Ecology, 79(6), 1998, pp. 1995–2012
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THE TROPHIC SIGNIFICANCE OF BACTERIA IN A DETRITUS-BASED STREAM FOOD WEB

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Abstract. We compared relative use of streamwater dissolved organic carbon (DOC) by bacteria and the trophic significance of bacteria to invertebrates in two headwater streams at Coweeta Hydrologic Laboratory in North Carolina: a stream with all leaf litter inputs excluded for 1 yr, and a reference stream. Leaf litter standing crop in the treatment stream was <1% that of the reference stream, and fine benthic organic matter (FBOM) was 50% lower than the reference. We used a whole-stream tracer addition of ^{13}C-1 sodium acetate for 3 wk to label bacteria and hence their consumers during both July and December. Bacterial δ^{13}C was measured by collecting respired bacterial carbon. We estimated the contribution of bacterial carbon to consumers using a mixing model for invertebrates and bacteria. The acetate label declined exponentially downstream with a 10-m uptake length in each stream and season. FBOM and biofilm were the only detrital samples to show a strong label; both were more labeled in the litter-excluded stream. Bacteria in the litter-excluded stream had 7–10 times more label than those in the reference stream during both seasons, showing their higher relative use of streamwater DOC. The percentage of invertebrate carbon derived from bacteria was >20% for many taxa. This was significantly related to the percentage of amorphous detritus in invertebrate guts, suggesting that bacterial carbon supporting higher trophic levels was associated with amorphous detrital particles. Predatory invertebrates were labeled, showing that bacterial carbon was important for higher trophic levels. Some invertebrates were more highly labeled than the bacteria. *Stenonema* in the treatment stream contained eight times more label than measured bacteria. This suggests that they were using an unmeasured bacterial source such as bacteria in exposed epilithic biofilms, which had higher δ^{13}C than all other detrital components. Invertebrates in the treatment stream did not appear to use more bacterial carbon than in the reference stream despite a lower standing crop of detritus. *Tallaperla,* a shredding stonefly, derived 20–40% of its carbon from bacteria in both streams, even though it was more labeled in the treatment stream. Our estimates of the percentage of invertebrate carbon derived from bacteria were higher than those found in laboratory-based studies. To investigate reasons for this difference, we examined the possibility that bacterial carbon was principally found in exopolymers, as our labeling method would have labeled exopolymers. We found 6 g/m^2 of colloidal carbohydrates in the reference stream, which was five times greater than bacterial biomass; thus the high use of bacterial carbon by invertebrates may be a consequence of the availability of these polymers.

Key words: bacteria; bacterivory; Coweeta Hydrologic Laboratory, North Carolina; δ^{13}C; detritivory; dissolved organic carbon; exopolymers; food webs; macroinvertebrates; microbial loop; streams.

INTRODUCTION

Detritus-based food webs differ from their green plant-based counterparts in that microbes, such as bacteria and fungi, can represent a strong link of energy and nutrients to higher trophic levels. In heterotrophic systems such as soil (Coleman 1995), forest streams (Cummins 1974), and estuaries (Findlay et al. 1992), microbes can be more productive than primary producers, and they represent the trophic base of the ecosystem. Even though bacteria can be an important trophic resource, we know less about their ecology than we do about plants. Small size and rapid turnover present methodological difficulties in quantifying the consumption of bacteria by grazers. Bacteria are often associated with particulate detritus, especially in benthic and soil habitats, which allows consumption by organisms that are much larger than the cells themselves (Meyer 1994). Invertebrates that feed on this assemblage are omnivores in that they receive energy both from detritus and from microbial consumers. The degree to which detritivores assimilate bacteria relative to detritus is significant in that it may regulate the amount of energy available for higher trophic levels. For example, if metazoans can directly assimilate detritus, the efficiency of the trophic transfer may be higher than if they only assimilate microbial carbon.

Manuscript received 12 February 1997; revised 15 September 1997; accepted 22 September 1997; final version received 14 October 1997.

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The microbial loop in stream sediments is quite different than that of pelagic systems (Meyer 1994). Most sediment bacteria are attached to larger particles, which means that meiofauna, macroinvertebrates, and protozoa can consume bacteria. Thus, there can be a significant trophic link between bacteria and larger consumers. In pelagic systems, much bacterial carbon may be lost by respiration during successive trophic transfers through Protozoa (Azam et al. 1983, Ducklow et al. 1986). However, there have been questions as to whether particle-associated bacterial carbon is important to deposit-feeding macroinvertebrates (Baker and Bradnam 1976, Findlay et al. 1986, Lopez and Levinton 1987). Lopez and Levinton (1987) suggested that while bacterial cells are easily digested, they were not ingested at high enough rates by deposit feeders to be an important carbon source. However, in sediments and biofilms, bacterial carbon is not found only in bacterial cells. Sediment bacteria, as well as attached algae, make copious amounts of exopolymers (Decho 1990), which can be consumed by macroinvertebrates with high efficiency (Couch et al. 1996). If there is up to 10 times the biomass of bacterial exopolysaccharides than bacteria in biofilm (Couch and Meyer 1992), then much of the bacterially derived invertebrate production in sediments could be from these bacterial exudates, and not just from bacterial cells.

Forest streams are systems where particle-associated bacteria may represent an important link between detritus and consumers because most carbon input is autumnal leaf fall. However, our understanding of the microbial loop in streams is limited relative to macroinvertebrate and algal interactions (Hildrew 1992). We have rates of bacterivory for several taxa (e.g., Borchardt and Bott 1995, Edwards and Meyer 1987) and measurements of dissolved organic carbon (DOC) utilization by bacteria (e.g., Moran and Hodson 1990, Leff and Meyer 1991), but no system-wide descriptions of the role of microbes, such as bacteria, in a stream food web. Studies examining bacterivory by stream macroinvertebrates have shown a wide range in the degree to which macroinvertebrates depend upon bacteria as a carbon source. Shredding craneflies and stoneflies were found to derive <1% of their carbon from bacteria (Findlay et al. 1986), while a scraping mayfly and a filtering blackfly derived between 20 and 67% of their carbon from bacteria (Edwards and Meyer 1987, 1990). Wotton (1980) found a smaller percentage of blackfly growth (14%) to be derived from bacterial carbon.

Because of limited methodology, techniques to determine bacterial consumption by grazers have only recently been developed. One approach to measuring bacterial consumption is to use visible bacterial tracers, such as fluorescently labeled bacteria (Sherr et al. 1987, Borchardt and Bott 1995, Hall et al. 1996). This method only measures ingestion; thus, one needs to assume assimilation efficiencies to determine the importance of bacterial carbon to a consumer. Another method is to label bacteria with an isotopic label and trace the label into the consumer (Findlay et al. 1986, Edwards and Meyer 1987, 1990, Pace et al. 1990). This method measures assimilation of cellular bacterial carbon by the consumer. However, these examples are all laboratory- or bottle-based studies that were conducted during a short time period.

We had two goals in this study. The first was to examine the fraction of invertebrate carbon derived from bacteria, and the second was to examine the effect of a large reduction of the detrital resource base on the trophic role of bacteria in the stream food web. We used a whole-system ^{13}C tracer method that enabled us to examine the fraction of invertebrate carbon derived from bacteria during a relatively long-term (3-wk) period. We labeled bacteria with ^{13}C-labeled sodium acetate; these bacteria were in turn consumed by invertebrates. By measuring bacterial $\delta^{13}C$ and invertebrate $\delta^{13}C$, we were able to determine the fraction of an invertebrate's carbon that was derived from bacteria. This method differed from that used in previous studies (Hall 1995) in that we measured bacterial $\delta^{13}C$ as well as invertebrate $\delta^{13}C$. Because this field method can measure bacterial use in many more taxa than is possible with laboratory methods, we can examine between-taxa differences in the fraction of carbon derived from bacteria. We predicted that shredder taxa, which fragment and consume leaves, would have lower use of bacterial carbon than gatherer taxa, which consume fine particles, because fungi dominate the microbial assemblage on leaf tissue (Weyers and Suberkropp 1996). Because this stable isotope technique labels more than cellular material, we determined the proportion of sediment bacterial carbon that was found in bacterial cells vs. that found in exopolymeric compounds to examine the possibility that ingested bacterial carbon may be in the form of exopolysaccharides (Couch and Meyer 1992, Wotton 1996).

The second goal was to examine the effect of a massive leaf litter reduction in a stream on use of streamwater DOC by bacteria, and use of bacteria by invertebrates. Bacterial biomass is often regulated by the quantity and quality of its carbon source (Bott et al. 1984), hence such a bottom-up manipulation would be likely to decrease bacterial biomass. Because leaf litter inputs were excluded for 1 yr prior to this study, we expected higher $\delta^{13}C$ of bacteria and invertebrates in the treated stream because of the reduction of particulate carbon sources for bacteria, and hence increased reliance on streamwater DOC derived from groundwater inputs. Additionally we predicted that there would be higher relative use of bacterial carbon in the treatment stream because of depleted stocks of detrital carbon, which can be a food source for invertebrates (e.g., Findlay et al. 1986).

METHODS

Study sites and leaf litter reduction

This study was conducted in two first-order streams at Coweeta Hydrologic Laboratory in Macon County, North Carolina (Catchments 53 and 55). They drain steep, forested catchments dominated by oaks, hickories, and yellow poplar. A dense understory of *Rhododendron maximum* shades the streams. The streams flow throughout the year. Stream substratum was mostly mixed silt, sand, pebble, and cobble, with some areas of bedrock outcrop. These streams are strongly heterotrophic because heavy shading by the overstory and evergreen rhododendron understory limits photosynthesis. Litter inputs are ~500 g AFDM·m^{-2}·yr^{-1} (Wallace et al. 1995), whereas primary production in a similar Coweeta stream is only 2.6 g AFDM·m^{-2}·yr^{-1} (Webster et al. 1983) (AFDM = ash-free dry mass).

One catchment (C55) had all leaf litter excluded using a 2.5-cm stretch mesh gill net, which was placed over the first 170 m of the stream in August 1993 (Wallace et al. 1997). Catchment 53 served as the reference stream. Fences were placed alongside the treatment stream to prevent litter from blowing or sliding in. There was a large space between the fence and the net to allow aerial adult insects to move into or out of the stream channel. Leaf litter was blown off the net once per week with a leaf blower during autumn, and when necessary during the rest of the year. There was no transport of litter from upstream of the net because the net extended beyond the springhead of this stream. This manipulation dramatically lowered leaf inputs and standing crop in the stream, as well as decreasing fine benthic organic matter (0.45 μm < FBOM < 1 mm particle size) stocks (Table 1). The mesh caused no measurable decrease in light reaching the stream, and chlorophyll *a* concentrations on tiles increased in the treatments during the manipulation (Table 1).

TABLE 1. Physical characteristics and organic matter standing stocks in the study streams. Organic matter stocks were averaged through June, July, and August 1994 and November, December, and January 1994–1995 (n = 12). Leaf litter and fine benthic organic matter (FBOM) were collected with four benthic cores each month in mixed substratum habitat. Chlorophyll *a* was collected from eight tiles incubated for 8 wk in each stream.

Variable	Date	C53, reference	C55, litter excluded
Channel length (m)†		135	170
Elevation (m)†		829	810
Release site location	Jul 1994	42	47
(m above flume)	Dec 1994	86	115
Sampling area	Jul 1994	0–35	0–40
(m above flume)	Dec 1994	50–80	70–105
Leaf litter‡	summer	255	2.6
(g AFDM/m^2)	winter	424	0.3
FBOM‡	summer	1248	476
(g AFDM/m^2)	winter	1399	461
Chlorophyll *a*‡	summer	1.6	4.1
(mg/m^2)	winter	0.5	4.3

† Data from Lugthart and Wallace (1992).
‡ Data from J. B. Wallace and S. L. Eggert, University of Georgia. AFDM = ash-free dry mass.

Overview of the stable isotope release method

We released a tracer amount of ^{13}C 1-sodium acetate to each stream during 1–27 July and 1–23 December 1994. The December release site, 50 m below the springhead in each stream, was 40–60 m upstream of the July release site in each stream so that there would be no contamination from previously added ^{13}C. We used Marriotte bottles and adjusted the flow once per day to maintain ^{13}C concentration in streamwater at ~1.0 μg/L. Given ambient DOC concentrations in the reference stream (0.97 mg/L in July, 0.70 mg/L in December), the isotopic enrichment of DOC was ~70–103‰ during the two experiments. DOC concentrations in the litter-excluded stream (0.54 mg/L in July, 0.58 mg/L in December) gave final enrichments of 145–135‰. We did not adjust acetate flow for storms; hence the concentration of ^{13}C would be lower during storms. However, discharge in these streams rapidly returns to baseflow after a storm, so there were not long periods when the label was diluted well below 1 μg/L. Because the total DOC enrichment was 2 μg/L relative to a total DOC concentration of 750 μg/L, there was no carbon enrichment effect from the tracer carbon addition.

Labeled acetate was assimilated by bacteria and increased their δ^{13}C well above background throughout the 3-wk trial. We measured bacterial δ^{13}C throughout the release experiment. At the end of the release, we sampled invertebrates below the addition site and analyzed them for δ^{13}C. We calculated the fraction of an individual invertebrate's carbon that was derived from bacteria using a mixing model:

$$\text{Fraction bacterial C} = (\delta I_{\text{labeled}} - \delta I_{\text{unlabeled}})/(\delta B_{\text{labeled}} - \delta B_{\text{unlabeled}}) \quad (1)$$

where δI and δB are the δ^{13}C values of the invertebrate and the bacteria. Bacterial δ^{13}C was determined throughout the release experiment. The δ^{13}C for labeled invertebrates was determined from samples taken at the end of the release. The δ^{13}C of unlabeled bacteria was not measured but was assumed to be the same as particulate detritus in the stream, which averaged −27‰.

Several assumptions are necessary in using this method to determine the fraction of invertebrate carbon derived from bacteria. First, we assume that bacteria and nothing else, biotic or abiotic, are being labeled. It is not likely that fungi took up this label because their half-saturation constants for labile dissolved carbon molecules are ~0.1–1 mmol/L, whereas bacteria have half-saturation constants that are much lower, ~0.001 μmol/L (Newell 1984). Because acetate concentrations were 0.1 μmol/L during this release, it is unlikely that fungi were taking up the label. Algae also exhibit similar acetate-diffusion kinetics to fungi; thus,

they should not be able to take up acetate at low concentrations (Wright and Hobbie 1966). We determined whether the mechanism of acetate uptake was biotic or abiotic using a laboratory incubation. A second assumption is that bacterial $\delta^{13}C$ does not change with time, and that invertebrates turn over most of their carbon during the 3-wk acetate addition period so that their $\delta^{13}C$ reaches an equilibrium. We verified this assumption by examining change in bacterial $\delta^{13}C$ and invertebrate $\delta^{13}C$ with time.

Sampling procedures

We collected detrital and invertebrate samples before and at the end of each 3-wk ^{13}C release experiment. Some unlabeled samples were collected well upstream of the release site after the experiment started. We did not collect any samples in the first 4–7 m below the release site to allow mixing of the isotopic label with streamwater. Additional samples were collected weekly during the release to examine turnover of the ^{13}C label.

FBOM was collected in the study reaches before the addition and weekly during the addition with a corer. The surficial 2 cm and the deeper 3–5 cm organic matter were elutriated separately from the sediment, poured through a 1-mm sieve, collected on a Gelman A/E filter, dried, and ground. Leaves were collected by hand, dried, and ground on a Spex ball mill to a fine powder. The soft outer layer of decomposing wood was scraped with a knife, dried, and ground. We used a wire brush to remove biofilm from rocks. The resultant suspension was pelleted with a centrifuge and dried. We filtered 4 L of streamwater with a Gelman A/E filter and scraped and dried the seston collected on the filter.

Invertebrates were collected in 10-m stream sections below the release site in July. Because we found that the label quickly attenuated below the release site, we collected from 5-m segments in December to reduce variability in label intensity. We collected invertebrates using a small mesh bag or by simply removing substratum from the stream. Animals were elutriated from the sediments, picked within 2 h of collection, and stored in 10% formalin solution. Formalin may have a small effect on invertebrate $\delta^{13}C$, but all samples were treated the same way, and most were strongly enriched, which would lessen the effect of formalin preservation. We removed the guts of all invertebrates except copepods and chironomids to avoid contamination from gut contents. This contamination would tend to lower the invertebrates' $\delta^{13}C$, because detritus was found to be less enriched than invertebrate tissue. All invertebrates were dried at 50°C. Invertebrates of >200 μg dry mass were analyzed individually using a mass spectrometer. Smaller taxa were analyzed together in groups of 2–70 depending on their size. Because we were collecting invertebrates from small streams that were part of a larger study examining ecosystem response to leaf litter reduction, we could not collect as many samples as we would have liked (>10 for each taxon) for fear of depleting the populations. This was especially true for the litter-excluded stream, where invertebrate abundance was strongly reduced by the litter exclusion (Wallace et al. 1997). Additionally, some taxa showed seasonal changes in abundance and were not found during both release periods. Hence, not every detritivore taxon was well represented in our sampling.

We analyzed most samples on a Europa Tracermass isotope-ratio mass spectrometer (Crewe, UK) interfaced with a Carlo-Erba NA 1500 carbon–nitrogen analyzer (Milan, Italy) following the same method as Hall (1995). To control for size effects of the sample on its $\delta^{13}C$, we grouped samples and standards with similar carbon content. Some samples were analyzed on a Europa 2020 mass spectrometer (Crewe, UK) using similar methods. The ratios are reported as the difference of the isotopic ratio of a sample relative to the Pee Dee Belemnite standard (Peterson and Fry 1987). Units are parts per thousand (‰). The $\delta^{13}C$ value for carbon is calculated by:

$$\delta^{13}C = ([R_{sample}/R_{PDBstandard}] - 1) \times 1000 \quad (2)$$

where R is the $^{13}C/^{12}C$ ratio. Samples with more ^{13}C will have a higher $\delta^{13}C$ value. We analyzed 1170 samples from four releases.

Test for biotic uptake of label

We tested whether acetate uptake was biotic or abiotic in stream sediments. Two sediment samples were collected from each stream on 6 May 1996, brought back to the laboratory, split into three 100-mL subsamples, and incubated in 500-mL flasks with 100 mL stream water. We added ^{13}C-sodium acetate to two subsamples to a final concentration of 1.0 mmol/L. $HgCl_2$ was added to one of these labeled replicates to a final concentration of 400 mg/L to arrest biological activity. The third subsample was an unlabeled control. After a 6-h incubation at 21°C on a shaker table, a small amount of the sediment was filtered on a glass-fiber filter, and rinsed with ~100 mL of water to remove excess label. The sediment was dried and analyzed as FBOM.

Bacterial $\delta^{13}C$ estimation

We used a modification of the respiration assay of Coffin and Cifuentes (1993) to estimate detritally associated bacterial $\delta^{13}C$. Essentially, we collected respired bacterial carbon dioxide in a NaOH trap, and analyzed it with a mass spectrometer. Our method differed from that used by Coffin and Cifuentes (1993) in that we controlled for inorganic CO_2 release from the sediments, because 30–90% of total CO_2 release occurred in killed detrital assemblages.

We collected detritus samples and immediately (<1 h) took them to the laboratory. A sample of sediment, leaves, or wood was evenly split into two subsamples and placed in two 4-L tissue culture flasks. We used ~200 mL of sediment, 30 g of wet leaves, or ~30 cm

of 1–3 cm diameter wood pieces. We added 200 mL of streamwater to each flask. To one of the paired flasks we added enough formalin or $HgCl_2$ to make a 5% formalin solution or 400 mg/L $HgCl_2$. Lee et al. (1992) found that both formalin and $HgCl_2$ were effective at shutting down biological activity in marine sediment traps. We switched to $HgCl_2$ for most of the December samples because of possible acidification effects of formalin solution affecting CO_2 release from the sediments. However, we did not see a difference in the quantity of CO_2 evolved from killed flasks using either formalin or $HgCl_2$. Live flasks received 100 mg/L cycloheximide to inhibit fungi. We inserted a test tube, stoppered the flask, and flushed it with air that had flowed through a soda-lime trap to scrub CO_2. After all the flasks were set up, we made 0.5 mol/L NaOH in a 60-mL syringe with freshly deionized water, and injected 10 mL through a tube in the stopper into the test tube in the flask. Between 12 and 16 h later, the NaOH in the flasks was removed with a syringe and injected into a 15-mL Vacutainer tube. Excess 20% $BaCl_2$ was added to the Vacutainer tube to precipitate $BaCO_3$. We centrifuged the precipitate, poured off the base, rinsed it with freshly deionized water, and centrifuged again. The $BaCO_3$ precipitate was dried under a stream of N_2 to prevent contamination with atmospheric CO_2 and weighed. Control flasks with no detritus were typically 10–15% of the $BaCO_3$ of flasks with living detritus. We corrected for the substantial abiotic release of CO_2 using the equation

$$\delta_{bacteria} = (\delta_{live} - \delta_{dead} \times b)/(1 - b) \quad (3)$$

where δ_{live} and δ_{dead} are the $\delta^{13}C$ values from the live and the killed sediments, and b is the ratio of carbon produced from killed vs. live treatments, which ranged from 0.3 to 0.9. We averaged b for each detritus type (e.g., leaves or sediment) within each stream and sampling period. Average b ($\pm$1 SD) was 0.78 $\pm$ 0.26 and 0.77 $\pm$ 0.3 for all summer and winter detritus samples in the treatment stream and 0.52 $\pm$ 0.17 and 0.66 $\pm$ 0.14 for summer and winter samples in the reference stream.

^{13}C acetate transport and uptake

Uptake lengths of acetate were calculated by determining the slope of the linear regression of the natural logarithm of $\delta^{13}C$ values of either biofilm or bacteria with distance downstream (x):

$$\ln(\delta^{13}C_x + 30) = \ln(\delta^{13}C_0 + 30) - kx \quad (4)$$

where $\delta^{13}C_x$ is the $\delta^{13}C$ value at distance x from the release site. Thirty was added to each $\delta^{13}C_x$ so we did not take a logarithm of a negative number. The inverse of the slope (k) is the uptake length (S), which is the average distance (in meters) downstream traveled by acetate before uptake (Newbold et al. 1981). The regressions were fitted with values from samples taken from the release location to the point where label had declined to background levels.

For each meter (x) of stream below the release site, the ^{13}C uptake rate, U (in milligrams of ^{13}C per meter per hour) was calculated as

$$U_x = (C_x \times Q \times 3600 \text{ s/h})/S \quad (5)$$

(Newbold et al. 1981). Concentration (C_x, in milligrams per liter) of acetate ^{13}C at each meter of stream below the release site was calculated with the time-averaged concentration at the release site (C_0) and uptake length S using Eq. 4. Time-average concentration at the release site was determined by dividing the release rate of ^{13}C by average baseflow discharge (Q, in liters per second) measured daily throughout the study using an H-flume. Release rate of ^{13}C was determined by dividing the total amount of ^{13}C released by the length of time for the release. U_x was summed for the 10-m study reach and divided by the area of the reach to determine average ^{13}C uptake per square meter.

We determined if acetate was preferentially removed by the upper sediment layer by comparing $\delta^{13}C$ of the top 2 cm of FBOM with the $\delta^{13}C$ of deeper FBOM using analysis of covariance with sediment depth as the factor and distance downstream as the covariate. Because variability in invertebrate $\delta^{13}C$ may be influenced by spatial variability of the ^{13}C in the sediments, we used a high α (0.1) to detect any possible trends.

Colloidal carbohydrates and bacterial biomass estimation

We measured colloidal carbohydrates in stream sediments on 9 May 1996 using the method described by Underwood et al. (1995). We use the term "colloidal carbohydrates," which is the fraction of carbohydrates released from sediment with some aqueous extractant (consistent with Underwood et al. 1995), in this case Na_2EDTA. Sediment samples were analyzed on the day of collection. Six sediment cores from a range of habitats in each stream were collected, and a 2.5-mL subsample was drawn from each core into an automatic pipet with the tip cut off. To each subsample we added 5 mL 100 mmol/L Na_2EDTA, and each subsample was incubated for 20 min and centrifuged at 4000 g for 15 min. Two milliliters of the resulting supernatant was analyzed for carbohydrates using the phenol-sulfuric acid assay (Underwood et al. 1995) with glucose as the standard. Replicate subsamples were dried and ashed so that colloidal carbohydrates could be expressed per gram organic matter.

Bacterial biomass was determined on 19 December 1994 and on 9 May 1996. A subsample of stream sediment was mixed with an equal volume of 0.1% sodium pyrophosphate and sonicated for 1 min to remove attached cells from particles. A 0.05-mL subsample was counted using the acridine orange direct count method (Hobbie et al. 1977). We estimated biomass by taking slide photographs of the field at 1250×

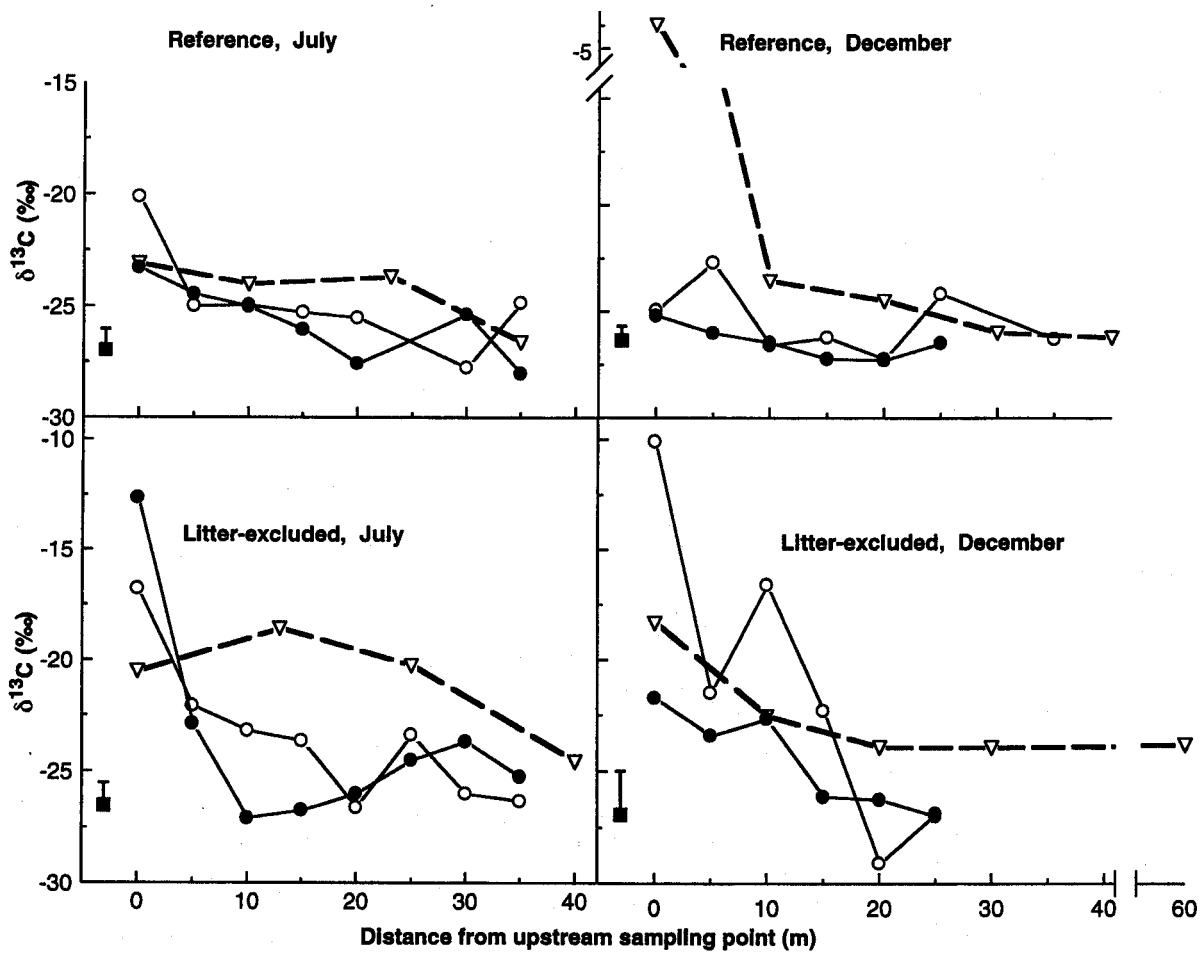

FIG. 1. $\delta^{13}C$ of fine benthic organic matter (FBOM) and seston in the study streams during the July and December release experiments. The upstream sampling point was 4–7 m below the release location. Open circles (○) are surficial (0–2 cm) FBOM, filled circles (●) are deep (>2 cm) FBOM, triangles (▽) are seston samples, and filled squares (■) are unlabeled upstream samples with a 95% one-tailed confidence interval.

and projecting them on a screen to determine size of cells. We measured diameter of cocci, and length and width of rods from >50 cells from each stream. Bacterial biomass was determined using a conversion factor of 2.2×10^{-13} g C/μm^3 and converted to dry mass assuming 50% carbon (Bratbak and Dundas 1984). We multiplied average biomass for rods and cocci by the number of each type of cell to determine bacterial biomass.

Invertebrate gut analysis

The percentage of amorphous detritus in the guts of detritivore taxa was determined by dissecting guts of 2–6 individuals of each taxon. Guts were suspended in deionized water, filtered onto a 0.45-μm cellulose-ester membrane filter, mounted onto a slide, and cleared with immersion oil (Cummins 1973). Amorphous detritus is made up of particles that comprise bacteria, microbes, polymeric fibrils, sediment particles, and tiny detrital fragments (Carlough 1994). Because it has no defined cellular structure, we distinguished it from algal cells, fungal hyphae, leaf fragments, or animal material found in the guts. Using phase-contrast microscopy, an amorphous detritus particle appeared as a diffuse aggregation of subcellular-sized particles. The percentage area of amorphous detritus relative to other gut materials was determined by digitizing the area of 50 particles per slide at 400× using a digitizing pad and a camera lucida attached to a Zeiss Universal compound microscope (Oberkochen, Germany).

RESULTS

Detrital and bacterial labeling

In both streams during both seasons, ^{13}C-labeled acetate was removed quickly from the water column by sediment bacteria, so that only the first 10 m of the study reach was well labeled. Thus there was a strong downstream gradient in both detrital and bacterial $\delta^{13}C$. Additionally, we found differences in $\delta^{13}C$ between different detrital types within each stream, ranging from leaves that had no observable label to highly labeled epilithon.

FBOM was not highly labeled in any of the experiments (Fig. 1); however, $\delta^{13}C$ of upstream samples was higher than the 95% confidence interval of unlabeled

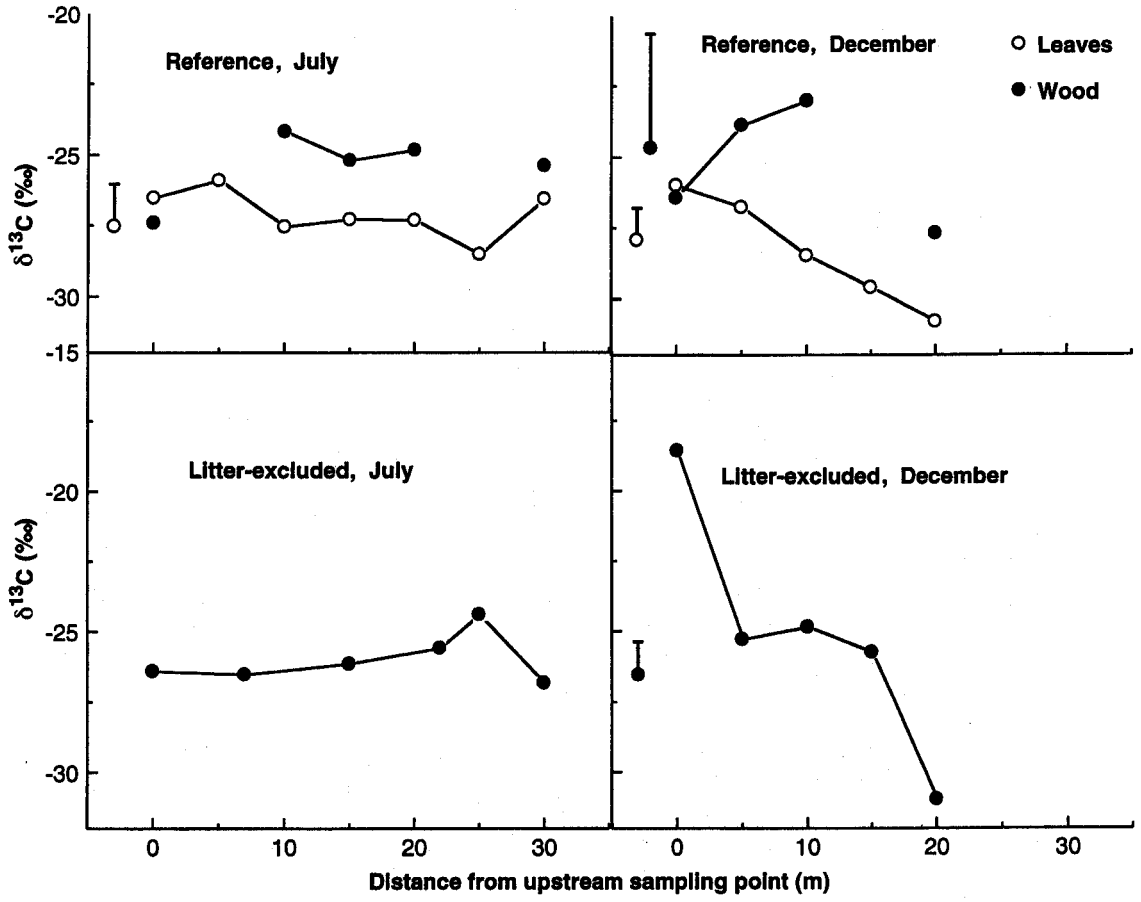

FIG. 2. $\delta^{13}C$ of leaves and wood scrapings in the study streams during the July and December release experiments. Circles with error bars are unlabeled samples with 95% one-tailed confidence intervals. There were no unlabeled wood samples in July.

samples. The litter-excluded stream had higher $\delta^{13}C$ of FBOM than the reference stream at the upstream sampling points (Fig. 1). An analysis of covariance for each of the releases, with distance downstream as the covariate, showed that the upper 2 cm of sediment FBOM was not uniformly more labeled than the lower 2 cm, suggesting that the isotope was reaching lower sediments (ANCOVA, $P > 0.1$). However, for the December release in the litter-excluded stream, $\delta^{13}C$ of the sediments varied with depth (ANCOVA, $P = 0.08$). Seston label was similar to FBOM (Fig. 1). Unlike FBOM, it did not decrease significantly downstream, except in the winter release in the reference stream.

Leaves and outer layers of wood had no detectable label in either stream, because no samples were above the 95% confidence interval for unlabeled samples, except the 0-m wood sample in the litter-excluded stream during December (Fig. 2). There were no leaves analyzed from the litter-excluded stream because no leaves were found there. Epilithon, on the other hand, was labeled in both streams during both releases (Fig. 3), and was more labeled than other measured detrital types. However, the litter-excluded stream's epilithon was more labeled than that of the reference stream, extending up to 150‰. Epilithon label strongly decreased downstream as did the FBOM label (Fig. 3).

Bacteria were more highly labeled than detritus in each stream (Fig. 4, Table 2). $\delta^{13}C$ of reference stream bacteria ranged from −20 to 20‰ while that of litter-excluded stream bacteria ranged from ~10 to 400‰, which averaged 7–10 times more label than the reference stream for the two release experiments. The bacterial label decreased downstream in a pattern similar to the detrital labeling in each stream. There appeared to be no trend in the bacterial label with time (Fig. 4), indicating that bacteria maintained a relatively constant $\delta^{13}C$ throughout the study. FBOM also showed no trend with time after the addition started.

We can estimate the ratio of bacterial carbon to total carbon in FBOM by dividing the increase in $\delta^{13}C$ of FBOM above background (−27‰) by increase in bacterial $\delta^{13}C$ above background. Higher values of this ratio indicate that a larger fraction of FBOM is bacterial carbon and that it is probably a better substrate for bacterial growth. The ratio was 0.18 in July and 0.07 in December in reference stream sediments, and 0.036 in July and 0.023 in December in litter-excluded stream sediments. The higher ratio in the reference stream suggests that there was more bacterial carbon per unit total carbon in reference-stream FBOM compared to treatment-stream FBOM. The bacterial direct counts support this conclusion: dry bacterial biomass per gram

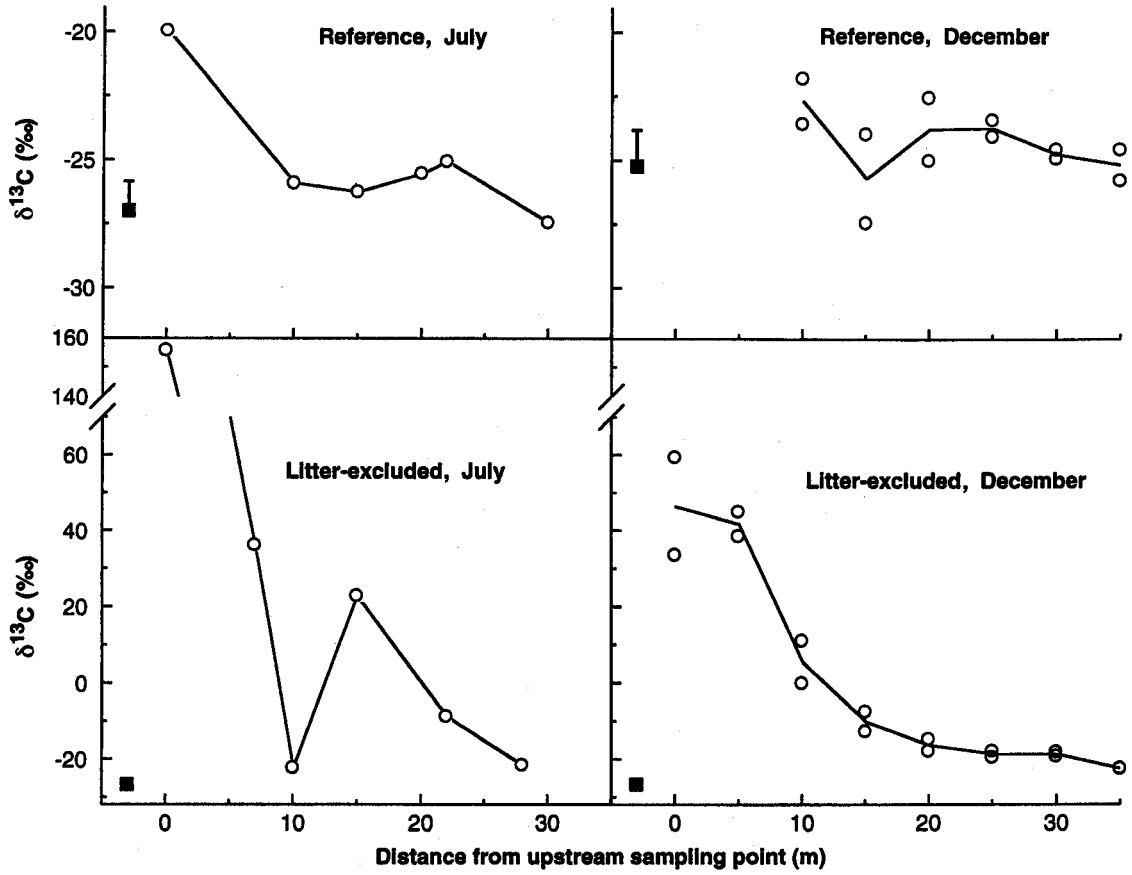

FIG. 3. $\delta^{13}C$ of rock biofilm in the study streams during the July and December release experiments. Note the axis break and the increase of scale for litter-excluded stream data. The filled square (■) indicates unlabeled samples with 95% confidence interval (not visible in the litter-excluded stream).

AFDM was 0.001 g/g in the reference stream and only 0.0005 g/g in the litter-excluded stream.

Using epilithon $\delta^{13}C$ (or leaf bacteria $\delta^{13}C$ for December reference stream release), we estimated acetate uptake lengths to be ~10 m for each release experiment in each stream (Table 2). Rock biofilm is preferable to leaf bacteria to measure acetate uptake because leaves are more easily transported downstream. However, in the reference stream during December there were no rocks within 10 m of the upstream sampling point. Because of these high acetate uptake rates, 33–56% of released acetate was taken up in the 4–7 m section between the release site and the upstream sampling point. Average concentration of acetate and acetate uptake rates were similar for all four releases (Table 2). Uptake of ^{13}C was not different in the two streams, even though there was less organic matter and less bacterial biomass in the litter-excluded stream (Tables 1, 3). There were fewer bacteria in the litter-excluded stream (Table 3), but they removed acetate from the water column as quickly as did bacteria in the reference stream. Hence, bacteria in the litter-excluded stream appeared to rely more on streamwater DOC and less on particulate carbon than did bacteria in the reference stream.

^{13}C-acetate uptake into sediments was almost completely biotic. Mean $\delta^{13}C$ of FBOM in the labeled treatment was +15.0‰, significantly higher than unlabeled controls (−26.7‰) (one-tailed paired t test, $P = 0.002$). FBOM in the $HgCl_2$ treatments (−25.8‰) was not higher than unlabeled FBOM (one-tailed paired t test, $P = 0.12$).

Invertebrate labeling and use of bacterial carbon

Invertebrates were labeled well above background, and like detrital and bacterial samples, invertebrate $\delta^{13}C$ decreased downstream from the release site (Fig. 5). For purposes of comparison, we calculated use of bacterial carbon only for individuals collected within 10 m of the upstream-most sampling point. There was strong between-taxon variability, which translated to differential usage of bacterial carbon. Taxa ranged from 0 to well over 100% of invertebrate carbon derived from bacteria (Table 4). Most, however, were between 0 and 100% (Table 4). Some taxa, such as *Stenonema* and *Wormaldia,* had significantly higher than 100% use of bacterial carbon, suggesting that we were not adequately measuring the signal of their food resources. While several taxa in the December reference stream release appeared to derive more than 100% of their carbon from bacteria, only harpacticoid copepods,

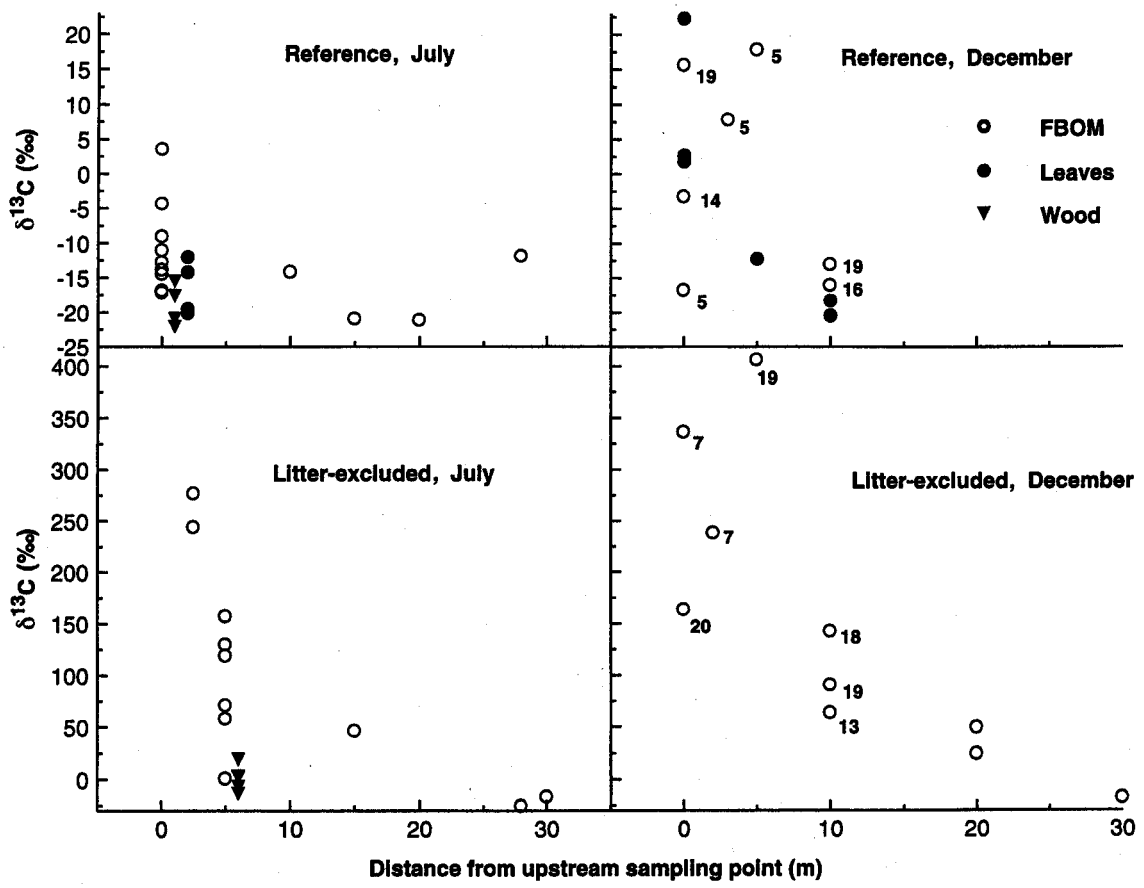

FIG. 4. $\delta^{13}C$ of bacteria in the study streams during the July and December release experiments. Note the increase in scale for litter-excluded stream data. Numbers next to winter sediment samples represent the number of days from the start of the release when that particular sample was collected. We only numbered those samples close to the release site.

Stenonema, and *Wormaldia* were significantly higher than 100% (Table 4).

There was no apparent pattern linking the fraction of invertebrate carbon derived from bacteria and the assigned functional feeding group of the organism. Some shredders, such as *Tipula, Fattigia,* and *Lepidostoma,* were uniformly low in their use of bacterial carbon, while *Leuctra* was much higher. Gatherers such as copepods and chironomids derived a large fraction of carbon from bacteria, while the fraction for oligochaetes was much lower. Scrapers such as *Stenonema* and *Epeorus* were uniformly highly labeled. Because assignment of functional feeding group (scraper, shredder, collector; see Table 4) describes how an invertebrate feeds, and not necessarily what it eats, we compared the percentage of invertebrate carbon derived from bacteria with the percentage of amorphous detritus in its gut. We found a significant positive relationship between the percentage of amorphous detritus in the guts of arthropod taxa and the percentage of invertebrate carbon derived from bacteria (Fig. 6). We excluded taxa that derived >140% of their carbon from bacteria because we do not know the $\delta^{13}C$ of bacteria consumed by taxa with values much greater than 100%.

Predatory taxa were also labeled (Table 5). We cannot absolutely determine their fraction of carbon derived from bacteria because they were two trophic levels away from bacteria, and thus do not consume bac-

TABLE 2. Acetate concentrations, uptake, and average bacterial $\delta^{13}C$ during each of the four release experiments.

Variable	Reference		Litter-excluded	
	July	December	July	December
Average ^{13}C concentration (mg/L)	0.00106	0.00107	0.00108	0.00094
Average discharge (L/s)	0.81	1.32	1.31	2.13
Average width† (m)	2.2	1.1	1.6	1.5
^{13}C uptake length (m)	11.0	7.6	10.0	11.6
^{13}C uptake rate ($mg \cdot m^{-2} \cdot h^{-1}$)	0.06	0.16	0.12	0.16
Bacterial $\delta^{13}C$	−8.0	4.5	166	190

† Width of stream in sampling area.

TABLE 3. Bacterial and colloidal carbohydrate standing stocks in the two study streams. Numbers in parentheses are standard errors; $n = 6$ in May and $n = 4$ in December.

Variable		Reference	Litter-excluded
Colloidal carbohydrates (mg carbohydrate/g AFDM)		4.83 (0.54)	1.27 (0.18)
Colloidal carbohydrates (g carbohydrate/m^2)		6.02 (0.67)	0.61 (0.09)
Bacterial biomass (g DM/m^2)	19 Dec 1994	0.23 (0.027)	0.14 (0.030)
	9 May 1996	1.24 (0.21)	0.23 (0.076)
Bacterial biomass (mg DM/g AFDM)	9 May 1996	0.99 (0.17)	0.48 (0.16)
Colloidal carbohydrates/bacterial biomass (mg carbohydrate/mg bacterial DM)		5.5 (0.9)	4.2 (1.1)

Notes: Colloidal carbohydrate standing stocks were determined by multiplying carbohydrate concentration per gram ash-free dry mass (AFDM) (Fig. 8) by summer fine benthic organic matter (FBOM) stocks. May bacterial biomass is higher than December biomass because we used 10 cm deep cores in May and 3 cm deep cores in December. May bacterial biomass was determined per gram AFDM of FBOM and was multiplied by summer FBOM stocks to determine biomass (in grams per square meter).

teria directly. Stoneflies, *Isoperla* and *Beloneuria,* were highly labeled, showing that bacterial carbon was reaching higher trophic levels.

We could detect no difference in use of bacterial carbon by invertebrates during the two seasons or between the two streams (Table 4). Even though invertebrates were more highly labeled in the treatment stream than the reference stream (see *Tallaperla* example in Fig. 5), this high labeling did not translate to a higher fraction of invertebrate carbon derived from bacteria. There were no taxa whose fraction of carbon derived from bacteria was higher in the litter-excluded stream than the reference stream during both December and July; for some taxa (e.g., *Leuctra, Diplectrona*) the values were lower in the litter-excluded stream. Our estimates of fraction of carbon derived from bacteria are not precise because we found such high variability within taxa, presumably because of feeding variations between individuals and spatial distribution of the label, such as the strong downstream decline in label. Hence, we cannot distinguish between fractions of carbon derived from bacteria that differ only slightly between taxa, streams, or season. Given a pooled standard deviation of 0.1 for *Tallaperla* using the mean of the fraction of carbon derived from bacteria for each of the four releases as replicates (Table 3), the difference in the fraction of carbon derived from bacteria between streams would have to be 0.63 to detect a significant difference ($\alpha = 0.1$).

Accrual of ^{13}C with time varied among taxa, perhaps as a function of turnover of carbon in the population. Invertebrates with faster carbon turnover will reach maximum $\delta^{13}C$ in less time. Chironomids in July appeared to reach an equilibrium label, suggesting that they had 100% carbon turnover during the 3-wk period. In December, chironomids in the litter-excluded stream were still accruing label at 21 d (Fig. 7), suggesting that they had not turned over 100% of their carbon. Other slower-growing taxa, such as *Stenonema* and *Pycnopsyche,* were still accruing labeled carbon at 21 d, showing that they had not yet turned over 100% of their carbon during the 3-wk addition.

Bacterial and colloidal carbohydrate standing stocks

Colloidal carbohydrates were positively related to organic matter in sediments from both streams (Fig. 8); however, colloidal carbohydrates per gram organic matter were 3.8 times higher in the reference stream relative to the litter excluded stream (*t* test, $P = 0.0001$; Table 3). Using this relationship, we extrapolated the standing stock of colloidal carbohydrates for each stream by multiplying standing stock of FBOM by the colloidal carbohydrate stock per gram AFDM of FBOM (Table 3). Bacterial biomass was less than 25% of colloidal carbohydrate stocks in both streams (Table 3). Bacterial biomass in the litter-excluded stream was between one-fifth and one-half the biomass in the reference stream, and colloidal carbohydrate concentration was 10 times lower. Standing stocks were lower in the litter-excluded stream because there was less FBOM per square meter and because there was less colloidal carbohydrates and bacterial biomass per gram AFDM of FBOM.

DISCUSSION

Detrital and bacterial labeling

Detrital $\delta^{13}C$ was much lower than bacterial $\delta^{13}C$ in both streams, showing that bacterial cells were concentrating ^{13}C-acetate relative to bulk detritus. Leaves and wood had no label, showing that little of their carbon was associated with bacteria. FBOM was labeled above background, with deep FBOM usually labeled as much as surface sediments. Unlabeled seston from upstream was probably diluting seston $\delta^{13}C$, so that we did not see strong labeling or a downstream trend in some of the experiments. Epilithon was more labeled than all other detrital sources because rocks were exposed to labeled water, and because epilithic bacteria are more likely to rely upon streamwater DOC

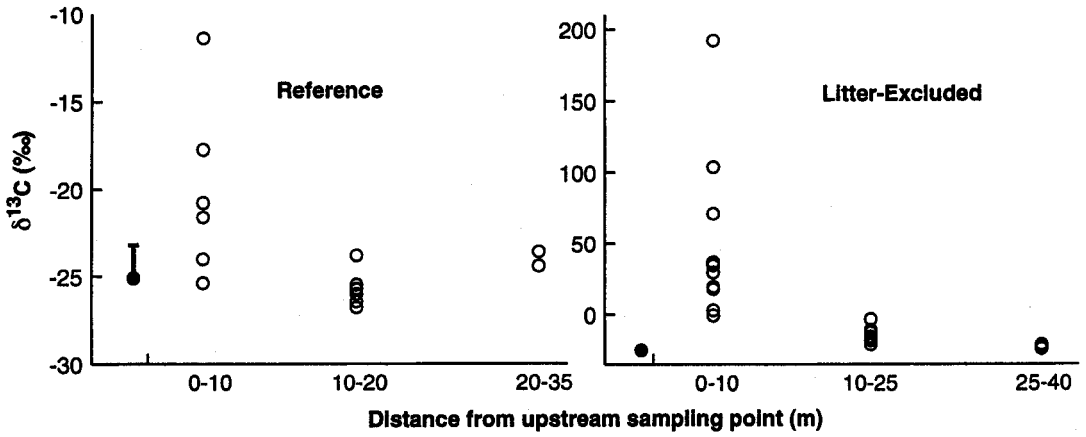

FIG. 5. $\delta^{13}C$ of *Tallaperla maria* during the July release experiment. Individuals were randomly collected in three 10–15 m sections below the release site. Note the increase in scale for litter-excluded stream samples. The filled circle (●) represents unlabeled individuals with one-tailed 95% confidence interval.

as a carbon source than bacteria that are attached to leaves or to fine benthic particles.

Our measurement of bacterial $\delta^{13}C$ represents the first use of this technique on sediment bacteria. Other methods of measuring bacterial $\delta^{13}C$ usually involve a nucleic acid extraction from bacterial cells (Coffin and Cifuentes 1993). However, it is difficult to extract nucleic acids from sediment bacteria because humic compounds are co-extracted with DNA. Because of the technical difficulty of nucleic acid extraction, we opted to use this respiration assay, although it may not be as precise as direct measurement of DNA. Nonbiological CO_2 release confounds the assay and requires correction. The calculated $\delta^{13}C$ is sensitive to the value of the correction factor (b); thus any means to minimize b will give more precise results. We minimized error by analyzing many bacterial samples throughout each release and averaging them.

Bacterial carbon in the litter exclusion stream was 7–10 times more labeled than bacterial carbon in the reference stream. This difference cannot be caused by higher isotopic enrichment of DOC in the litter-excluded stream as it was only twice as high. Also, acetate is likely to be much more labile than most streamwater DOC, thus bacterial $\delta^{13}C$ may be higher than the average $\delta^{13}C$ of DOC. We suggest that because little new

TABLE 4. Fraction of invertebrate carbon derived from bacterial carbon for 19 taxa in four functional groups during July and December experiments in the two study streams. Numbers in parentheses are standard errors of the mean.

Functional feeding group	Insect order	Taxon	Fraction of carbon derived from bacteria			
			Reference, July	Reference, December	Litter-excluded, July	Litter-excluded, December
Shredders	Plecoptera	*Leuctra* spp.	0.77 (0.21)	1.15 (0.26)	0.40 (0.18)	···
	Plecoptera	*Tallaperla maria*	0.38 (0.18)	0.31 (0.11)	0.43 (0.09)	0.19 (0.03)
	Trichoptera	*Fattigia pele*	···	0.15 (0.03)	···	−0.01 (0.02)
	Trichoptera	*Lepidostoma* spp.	0.11 (0.08)	0.25 (0.11)	0.27 (0.01)	···
	Trichoptera	*Pycnopsyche* spp.	···	1.13 (0.28)	···	···
	Diptera	*Tipula* spp.	0.19 (0.06)	0.10 (0.10)	0.25 (0.07)	0.01 (0.01)
Gatherers	Ephemeroptera	*Paraleptophlebia* spp.	···	2.35 (0.81)	0.69 (0.38)	···
	Plecoptera	*Amphinemura wui*	···	1.15 (0.26)	···	0.46 (0.03)
	Trichoptera	*Lype diversa*	0.19 (0.01)	···	···	···
	Diptera	Chironomidae	0.34 (0.09)	1.89 (0.87)	0.79 (0.17)	0.51 (0.10)
	Diptera	*Sciara* spp.	0.09 (0.08)	···	···	···
		Harpacticoida	0.54 (0.07)	4.19* (0.86)	···	···
		Oligochaeta	···	0.32 (0.20)	0.16 (0.03)	0.09 (0.04)
Filterers	Trichoptera	*Diplectrona modesta*	···	0.53 (0.19)	···	0.09 (0.01)
	Trichoptera	*Parapsyche cardis*	···	0.52 (0.14)	···	···
	Trichoptera	*Wormaldia moesta*	10.8* (4.22)	13.1* (2.63)	···	1.14 (0.32)
Scrapers	Ephemeroptera	*Epeorus* sp.	···	···	···	1.24 (0.16)
	Ephemeroptera	*Stenonema* spp.	···	4.72* (0.90)	8.43* (2.03)	1.28 (0.16)
	Coleoptera	*Ectopria thoracica*	···	···	···	0.04 (0.03)

Notes: The fraction of carbon derived from bacteria is calculated using Eq. 1. Ellipses (···) indicate taxa missing from upper 10 m of the release site for that particular release experiment. Sample size ranges from 2 to 16 for each taxon during each release experiment. Note that some shredder taxa were missing from the litter-excluded stream because of the manipulation.

* Fraction of bacterial carbon significantly >1 (one-tailed t test, $\alpha = 0.05$).

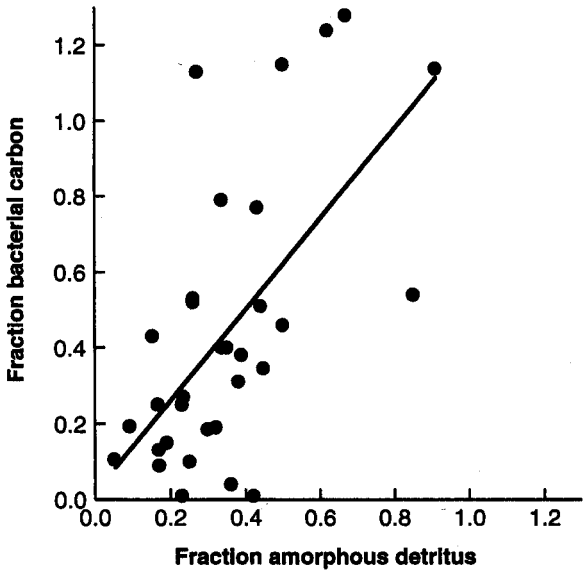

FIG. 6. Relationship of fraction of invertebrate carbon derived from bacteria vs. fraction of amorphous detritus in invertebrate guts. Points are averages of fraction amorphous detritus (n = 2–5) and fraction of invertebrate carbon derived from bacteria (n = 2–16). Due to measurement error, some fractions are greater than 1. A taxon may appear in this graph up to four times—once for each release. The line is a least-squares fit regression line ($y = 1.21x + 0.02$, $r^2 = 0.39$, $P < 0.0001$).

particulate carbon entered the litter-excluded stream during the year before the ^{13}C release, the quantity of benthic organic matter decreased (Table 1), causing attached bacteria to derive more of their carbon from water-column DOC. Uptake of acetate per bacterial cell was higher in the litter-excluded stream than in the reference stream because acetate uptake per unit area was similar despite fewer bacteria in the litter-excluded stream (Table 3). Other research has shown that streamwater DOC can be a significant source of carbon to bacteria; Bott et al. (1984) demonstrated that a 40% decrease in DOC concentration caused standing stock of bacterial cells to decrease by 53%, and that bacterial biomass on ashed sediments was 20-fold lower than on organic-rich sediments. Thus both water column and sediment sources of organic matter can influence bacterial biomass (Bott et al. 1984). We cannot determine the absolute amount of bacterial production derived from streamwater DOC, because we know nothing about the availability and use of the unlabeled DOC fraction relative to acetate.

Mechanism for invertebrate uptake of ^{13}C label

The ^{13}C label was incorporated by bacteria prior to consumption by invertebrates. Biotic uptake of the label corroborates other studies showing that DOC uptake in sediments is largely biotic, i.e., taken up by metabolically active microbes (Lock and Hynes 1976, Dahm 1981, Kuserk et al. 1984), even though some abiotic adsorption of DOC has been found by others (McDowell 1985, McKnight et al. 1992).

Bacterivorous Protozoa may have been consumed by detritivorous invertebrates, thus creating an alternate path for the ^{13}C transfer. However, protozoan consumption of bacteria is low in stream sediments from the litter-excluded stream (prior to the treatment) and an adjacent first-order stream (L. A. Carlough and J. L. Meyer, *unpublished data*). Only 1% of bacterial standing stock was grazed per day by flagellates and ciliates because there were too few Protozoa (4–6 × 10^4 flagellates/mL, 3–6 × 10^2 ciliates/mL) relative to bacterial densities (2 × 10^9 bacteria/mL) (L. A. Carlough and J. L. Meyer, *unpublished data*). Nonetheless, protozoan $\delta^{13}C$ would likely track bacterial $\delta^{13}C$ because of quick protozoan biomass turnover and reliance upon bacteria as a food source.

Fungi have half-saturation constants for organic substrates 1000 times higher than those of bacteria (Newell 1984); hence, they were probably not labeled in these experiments, even though their biomass is high in Coweeta streams (Paul and Meyer 1996). Our evidence is that leaves were not labeled above background, although a large percentage of their biomass (2–5%) was composed of fungi (Paul and Meyer 1996). If fungi were labeled, we would expect to see leaf $\delta^{13}C$ above background, yet leaves were uniformly unenriched. Suberkropp and Weyers (1996) found no significant fungal uptake of leucine at four times the concentration of acetate of this study. Algae take up acetate much

TABLE 5. $\delta^{13}C$ (‰) of some predator taxa from study streams during July and December. Each value is a single measurement from a composite sample of three or more individuals.

		Reference stream		Litter-excluded stream	
Order	Species	Jul	Dec	Jul	Dec
Plecoptera	*Beloneuria* sp.	−21.0	−0.85	122.4	−12.1
Plecoptera	*Isoperla* spp.	···	47.0	219.3	38.5
Odonata	*Lanthus vernalis*	−22.3	−1.9	···	−18.6
Odonata	*Cordulegaster* sp.	···	−12.7	32.0	···
Diptera	*Hexatoma* spp.	−23.4	−22.2	···	−7.5
Urodela	*Desmognathus* spp.	···	···	···	−16.6

Notes: Samples were taken within 10 m of the sampling site that was farthest upstream. Entries with ellipses (···) indicate taxa for which no data were available. We have no data on unlabeled predators in this study; however, the range of unlabeled detritivores was from −35‰ to −23‰, and means of unlabeled predator taxa in another Coweeta stream ranged from −25‰ to −23‰ (Hall 1995).

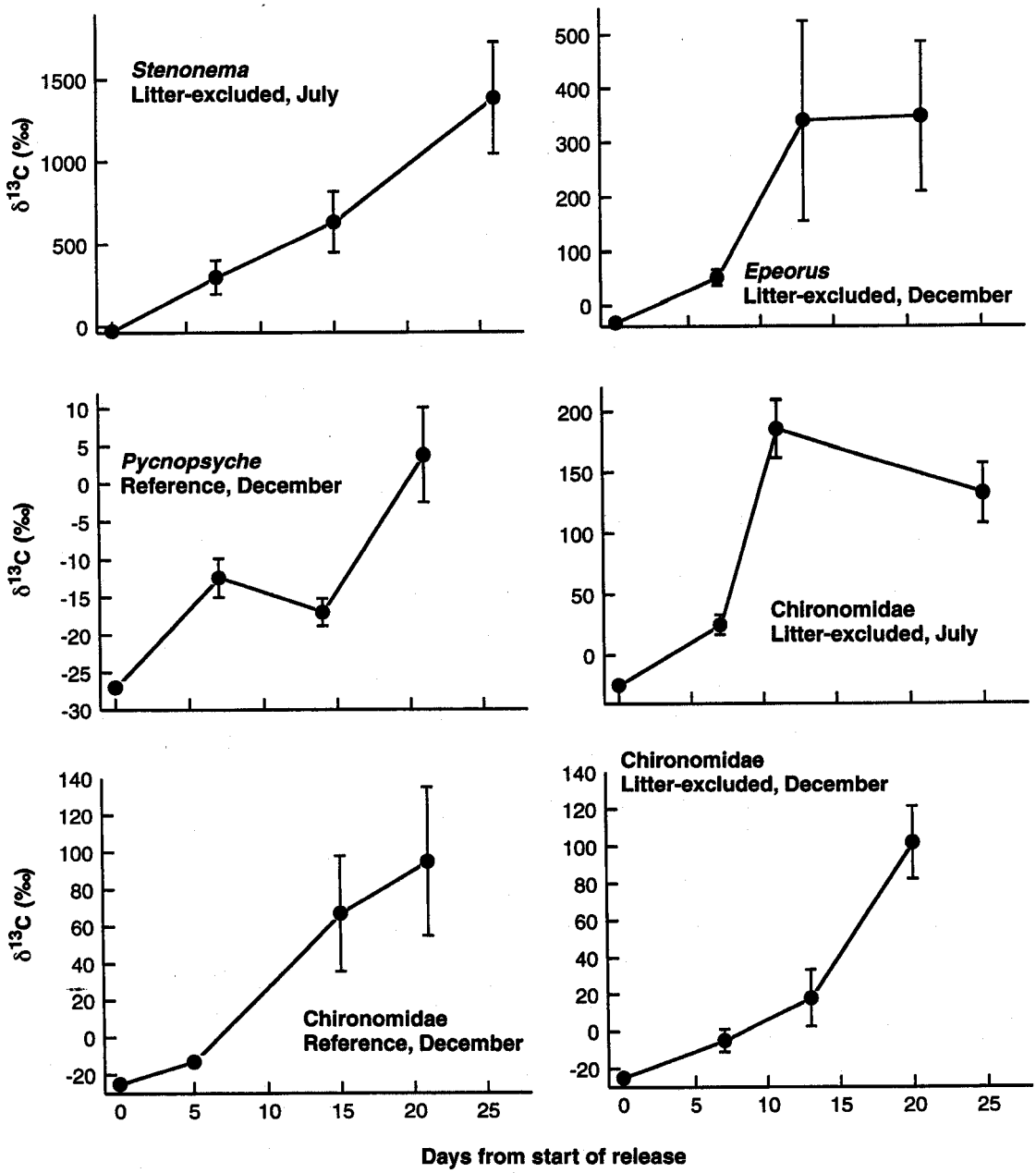

FIG. 7. Time course of ^{13}C label in various invertebrates. Error bars show one standard error for n ranging from 3 to 4.

more slowly than bacteria at low concentrations (Wright and Hobbie 1966). Much less than 10% of natural acetate uptake in a lake was from algae, even though algal biomass was higher than bacteria (Wright and Hobbie 1966). Hence, it appears that most incorporation of acetate was via bacteria and their exopolymers.

Some invertebrates had higher $\delta^{13}C$ than the respired bacterial carbon; thus they appeared to derive >100% of their carbon from bacteria. This cannot be true; it is more likely that invertebrates were consuming bacterial carbon that we could not measure, such as sestonic and epilithic bacteria, or that they were consuming flocculated DOC that incorporated the labeled acetate. For instance, *Wormaldia* was highly enriched relative to sediment bacteria. *Wormaldia* is a net-spinning filter feeder that is capable of filtering bacterial-sized particles from the water because its net mesh openings are <1 μm (Wallace and Malas 1976). Bacteria trapped in the net may also take up acetate from the water column. *Stenonema,* a biofilm scraper, also was more enriched than bacteria. However epilithon was 10 times more labeled than FBOM, suggesting that epilithic bacteria were much more labeled than sediment bacteria or leaf bacteria. In a laboratory study, *Stenonema* derived 47% of its carbon from bacterial cells (Edwards and Meyer 1987). It is also possible that invertebrates could directly consume DOC either by DOC adsorbing onto biofilms or by flocculation of DOC into small particles, thus bypassing the bacterial intermediate (Hershey et al. 1996, Wotton 1988, 1996). Direct consumption of labeled acetate could explain invertebrate

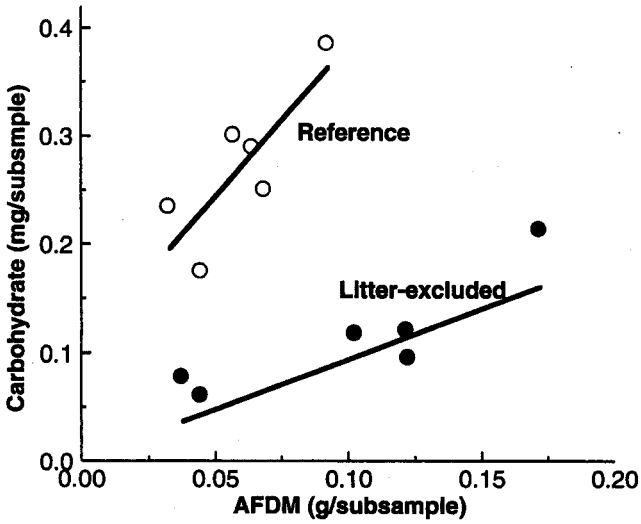

FIG. 8. Amount of colloidal carbohydrate per 2.5-mL sediment subsample plotted against the amount of ash-free dry mass (AFDM) per 2.5-mL sediment subsample. Lines are least-squares fit regression lines. The reference stream line is $y = 2.81x + 0.11$, $r^2 = 0.66$, $P = 0.048$; the litter-excluded stream line is $y = 0.092x + 0.02$, $r^2 = 0.77$, $P = 0.021$.

$\delta^{13}C$ that is higher than bacterial $\delta^{13}C$ (e.g., *Stenonema*, Table 3). Blackflies have been shown to assimilate radiolabeled DOC directly; although assimilation rates have not been determined (Hershey et al. 1996). Blackflies can also consume colloidal particles to which DOC can be adsorbed (Wotton 1976, 1988). However, we found no abiotic uptake of acetate, so it is not likely that acetate was adsorbed onto biofilms and consumed directly by invertebrates.

Invertebrate use of bacterial carbon

We found a positive relationship between the fraction of carbon derived from bacteria and the amount of amorphous detritus in invertebrate guts, showing that differences in bacterial assimilation are related to behavioral feeding differences between taxa. Amorphous detritus in seston of a blackwater river was composed of polymeric fibrils, microbes, and clays, indicating that this material is largely of microbial origin (Carlough 1994). Bacterial carbon consumed by invertebrates most likely came from amorphous detritus, and not leaf tissue. Biomass and production of bacteria on leaves is much lower than in sediments where most amorphous detritus is found. Bacterial production in the top 1 cm of sediments in another Coweeta stream was 26.4 $mg \cdot m^{-2} \cdot d^{-1}$ while production on leaves was 1.5 $mg \cdot m^{-2} \cdot d^{-1}$ (Meyer et al. 1988), which suggests that associated leaf bacteria may not be trophically important, a prediction that was verified by Findlay et al. (1986). However, we found at least 15% amorphous detritus in guts of all but two taxa (Fig. 8), including shredders. This amorphous detritus may be the source of bacterial carbon consumption detected with the isotope release. "Shredders" do not necessarily eat only leaves, but rather comminute leaves as part of their feeding process, and amorphous detritus may be associated with the leaves. Stream insects can be quite variable in their diets relative to their assumed functional feeding group and can consume a wide variety of particles (Mihuc and Minshall 1995); thus we find a measurable fraction of an invertebrate's carbon to be derived from bacteria in all taxa, regardless of their functional group.

A large fraction of invertebrate carbon was derived from bacteria, higher than that reported in previous studies. *Tallaperla* and *Tipula* from Coweeta derived <1% of their carbon from bacterial cells occurring on leaf tissue (Findlay et al. 1986). In this study we found that *Tallaperla* and *Tipula* derived a much larger percentage of their carbon (mean = 32% and 14% respectively) from bacteria. *Tipula* derived less of its carbon from bacteria relative to most other taxa (Table 4), suggesting that it does indeed assimilate mostly nonbacterial carbon.

That some predators were labeled indicates that bacterial carbon was reaching higher trophic levels. Bacteria represent a link of organic matter to higher trophic levels in these streams, which contrasts with some findings from marine pelagic ecosystems, where bacterial carbon is lost though several trophic transfers and thus is unimportant to higher trophic levels (Ducklow et al. 1986).

When using a mixing model (Eq. 1) to determine the fraction of carbon derived from either of two food sources, we assume (1) that the food source $\delta^{13}C$ does not change with time; and (2) that the consumer $\delta^{13}C$ is at equilibrium with respect to its diet, i.e., the consumer $\delta^{13}C$ is also stable. We met the first assumption: $\delta^{13}C$ of bacteria and FBOM showed no temporal trend after the first week, indicating that bacteria reached an equilibrium in less than 1 wk. Carbon turnover in invertebrates was longer. Some invertebrates had not equilibrated with their food at the end of the 3-wk period, which makes our estimate of fraction of carbon derived from bacteria an underestimate of the true value. Hence, by not correcting for invertebrate growth, we have determined more conservative estimates of bacterial consumption. Chironomid assemblages have a turnover time (biomass/production) of ~8 d at Coweeta at 15°C (Huryn and Wallace 1986); therefore, by the end of the 3-wk period all chironomids should have been exposed to the label for their entire life. However, chironomids in the litter-exclusion stream during December did not yet appear to have reached equilibrium, even though the temperature of the stream (9°C) was warm enough to allow a turnover time of 11 d. Peterson et al. (1997), using 6-wk tracer release of ^{15}N, found that invertebrates reached equilibrium $\delta^{15}N$ between 21 and 35 d after the start of the release. Fry and Arnold (1983) found rapid turnover in growing shrimp, with 50% of initial carbon remaining after 4–19 d. Although our data indicate that most invertebrates had not turned over 100% of their carbon at the end of the 3-wk period,

results from other isotope studies imply that most of their carbon turned over. The implications are that our estimates of invertebrate carbon derived from bacteria are lower than would have been measured if the animals had more time to accrue the ^{13}C label.

We saw no between-stream difference in invertebrate use of bacterial carbon, despite the severe depletion of other sources of organic matter in the litter-excluded stream. Although invertebrates were more labeled in the litter-excluded stream than the reference stream, ^{13}C label in bacteria from the litter-excluded stream was also higher than in reference-stream bacteria. The ratio of bacteria and colloidal carbohydrates to total matter was lower in the litter-excluded stream than in the reference stream (Table 3). Thus, it is possible that invertebrates in the litter-excluded stream were ingesting more unlabeled organic matter relative to labeled bacteria and exopolymer, which may decrease their fraction of carbon derived from bacteria.

Exopolymers and the microbial loop in Coweeta streams

Much of what we refer to as "bacterial carbon" was probably made up of bacterial exopolymers. We have two lines of indirect evidence for the trophic significance of exopolymers. First, we found large quantities of colloidal carbohydrates, a portion of bacterial exopolymers, which had five times more mass than bacterial cells in sediments. High exopolymer stocks have been observed in other systems. Wood biofilm in a blackwater river was composed of 7% exopolysaccharides, which was nine times the biomass of bacterial cells (Couch and Meyer 1992). Marine sediment consisted of 3–13 mg colloidal carbohydrates per gram sediment (Underwood et al. 1995). The second argument for the trophic significance of exopolymers is that invertebrates can assimilate this material. Marine harpacticoid copepods assimilated exopolymers with high efficiency (64–91%) (Decho and Moriarity 1990). Blackflies (*Simulium* spp.) can also assimilate exopolymers with efficiencies ranging from 80 to 90% (Couch et al. 1996). Wotton (1996) recently suggested that colloidal exopolymers are potentially important for the nutrition of suspension feeders in streams, both as food itself and as a means to adsorb DOC.

How do our estimates of bacterial consumption match with measures of bacterial and carbohydrate standing stocks and production in detritus? We have no direct measures of the production rate of exopolymers. If we assume that exopolymer turnover rate is only 10% that of bacterial cells (70 yr^{-1} in another Coweeta stream; Crocker and Meyer 1987), then exopolymer production would be 42 $g \cdot m^{-2} \cdot yr^{-1}$. Assuming a 40% production efficiency (Benke and Wallace 1980) and a 80% assimilation efficiency (Couch et al. 1996), measured invertebrate detritivore production (8 $g \cdot m^{-2} \cdot yr^{-1}$; Lugthart and Wallace 1992) could be derived from 25 $g \cdot m^{-2} \cdot yr^{-1}$ of exopolymer production. Hence, there is probably enough of these polymers in the stream sediments to support invertebrate production.

Exopolymers represent a small fraction of total organic matter in stream sediments, and invertebrate consumption of these polymers may be limited by the dilute nature of these compounds with respect to more refractory particulate carbon. Chironomids feeding on FBOM with a 1–4% assimilation efficiency (Schurr 1989) must consume 25–100 mg of FBOM for each milligram of assimilated biomass. We measured 4.8 μg colloidal carbohydrates/mg AFDM and 1.0 μg bacterial biomass/mg AFDM in the reference stream; so 25–100 mg of FBOM would contain 0.14–0.57 mg of bacterially derived carbon. If this material has an assimilation efficiency of 80% (Couch et al. 1996), then 11–44% of chironomid carbon requirements could come from bacterially derived carbon, which is lower than found in this study (34–100%). Because colloidal carbohydrates represent only a fraction of the extracellular material secreted by bacteria and algae (Decho 1990, Underwood et al. 1995), the fraction of assimilable bacterial organic material in sediments is probably higher than that used in these calculations.

Our findings support Cummins' (1974) early speculation that detritivorous macroinvertebrates derive much of their carbon from microbes in streams (the so-called "peanut butter"), instead of from the detritus itself (the "cracker"), but we add that exopolymers may strengthen this bacterial–invertebrate trophic link. Exopolymer consumption may be the reason why our values of fraction of carbon derived from bacteria are higher than the low percentages (~1%) found by Findlay et al. (1986) for some of the same species. Their method (tritiated thymidine) only labeled bacterial cells, whereas our method probably labeled both cells and exopolymers. Thus, the role of bacterially derived carbon in invertebrate nutrition may be higher than suggested by Lopez and Levinton (1987) because exopolymers may represent a more important carbon source than bacterial cells themselves.

Conclusion

This study demonstrates that there is a large bacterial component in detrital food webs of Coweeta streams. Using an isotopic tracer, we found that invertebrates could derive from <10 to 100% of their carbon from bacteria, and that this percentage was directly related to the proportion of amorphous detritus found in invertebrate guts. Bacterial carbon was transferred up the food web and was detectable in invertebrate and vertebrate predators. Much of this assimilated carbon may be from bacterially produced exopolymers, which were more abundant than bacterial cells. We found that leaf litter exclusion caused bacteria to rely more upon water column DOC; however, we found no difference in the significance of bacterial carbon to invertebrates in the two streams.

High consumption of bacteria is not limited to heterotrophic stream systems. In northern lakes, benthic bacterial production was estimated to account for ~53% of macrobenthic production, suggesting a tight linkage between macroinvertebrates and bacteria (Schallenberg and Kalff 1993). In an agricultural soil food web, the dominant flows were from bacteria to protozoan and metazoan consumers, with little consumption of live plant tissue (de Ruiter et al. 1995). Even in pelagic zones of lakes, bacterial production averages 30% of areal primary production (Cole et al. 1988), with bacterial respiration sometimes higher than phytoplankton production (del Giorgio et al. 1997); thus, there is a strong bacterial component to this food web. Because bacteria are often energetically dominant consumers in a food web, they must be considered quantitatively in ecosystem food webs. Much food web theory and research has focused on primary producers rather than on bacterial production (e.g., Oksanen et al. 1981). In heterotrophic Coweeta streams, primary producers are unimportant relative to bacterial and fungal decomposers.

The field method we have used is a complementary approach to more precise laboratory-based studies (Findlay et al. 1986) or inferential studies (Schallenberg and Kalff 1993) of bacterial consumption. While we found high variability because of rapidly decreasing label concentration downstream, our experiment extended through a longer time span (3 wk) and larger spatial scale (10-m stream reach) than laboratory studies. We were also able to measure many more taxa than has been done in laboratory studies. On the other hand, laboratory studies can provide a more controlled environment, which reduces variability; however, the range of estimates of percentage of invertebrate carbon derived from bacteria was also large (20–67%) for *Simulium* in a laboratory study because of natural variability in bacterial abundance in river water (Edwards and Meyer 1987). The method reported here is not limited to benthic systems. For example, Kling (1994) used whole-system carbon isotope tracers to label bacteria in lake microcosms. The method may also be useful in soils, which are similar to stream sediments in that their food webs are microbially based (Coleman 1995).

A whole-ecosystem tracer addition permits a larger perspective on the role of DOC and bacterivory in a food web because it integrates several different processes, e.g., uptake of DOC by bacteria, and feeding on extracellular material by invertebrates. This perspective will enable ecologists to more accurately assess the role of bacteria in food webs.

Acknowledgments

Thanks to Chris Peredney and Kate Dowell who assisted with much of the field and laboratory work during this study. Sue Eggert, Brian Keeley, and Bruce Wallace supplied organic matter data and maintenance of the litter exclusion net. Tom Maddox assisted with the Carlo-Erba and mass spectrometer and Curt Dell analyzed some of the isotope samples at Kansas State University. Dave Coleman, Karen Porter, Mike Paul, Cathy Pringle, Bruce Wallace, Roger Wotton, and an anonymous reviewer commented on earlier drafts of this manuscript. Discussions with Bruce Wallace, Bill Sobczak, and Stuart Findlay improved this paper. This study was funded by a NSF Dissertation Improvement Grant DEB 93-21566 to R. O. Hall and J. L. Meyer; NSF grant DEB 92-07498 to J. B. Wallace, J. L. Meyer, and J. R. Webster; and Coweeta LTER site (BSR 91-11661). Additional support while writing this paper was provided by a grant from the Andrew W. Mellon Foundation to G. E. Likens.

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