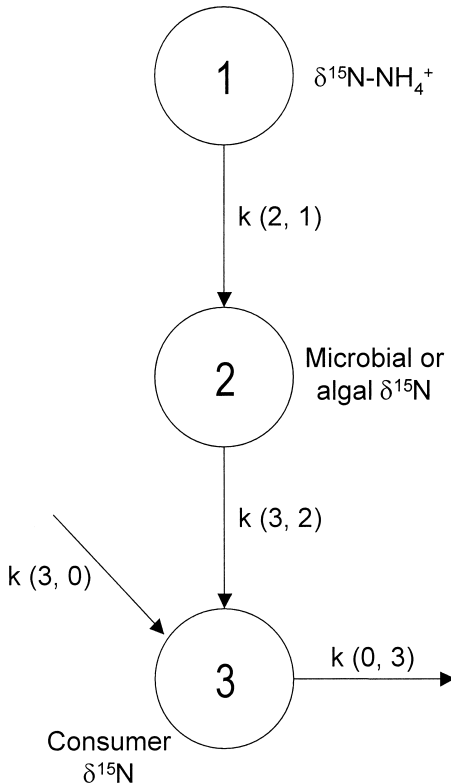FIG. 1. Schematic diagram of the compartment model of tracer ^{15}N flow from the dissolved ^{15}N-NH_4^+ added to the stream (compartment 1) through a 1° uptake compartment consisting of algae or microbes (compartment 2) to a 1° consumer compartment such as a benthic invertebrate (compartment 3). The ^{15}N content of the compartments was modeled in units of $\delta^{15}N$, and k (2, 1) and k (3, 2) represent the rate constants for the flow of tracer ^{15}N between compartments. The consumer compartment also received N from unlabelled sources (denoted by 0). See text for additional details.

ous material was k (3, 0). The total N turnover rate constant of the 1° consumer was k (0, 3). The compartments were assumed to remain fixed in N content (i.e., the total N in the biomass) and, therefore, the inputs and outputs were in balance.

The ratio of k (3, 2) to k (0, 3) was the proportion of the total N in the diet supplied by the tracer-labelled algae or microbes, the instream fraction (IF). IF was the fraction of total N in a consumer's diet derived from N in organic material synthesized in the study reach during the tracer addition; the remainder of the

dietary N could have consisted of allochthonous inputs, material transported from upstream, or relatively inactive organic matter synthesized within the reach at an earlier time.

The assumptions of the compartment model can be summarized as follows: 1) biomass of consumers and N flowing through the 1° uptake and 1° consumer compartments remained at steady state during the experiment; 2) invertebrates at the 1° consumer level fed only on algae or heterotrophic microbes and did not feed selectively within these categories; and 3) consumers of detritus assimilated an equal mixture of fungi and bacteria associated with leaves and FBOM (i.e., fungi and bacteria were combined into a single dietary category).

Model demonstration.—The utility of the compartment model for understanding the kinetics of isotope addition experiments was demonstrated with a hypothetical experiment in which $\delta^{15}N$-NH_4^+ was raised to a level 500‰ above background for 6 wk (Fig. 2). Equations 2 or 3 described the responses of microbes or algae that took up the $^{15}NH_4^+$ (Fig. 2A). N turnover rate constants were set to 0.18/d for microbes (the mean rate for leaves and FBOM in Eagle Creek; Hamilton et al. 2001) and 0.07/d for algae (the rate for epilithon in Eagle Creek; Hamilton et al. 2001). Note that the microbes reached steady state with respect to the $\delta^{15}N$-NH_4^+ over the course of the tracer addition, but the algae remained >10% below their eventual steady state plateau until the last week of the addition.

Water-column $\delta^{15}N$-NH_4^+ was replaced in equation 2 or 3 with the $\delta^{15}N$ of the algae to simulate the response of a consumer that ate only algae (algivore) and whose $\delta^{15}N$ would eventually level out at 500‰ (Fig. 2B). The response also was simulated for a consumer that ate ½ algae and ½ unlabelled food (i.e., k (3, 2) = 50% of k (0, 3); IF = 0.5) and whose $\delta^{15}N$ would eventually level out at 250‰. The whole-body N turnover rate constant k (0, 3) was 0.1/d for both consumers. The $\delta^{15}N$ values of the algivores reached 88% of their eventual plateaus by day 42. This time lag for approach to isotopic equilibrium showed the minimum duration of enrichment required to reach equilibrium. Isotopic enrichment of a 2° consumer would have been further delayed. Given these turnover rate constants, interpretation of tracer $\delta^{15}N$ measurements of a predator feeding on a consumer would be difficult unless the isotope addition
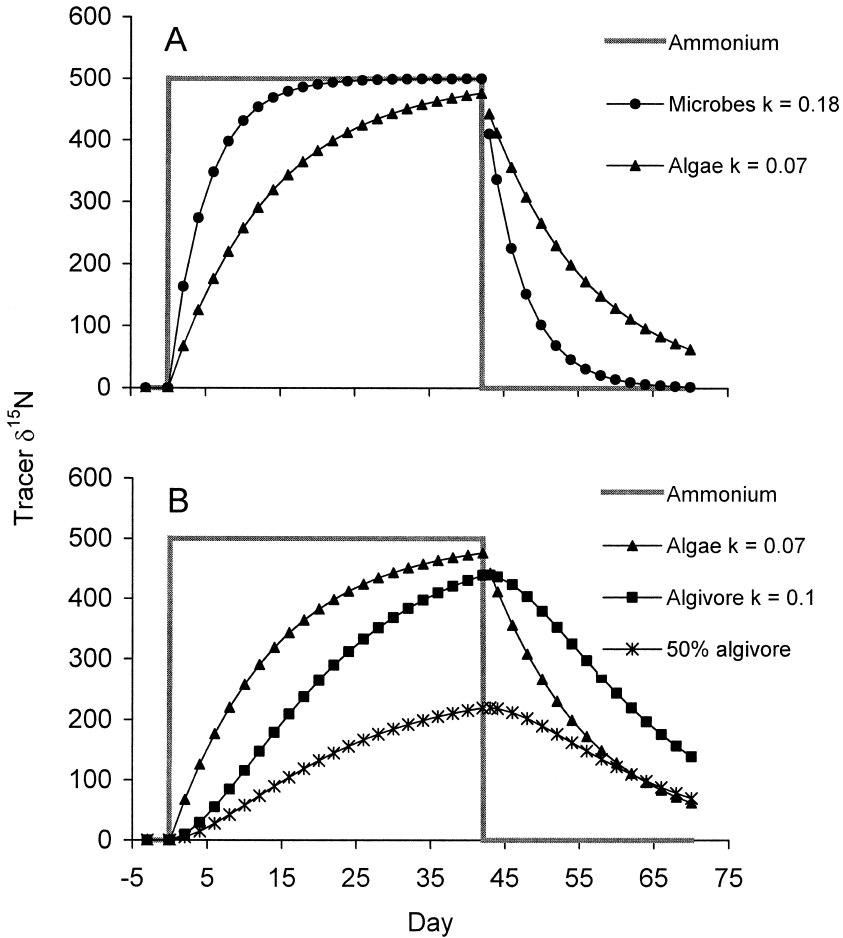
FIG. 2. Compartment model results for a hypothetical isotope addition experiment in which the concentration of the tracer was maintained at a constant level for 6 wk, and the algae or microbes assimilated only dissolved NH_4^+ as their N source. Turnover rate constants for microbes and algae were determined from the ^{15}N depuration in Eagle Creek after the tracer addition ceased (Hamilton et al. 2001). A.—Simulated responses of microbes and algae. B.—Simulated responses of algae, a 1° consumer that fed entirely on algae (algivore), and a 1° consumer that fed ½ on algae and ½ on unlabeled N sources such as organic detritus of allochthonous origin. K = 0.1 for 50% algivore.

lasted a much longer time than in our experiment. However, the model could be used to assist in interpreting the observed isotopic enrichment, provided the increase was large enough to be measured.

Estimation of and accounting for variation in the $\delta^{15}N\text{-}NH_4^+$ is important in tracer addition experiments because such variation affects uptake kinetics. Equation 3 was used to simulate the response of algae, microbes, and 1° consumers under conditions of variable ^{15}N enrichment of NH_4^+ (Fig. 3A, B). The temporal pattern in $\delta^{15}N\text{-}NH_4^+$ in our simulation was calculated for

the Eagle Creek LINX experiment, in which the rate of addition was nearly constant, and variation in the isotopic enrichment of the NH_4^+ was driven by changes in discharge and ambient NH_4^+ concentration (Hamilton et al. 2001). Additional simulations were conducted using the same turnover rate constants but variable isotopic enrichment to show how algae and microbes would be expected to respond to the variable isotopic enrichment. In our example, the ^{15}N enrichment of the consumer remained low and attenuated compared to changes in $\delta^{15}N\text{-}NH_4^+$ (Fig. 3B). The response was attenuated be-
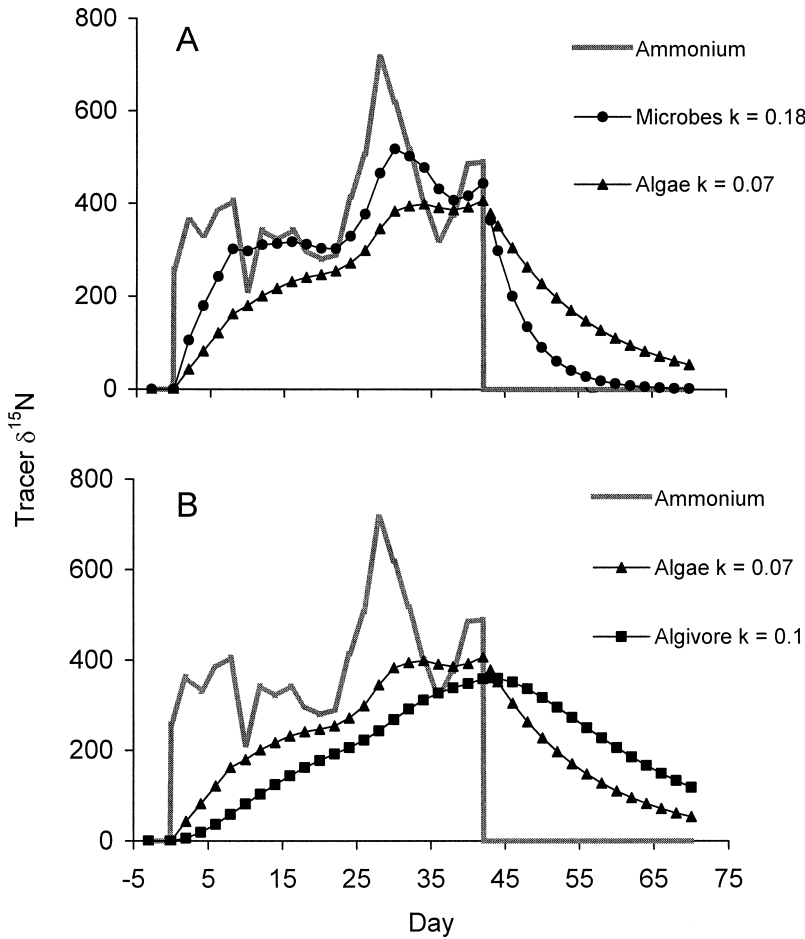
FIG. 3. Compartment model results for the Eagle Creek isotope addition experiment. The $\delta^{15}N$-NH_4^+ was the mean across the study reach, as calculated from discharge, ambient NH_4^+ concentration, tracer addition rate, and the downstream depuration (Hamilton et al. 2001). The variation over the 6 wk was a consequence of fluctuating stream discharge and ambient NH_4^+ concentration. A.—Simulated responses of microbes and algae. B.—Simulated responses of algae and a 1° consumer that fed entirely on algae (algivore).

cause the tracer ^{15}N was diluted with preexisting N at each step during its passage through the primary uptake compartment and into the consumer's body. Previous studies, recognizing the importance of uptake kinetics, have judged whether consumers had reached steady state by the end of tracer addition by comparing consumer $\delta^{15}N$ with the $\delta^{15}N$ of presumed food sources (e.g., Mulholland et al. 2000a, Tank et al. 2002) or by showing that consumer $\delta^{15}N$ values changed relatively little between the last 2 sampling dates during the tracer addition (e.g., Tank et al. 2000).

Parameter estimation in the compartment models.— The objective of the modeling was to analyze rates of ^{15}N transfer from basal sources into the food web to indicate the food sources used by consumers. The compartment model simulated the isotopic kinetics of a 1° consumer feeding on algae or microbes and other unlabelled material, but it did not account for diets composed of mixtures of foods differentially labelled with tracer ^{15}N. Identification of basal sources would have been difficult if a consumer had multiple food sources that became variably enriched with tracer ^{15}N on distinctly different temporal tra-

jectories. However, instream production in Eagle Creek was almost entirely from microalgae in the epilithon (cobbles in riffles) and heterotrophic bacteria and fungi in the FBOM and leaves (detrital accumulations along the edges of the stream). The 1° uptake compartments were spatially segregated in the stream, and the consumers tended to inhabit either riffles or detrital accumulations, but not both. Therefore, 1° consumers were assumed to feed on one of these instream producers, but not both. In either case, consumers could have derived additional nutrition from allochthonous or upstream inputs of organic matter or from organic matter synthesized in the stream prior to the tracer experiment.

The compartment model was fit to the observed tracer ^{15}N accumulation and depuration in a 1° consumer by specifying 1 of the 2 hypothesized food sources (1° uptake; compartment 2) as a forcing function and fitting the model against the observations (1° consumer; compartment 3) by adjusting the rate constants (3, 2) and k (0, 3) within limits of 0 to 1. Theoretical δ^{15}N values for algae and microbes in Eagle Creek (Fig. 3A) were used as alternative forcing functions. Parameters of the compartment model were estimated using the Windows version of Simulation Analysis and Modeling (WinSAAM 3.0.1), an equation-solving package developed for biomedical research by the US National Institutes of Health, Bethesda, Maryland (available from: www.winsaam.com). The observations (mean δ^{15}N of a consumer on a given date) were given equal weighting for the fit. WinSAAM iteratively sought a generalized least squares fit of the model to the data; the goodness of fit was indicated by the residual sum of squares.

The most plausible food source for the consumer was that which produced the best fit. One of the 2 alternatives was discarded if a fit could not be made or if the resultant values of the parameters were unrealistic. Both results were presented when both algae and microbes yielded plausible model fits, but information on the biology of the particular species of consumer was considered in choosing the most likely of the alternative diets.

Results

Biomass of producers and consumers in Eagle Creek

The largest N pool at the time of the experiment (June–July) was detrital organic matter that occurred as FBOM and small woody debris (Table 1). Freshly fallen leaves were scarce in the stream because most inputs from deciduous trees occur in the fall (October–November). The invertebrate fauna included diverse functional feeding groups, and an assemblage of 12 species of mussels accounted for >½ of the total consumer biomass (Raikow and Hamilton 2001). Crayfish and fish were also important in terms of biomass; each exceeded the combined biomass of all insects and amphipods.

Tracer ^{15}N in consumers

The temporal course of tracer δ^{15}N in representative consumers usually showed the expected pattern of accumulation and depuration, although each consumer varied in the rate of change and the degree of ^{15}N enrichment (Fig. 4). δ^{15}N did not decrease consistently with distance from the addition point on days 7 and 42, although spatial variability was observed across the study reach. Only hydropsychids and heptageniids showed statistically significant longitudinal trends ($p < 0.05$) on day 42, when enrichment should have been maximal, and the 2 longitudinal trends were in opposite directions. The absence of longitudinal patterns in δ^{15}N was not surprising because the study reach was <40% of the uptake length for NH_4^+ in our experiment, and invertebrates and their food sources were subject to downstream drift.

Peak tracer δ^{15}N values reached during the experiment varied greatly among the consumers. Baetid mayflies had the greatest ^{15}N enrichment, and the mussel *Pleurobema sintoxia* had the least enrichment (Table 1). Most consumers reached their maximum enrichment between days 35 and 49, near or shortly after the end of the tracer addition. The reason for the increase in δ^{15}N in *Stenelmis* larvae after the tracer addition ended is unknown. However, these elmids were not abundant, so composite samples consisted of fewer individuals than for the other taxa. Some of the taxa included in Table 1 were sampled only once or a few times near the end of the addition, and data for those taxa were insufficient for modeling.

The digestive glands of adult crayfish and *P. sintoxia* clearly responded to the ^{15}N-enriched diet much faster than the muscle tissue (Fig. 4). The divergent data pairs for juvenile crayfish on days 7 to 35 represent divergence between 2

TABLE 1. Compartments sampled for stable isotope analysis, showing reach-weighted mean biomass as ash-free dry mass (AFDM) of each general category, generalized functional feeding groups (FFG; Cummins and Merritt 1996), and the maximum tracer $\delta^{15}N$ (mean across sampling sites, background-corrected) observed during the experiment.

Compartment	AFDM (g/m²)	FFG	Maximum tracer $\delta^{15}N$
Epilithon	5.84	–	155
Fine benthic organic matter (upper cm)[a]	164	–	5.3
Small woody debris (<2 cm diameter)	198	–	37
Leaves	0.90	–	29
Insects + amphipods	0.34		–
Gammarus (Gammaridae)	–	Shredder	51
Stenomena + Stenacron (Heptageniidae)	–	Scraper	168
Baetis (Baetidae)	–	Scraper	258
Macrostemum + Hydropsyche (Hydropsychidae)	–	Filterer	45
Helicopsyche (Helicopsychidae)	–	Scraper	182
Psephenus (Psephenidae)	–	Scraper	113
Stenelmis (Elmidae) larvae	–	Scraper/collector-gatherer	38
Simulium (Simuliidae)	–	Filterer	27
Gerridae	–	Predator	15
Boyeria (Aeschnidae)	–	Predator	53
Anisoptera	–	Predator	63
Corydalus (Corydalidae)	–	Predator	60
Snails	–	Scraper	91
Limpets	–	Scraper	226
Bivalves (Unionidae)	1.93	–	–
Pleurobema sintoxia	–	Filterer	1[b], 7.5[c]
Orconectes propinquus (Cambaridae)	0.55	Omnivore/predator	52[d], 58[c,e]

[a] Measured in July 1999
[b] Muscle tissue
[c] Digestive gland
[d] Juveniles
[e] Adults

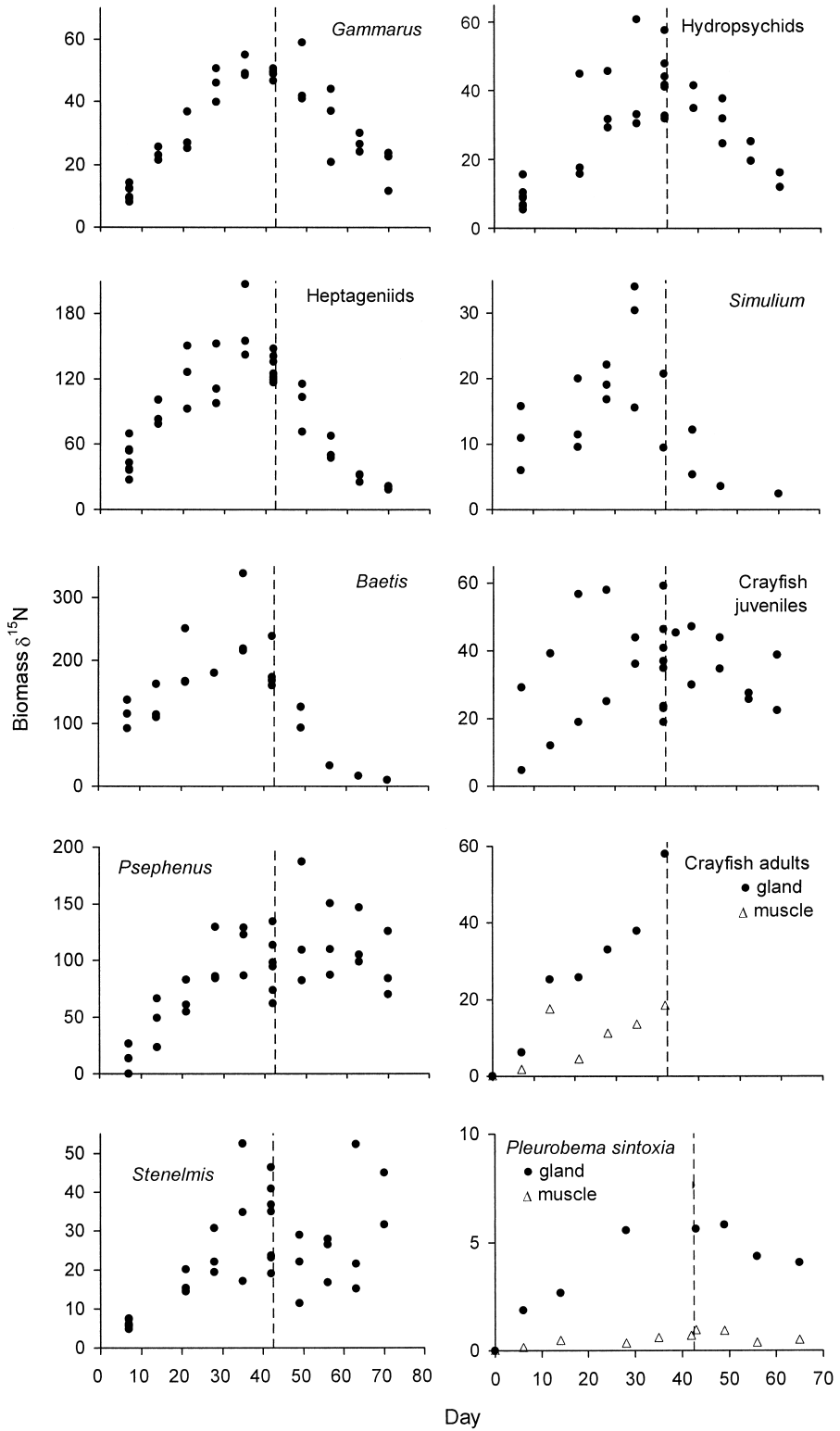sites (300 and 400 m downstream of the addition).

Model fits and parameter estimates

The compartment model simulated the observed time course of mean $\delta^{15}N$ increase and decrease in each of the consumer taxa (Fig. 5). Downstream patterns in ^{15}N enrichment have been observed in the other LINX experiments. However, no consistent spatial pattern in the consumer $\delta^{15}N$ was detected in our e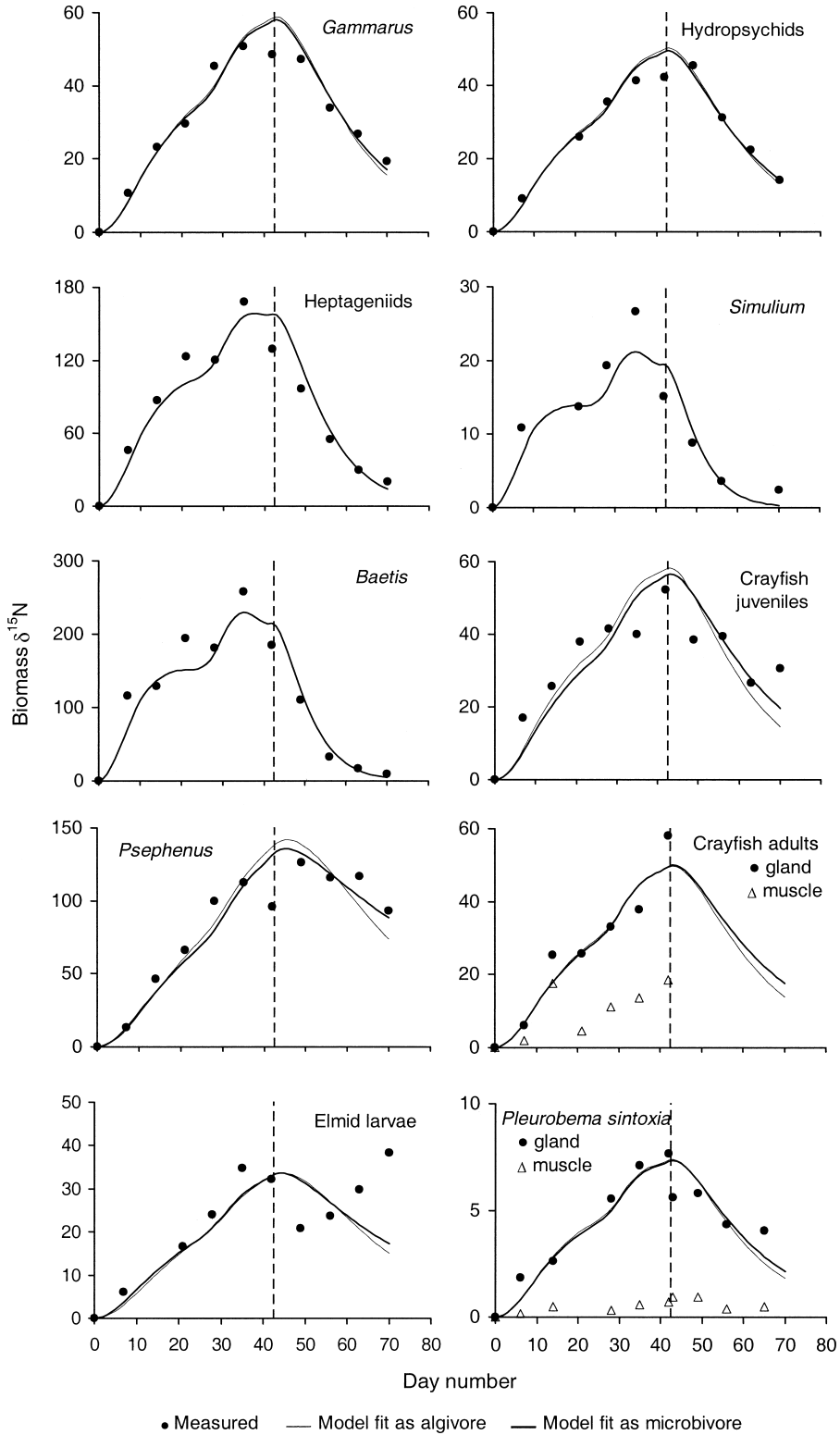xperiment, and measurements from all stations were pooled on each date to produce reach-wide means for modeling purposes. The model could have been applied at individual sampling stations had a downstream pattern been detected. The $\delta^{15}N$ measurements made on the digestive glands of mussels and adult crayfish were used for the modeling because those tissues were more responsive than muscle to dietary changes.

The fits of the models against the observations helped to indicate whether an algal or microbial diet was most likely (Table 2). The only model that could be fit for baetid mayflies and *Simu-*

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FIG. 4. Temporal patterns of ^{15}N enrichment in consumers during and after the addition of $^{15}NH_4^+$ to the stream. The vertical dashed lines mark day 42, when the ^{15}N addition ended. Each point represents a different sampling site along the reach.

Measured ●   — Model fit as algivore   — Model fit as microbivore

lium used a microbial diet. The model that used an algal diet for heptageniid mayflies had an unrealistic turnover rate constant (1.0/d), so those results were discarded in favor of the more realistic fit to the microbial diet. The model could be fit satisfactorily using either diet for *Gammarus*, hydropsychid caddisflies, *Psephenus*, *Stenelmis* larvae, *P. sintoxia*, and the crayfish *O. propinquus*; therefore, the model did not identify which of the 2 basal food sources was most likely for these taxa.

Knowledge of habitat and feeding biology was used to help determine which diet was more likely for consumers whose ^{15}N enrichment could be modeled equally well using either diet. *Gammarus* inhabits leaf packs and shreds decomposing leaves to feed (Cummins and Klug 1979), so a microbial diet was chosen as most likely. Net-spinning hydropsychid caddisflies feed primarily on suspended material and algae that grow on their nets, although Wallace (1975) showed that the 2 genera found in Eagle Creek tended to feed on particles of distinct sizes. Algae was chosen as the more likely diet because suspended material in the stream contained significant amounts of chlorophyll *a*. Filter-feeding mussels were not common in local streams that lacked upstream lentic environments (Raikow and Hamilton 2001), so algae seemed likely to be more important than microbes in their diets. *Psephenus* scrapes material from cobbles and was more likely to be algivorous (White and Brigham 1996). *Stenelmis* larvae always were found on woody debris where they were likely to feed on heterotrophic biofilms colonizing wood, so they were judged to be microbivores (White and Brigham 1996).

The diet of crayfish was especially difficult to characterize even though the species in Eagle Creek also had been studied in nearby Augusta Creek (Creed 1994). Algae was selected as their primary food because crayfish were collected from riffle habitat in our study and because crayfish graze on algae in Augusta Creek (Creed 1994). However, crayfish are mobile and

capable of feeding on a wide variety of living and nonliving organic matter (Momot 1995, France 1996, Whitledge and Rabeni 1997), and significant predation by crayfish would make them 2° consumers, in which case modeling them as 1° consumers was invalid.

The model estimates of the importance of IF in consumers' diets were not affected by ambiguity in the choice of algal or microbial diet (except in the case of *Psephenus*). However, consumer turnover rate constants modeled with the microbial diet were ~40% lower than rate constants modeled with the algal diet. This difference reflected the higher N turnover rate constant and greater ^{15}N enrichment of microbes relative to algae (Table 2). IF varied more than an order of magnitude among taxa, but it was generally <50%. The turnover rate constants of body N (or digestive gland N) also varied widely, ranging between 0.06 and 0.26/d. IF was related to the maximum ^{15}N enrichment, but enrichment also was affected by the wide variation in turnover rate constants. For example, baetid mayflies reached a much higher δ^{15}N than *Psephenus*, but the estimates of IF were similar because of the difference in turnover rate constants of these 2 consumers.

Discussion

The compartment model simulated the kinetics controlling the accumulation and depuration of tracer ^{15}N in 1° uptake and 1° consumer compartments during the stable isotope addition experiment. The model accounted for differences in N turnover rates among consumers and provided an estimate of the ^{15}N contribution to the diet even in cases where the organisms were far from isotopic steady state by the end of the ^{15}N addition. Such a modeling approach is essential for interpretation of data in the face of temporal variation in the degree of dilution of the added tracer in the stream and the fact that most consumers do not reach isotopic steady state with

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FIG. 5.   Model fits to the mean δ^{15}N values for consumers sampled across the study reach. The lines show the model fits (see Table 2 for the parameter estimates). δ^{15}N measurements were made at all 7 stations on days 7 and 42 and at 3 stations on the other dates. δ^{15}N of samples from all sampling stations on days 7 and 42 showed no significant decrease in ^{15}N enrichment in the downstream direction, and therefore measurements from all stations were pooled.

TABLE 2. Parameters of the models fit to the observations of consumer $\delta^{15}N$ for those taxa that were abundant enough to model. The food source marked in bold was considered most likely based on model results or additional information (see text for explanation). The residual sum of squares (SS) indicated the goodness of fit of the model against the observations and was used to compare 2 alternative fits for the same consumer. The transfer rates k (3, 2) and k (0, 3) represent inputs of tracer ^{15}N and outputs of total N, respectively (see Fig. 1 and text for explanation). The ratio of k (3, 2) to k (0, 3) yielded the fraction of the diet supplied by the hypothesized food source (instream fraction). Fractional standard deviations of the fitted parameters are given in parentheses.

	Potential food source	Residual SS	k (3, 2)	k (0, 3)	Instream fraction
Gammarus	**Microbial**	73	0.0093 (0.08)	0.058 (0.08)	0.16 (0.02)
	Algal	103	0.023 (0.12)	0.14 (0.12)	0.16 (0.03)
Stenomena + Stenacron	**Microbial**	612	0.044 (0.09)	0.12 (0.07)	0.38 (0.04)
	Algal	323	0.37 (0.65)	1.0 (0.59)	0.37 (0.33)
Baetis	**Microbial**	439	0.11 (0.11)	0.22 (0.07)	0.50 (0.06)
	Algal	No fit			
Macrostemum + Hydropsyche	Microbial	30	0.0080 (0.10)	0.059 (0.10)	0.14 (0.02)
	Algal	41	0.020 (0.13)	0.15 (0.13)	0.13 (0.02)
Psephenus	Microbial	342	0.013 (0.09)	0.022 (0.21)	0.60 (0.13)
	Algal	494	0.028 (0.13)	0.061 (0.22)	0.45 (0.11)
Stenelmis	**Microbial**	199	0.0039 (0.14)	0.033 (0.23)	0.12 (0.03)
	Algal	246	0.0075 (0.15)	0.074 (0.19)	0.10 (0.02)
Simulium	**Microbial**	31	0.012 (0.14)	0.26 (0.11)	0.045 (0.008)
	Algal	No fit			
Pleurobema sintoxia[a]	Microbial	6.9	0.0012 (0.13)	0.058 (0.02)	0.020 (0.004)
	Algal	7.5	0.0031 (0.17)	0.16 (0.18)	0.019 (0.005)
Orconectes propinquus juveniles	Microbial	641	0.0083 (0.09)	0.050 (0.11)	0.16 (0.02)
	Algal	738	0.024 (0.17)	0.15 (0.18)	0.15 (0.04)
Orconectes propinquus adults[a]	Microbial	28	0.0073 (0.18)	0.050 (0.45)	0.15 (0.07)
	Algal	27	0.018 (0.20)	0.13 (0.31)	0.14 (0.05)

[a] Digestive gland measurements used in modeling

their diets over the typical 1 to 2 mo duration of stable isotope addition experiments.

Without the model, judgment regarding whether isotopic steady state had been reached in our experiment could have been made only on the basis of visual or statistical comparisons of the last few samplings before the end of the tracer addition. Moreover, comparisons between consumers and their potential diets would have been based on final $\delta^{15}N$. The example noted above (baetid mayflies cf. *Psephenus*) points to the hazard of this approach. We could have argued that both of these consumers reached isotopic steady state with their diets based on the last 3 sampling dates during the addition, then proceeded to match them with similarly ^{15}N-enriched foods or to apply isotopic mixing models. The large difference in apparent plateau $\delta^{15}N$ values (~200 vs 100‰) would have led to the conclusion that these taxa had isotopically distinct diets. However, the model showed that differences in turnover rate in the bodies of these consumers alone could explain the differential ^{15}N enrichment.

Insights into the food web of woodland streams

The model results are summarized in Fig. 6, which compares maximum isotopic enrichment (Fig. 6A), IF values (Fig. 6B), and rate constants (Fig. 6C). The 1° consumers in Eagle Creek derived <50% of their N from instream production (i.e., IF < 0.5), with most of the consumers showing lower IF values (Fig. 6B). Maximum isotopic enrichment (Fig. 6A) was correlated with IF values, but turnover rate constants were not (Fig. 6C). The balance of consumer diets probably consisted of allochthonous sources of
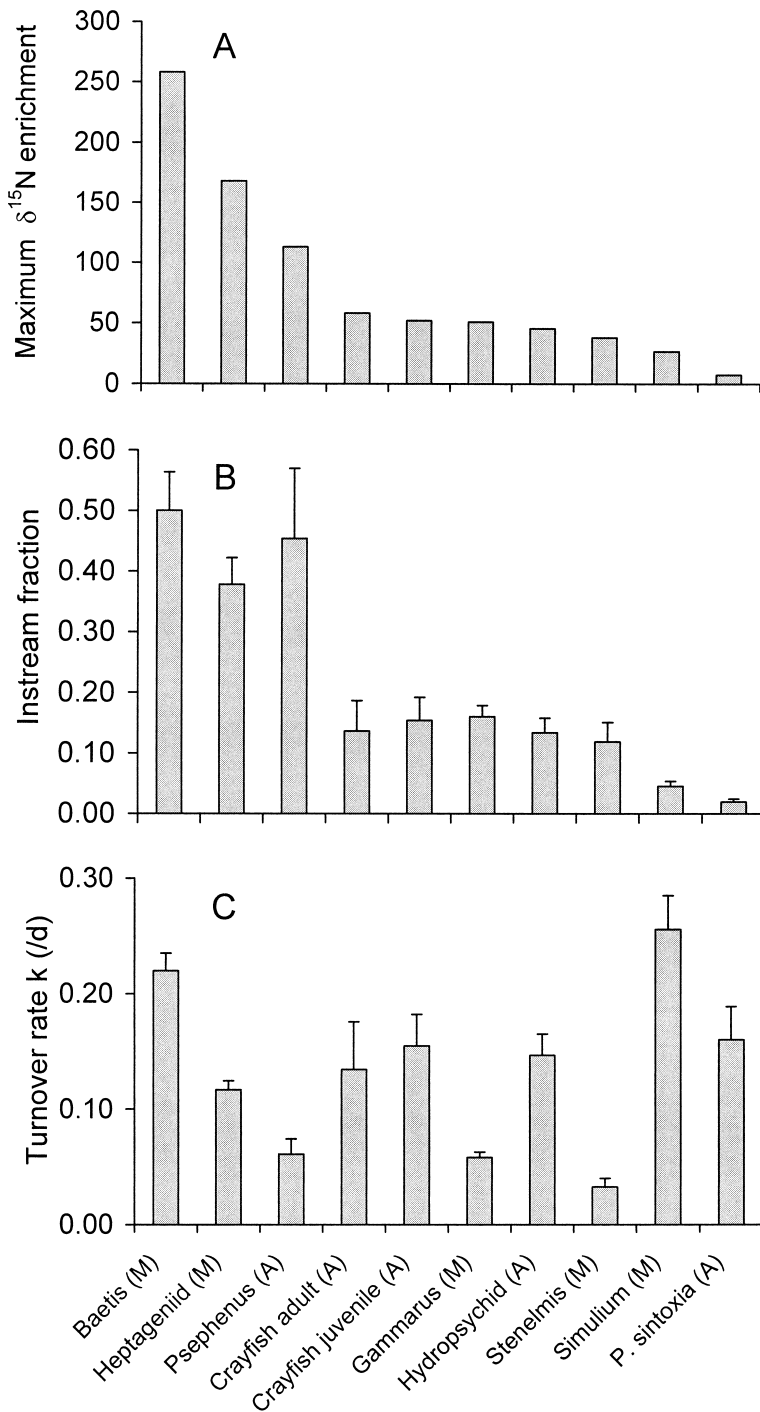
FIG. 6.   Maximum ^{15}N enrichment (A), mean (+ SD) instream fraction (B), and mean (+ SD) total N turnover rate constant (C) for 1° consumers. Modeled diets for each taxon are indicated as either microbial (M) or algal (A). Error bars for the instream fraction were propagated from the parameter estimation errors for k (3, 2) and k (0, 3). *P. sintoxia* = *Pleurobema sintoxia*.

detrital organic matter that were not labelled with tracer ^{15}N or of microbes deriving N from these unlabelled allochthonous sources. This finding is consistent with observations of the feeding behavior of individual taxa (e.g., Cummins and Klug 1979) and with studies that have used natural abundances of C and N isotopes to examine food webs in woodland streams (e.g., Rosenfeld and Roff 1992, Lester et al. 1995, Hicks 1997). Therefore, allochthonous inputs of organic matter appear to be important in stream food webs when the riparian canopies limit light reaching the stream and produce high rates of detrital input.

Small woodland streams such as Eagle Creek probably represent an extreme case of dependence on allochthonous detrital inputs compared to most aquatic ecosystems that are dependent on autochthonous production (Vannote et al. 1980). Stable isotope studies have documented that algal production is generally the major source of organic matter sustaining metazoan food webs in floodplains and wetlands where detrital inputs from vascular plants are high but conditions are more conducive to algal growth (Lewis et al. 2001, Thorp and Delong 2002). Our experiment was done during midsummer when shading from the canopy was maximal. Small woodland streams may support more algal growth and more 2° production based on algae in seasons when leaf cover is reduced or absent or in reaches where riparian banks are not forested than when reaches are shaded by leaves. However, large autumn inputs of fresh leaf litter may more than compensate for any seasonal increase in algal growth that results from increasing light during leaffall. The results of other LINX experiments have provided some insight into seasonal changes in food sources in streams flowing through deciduous forest. The importance of epilithic algae diminished as light decreased in a LINX experiment that spanned spring leaf emergence (Mulholland et al. 2000a). The influence of freshly fallen litter inputs changed the importance of algae–grazer trophic links in a LINX experiment during autumn leaffall, although decreasing temperatures after leaffall also may have reduced consumer activity (Tank et al. 2000). Comparable experiments conducted during different seasons in the same stream have yet to be reported.

The apparent importance of microbes, rather than algae, in the diets of mayflies (baetids and heptageniids) that scrape their food from surfaces was an unexpected result. Such mayflies have been among the most ^{15}N-enriched consumers in the other LINX experiments (e.g., Mulholland et al. 2000a, Tank et al. 2000). These consumers may have selected fast-growing algal cells with turnover rates comparable to those of heterotrophic microbes, and this possibility is one of the caveats discussed below.

The model estimates of N turnover rates for consumers ranged from 6 to 26%/d (Table 2, Fig. 6C). These estimates seem reasonable based on the scant information available in the literature. Energy balances for stream invertebrates can be equated roughly with N balances by assuming that ~½ of ingested N is assimilated and a minority of that N ultimately is used in growth (Grimm 1988). For example, Howard (1975) showed that in nearby Augusta Creek (Michigan) the caddisfly shredder *Pycnopsyche* ingested leaf matter at a rate of 2.3 g g^{-1} d^{-1} as dry mass (normalized to its biomass). The N turnover rate would have been 16%/d assuming a 50% assimilation efficiency of leaf fragments that are 1% N and assuming that invertebrate biomass is 7% N. However, this estimate is a maximum because it does not account for net N retention through growth. Wollheim et al. (1999) used measured growth rates and knowledge of feeding biology to estimate N turnover rates for 4 invertebrates representing different functional feeding groups in the Kuparuk River of Alaska and obtained values of 2, 13, 14, and 37%/d. In contrast, Mulholland et al. (2000a) reported an N turnover rate of only 0.3%/d for the stream snail *Elimia* in laboratory studies. Other LINX studies have estimated turnover rates based on ^{15}N dynamics that were similar to the range reported in our present study (e.g., Dodds et al. 2000).

Caveats for interpretation of model results

Our modeling results must be considered tentative because they are subject to a number of caveats. These caveats should be addressed in isotope addition experiments, and we point them out here to underscore the preliminary nature of our conclusions and to stimulate further work on resolving them. Some of these problems could be addressed by improving the design of isotope addition experiments.

The compartment model simulates N turn-

over in organisms assuming that each organism is a single well-mixed pool, when, in reality, organisms are composed of various kinds of tissues with vastly different N turnover rate constants. Whole-body measurements represent a weighted average of these constants (Tieszen et al. 1983). Researchers using ^{14}C-labelled algae to study the nutrition of zooplankton over much shorter time scales have proposed that the uptake and release of ^{14}C by zooplankton is best modeled using distinct metabolic and structural compartments with short and long turnover times, respectively (Conover and Francis 1973, Lampert and Gabriel 1984). The tracer ^{15}N kinetics of the invertebrates sampled at Eagle Creek could have reflected assimilation of ^{15}N into faster turnover tissues while the overall body $\delta^{15}N$ was affected by relatively stagnant N pools in other tissues. The differences in ^{15}N enrichment between digestive glands and muscle tissues in the mussel and adult crayfish illustrate this phenomenon; digestive glands may provide a better indicator of short-term dietary composition than the muscle or whole-body samples that commonly are analyzed in stable isotope studies of food webs (Hesslein et al. 1993). The importance of relatively stagnant N pools in some tissues should be investigated further in controlled laboratory studies, and methods for sampling fast-turnover tissues in small invertebrates must be developed for isotope addition studies. This problem also affects studies of natural abundances of stable isotopes when the diets of organisms are subject to seasonal changes in isotopic composition or to changes caused by experimental manipulation. The isotopic fractionation between various kinds of tissues and the diet, if significant, should be taken into account when sampling particular tissues for natural abundance measurements. Subtraction of comparable background $\delta^{15}N$ measurements makes this correction unnecessary in isotope addition studies.

The assumption of steady state turnover of N in compartments would be violated if there were substantial growth in biomass in a compartment during the tracer addition (Hesslein et al. 1993). Our model did not account for growth because a large change in the biomass of individual consumers was not noticed at Eagle Creek over the course of the experiment, and occasional measurements did not indicate changes in algal and microbial biomass (Ham-

ilton et al. 2001). Regardless, growth could have been readily taken into account in the model of isotopic turnover in organisms, as has been done in some previous studies (e.g., Hesslein et al. 1993, Wollheim et al. 1999, Dodds et al. 2000).

Downstream transport of organic matter into the study reach can confound tracer labeling of consumers. Unlabelled material, including algae and allochthonous organic matter colonized by heterotrophic microbes, that is carried from upstream into the study reach can be ingested by consumers that filter the water or feed on deposited organic matter. This unlabelled material may include substantial instream production that will not be reflected in the isotopic enrichment of the consumers, leading to overestimation of the importance of allochthonous (terrestrial) sources. The relatively low IF values of hydropsychid caddisflies, *Simulium*, and *P. sintoxia* could be explained by dependence on material originating from upstream, where beaver ponds and wetlands could have exported plankton and detritus of high nutritional value into the stream. Other stable isotope addition studies have shown that concentrations of tracer ^{15}N in *Simulium* peaked downstream from the zone of greatest benthic ^{15}N enrichment, and the investigators proposed that resuspension and downstream transport of benthic particulate matter explained the downstream shift (Wollheim et al. 2001). This phenomenon could not be quantified in filter feeders in Eagle Creek because the study reach was not long relative to its NH_4^+ uptake length, and water column $\delta^{15}N$-NH_4^+ did not decline dramatically throughout the reach.

Modeling algae and microbes as single compartments with characteristic turnover rate constants is an oversimplification because algae, bacteria, and fungi are complex assemblages of organisms that range widely in cell size and turnover rate. For example, the apparent microbial diet of consumers such as baetid or heptageniid mayflies in our study could have reflected preferential feeding on, or assimilation of, high-turnover algal cells within the epilithon rather than a diet of heterotrophic microbes. Evaluation of this possibility requires additional information on the feeding biology of the consumer but, even when available, such information usually is limited to observations of ingestion, which may not reflect assimilation, or visual analysis of gut contents, in which quantification of the composition of fine particulate

matter is difficult and judgment of assimilation is impossible.

Finally, many aquatic invertebrates are omnivorous, despite classification into a particular functional feeding group (Mihuc 1997). Diet inferred from ^{15}N tracers may not correspond with dietary sources of energy in cases where organisms obtain their N and energy (C) from different sources. For example, some predominantly detritivorous caddisflies occasionally consume other caddisflies to the extent that the protein-rich prey significantly augments their overall N nutrition (Wissinger et al. 1996).

Design of future stable isotope addition experiments

The compartment model approach developed in our study demonstrated the kinetics of isotope accumulation and depuration in 1° uptake compartments and consumers, but the model results pointed to a number of new questions regarding the conclusions that can be drawn from analysis of data from short-term isotopic tracer additions. The problems encountered in stable isotope tracer studies resemble those recognized several decades ago when radioisotopes first became widely used for studies of ecological processes, although the time scales of experimentation with radioisotopes were generally much shorter than those in stable isotope addition experiments (Conover and Francis 1973, Smith and Horner 1981). The duration of current stable isotope studies (<50 d) may be too short for ascertaining the diets of macroinvertebrates. Modeling of isotopic tracer dynamics can help alleviate this problem but, for future stable isotope addition experiments, we recommend consideration of the following guidelines for experimental design: 1) the dynamics of tracer dilution in the body of water should be monitored at frequent intervals to facilitate modeling the kinetics of tracer accumulation and depuration in organic compartments; 2) tissues with fast turnover rates should be sampled instead of whole organisms whenever possible (although the use of specific tissues as dietary indicators should be investigated in laboratory studies); 3) biomass of consumers should be monitored during experiments so that growth can be incorporated into the modeling; 4) isotope addition should continue for as long as possible without spanning periods of changing conditions, such as leaffall, that affect food

webs; 5) tracer input rates should be adjusted when possible to accommodate changes in discharge and tracer dilution so that enrichment is kept constant (adjustment is more important for gradual changes than for brief episodes such as spates); 6) whenever possible, the use of modeling to estimate tracer depuration rates in consumers should be avoided by transferring consumers to a comparable field or lab environment containing the same potential foods but lacking the tracer, thereby allowing direct observation of depuration; and 7) modeling of the kinetics of tracer accumulation and depuration should be used to facilitate interpretation of the observations.

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