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Effects of labile carbon addition on a headwater stream food web

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Abstract

We added dextrose for two 8-week periods (summer and autumn) to a highly heterotrophic headwater stream in North Carolina, U.S.A., to examine the responses of its benthic food web to increased labile carbon. We hypothesized that addition of labile carbon would elevate microbial abundance and activity, resulting in greater resource availability and higher macroinvertebrate production and that the effect of dextrose addition would be less marked during the autumn due to lower ambient stream temperatures and large seasonal inputs of leaf litter. Bacterial densities were significantly higher in the treatment reach during both additions. Thick microbial mats of sheathed bacteria and the aquatic hyphomycete *Lemonniera pseudofloscula* developed on bedrock outcrops. Increased microbial growth led to higher respiration rates on leaf disks and a threefold increase in instantaneous growth rates of chironomid larvae. The abundance and biomass of invertebrate collector–gatherers and predators increased significantly on bedrock during the summer addition but not in the autumn; however, shredder biomass increased significantly in the autumn. On mixed substrates, shredder abundance and scraper biomass increased significantly during the autumn addition. During both additions, all functional feeding groups, including predators, assimilated isotopically distinct dextrose, despite high standing stocks of coarse particulate matter during the autumn addition. Consumers of epilithon and fine particles showed the greatest response. Assimilation of dextrose and increases in invertebrate abundance and biomass suggest that the added carbon stimulated biological activity even in a stream with abundant organic carbon.

Dissolved organic carbon (DOC) is a major source of organic matter in stream food webs and may comprise up to 98% of a stream's total organic matter inputs (Meyer 1994; Webster and Meyer 1997). While much of the dissolved organic matter present in running waters is refractory (Thurman 1985) and of limited importance in supporting food webs, smaller fractions of labile carbon are extremely important in heterotrophic energy pathways (Rounick and Winterbourn 1983; Hall and Meyer 1998). Bacteria and fungi are often limited by carbon quantity and quality in aquatic ecosystems (Bott and Kaplan 1985; Findlay and Sobczak 2000; Kaplan and Newbold 2000). Higher trophic levels have been shown to benefit directly from bacterial biomass produced by labile DOC uptake (Hall and Meyer 1998). Microbes are consumed by flagellates and ciliates, which in turn are grazed by macroinvertebrate consumers (Bott and Kaplan 1990; Carlough and Meyer 1990). The use of waste products, exudates, and decomposing consumers by microbes completes the microbial loop in small streams. Thus, carbon limitation (quantity and/or quality) may play a key role in structuring lotic food webs (Bott and Kaplan 1985; Findlay et al. 1993; Jones 1995).

Food web studies using isotopic tracer additions have found that stream invertebrates derive from <10% to 100% of their carbon from bacteria (e.g., Hall and Meyer 1998). Differences in bacterial assimilation are generally related to feeding differences among taxa. Invertebrate filterers, collector–gatherers, and shredders all derive a significant proportion of their carbon from bacteria (Hall and Meyer 1998). Epilithon, partly derived from DOC and consisting of bacteria, algae, and other organisms in a mucopolysaccharide matrix attached to hard substrates, is a valuable food for

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stream organisms, particularly for taxa within the collector–gatherer and scraper functional groups (Lock et al. 1984). Finally, invertebrate predators ingest primary consumers that consume significant amounts of material derived from DOC. Thus, a measurable fraction of the total carbon intake of stream invertebrates can be linked to bacterial production, regardless of functional feeding group.

Anthropogenic activities may alter the sources and cycling of aquatic organic carbon. Land use change, pulp mills, domestic and industrial wastewater, agriculture, and food-processing waste all potentially increase organic material inputs to streams, with potentially large consequences for stream biota (Jones 2001). Increased availability of carbon may encourage growth of nuisance bacteria or fungi, alter water chemistry, and impact higher trophic levels (Warren et al. 1964; Hedin 1989; Bernhardt and Likens 2002). For example, organic enrichment and subsequent increases in food availability may alter aquatic invertebrate community composition and ultimately lead to increases in densities of tolerant taxa while decreasing those taxa sensitive to enrichment (Hynes 1960).

Although previous studies have added labile forms of carbon to streams (Warren et al. 1964; Hedin 1989; Bernhardt and Likens 2002), few have examined the responses of invertebrate consumers or the possibility of carbon limitation in highly heterotrophic systems with large natural standing stocks of carbon. A long-term exclusion of leaf litter inputs to a forest stream showed insect production was strongly linked to particulate organic matter standing stocks (Wallace et al. 1999). In the present study, we examined the role of dissolved organic matter availability in microbial activity and macroinvertebrate growth and abundance. Based on earlier findings, we hypothesized that addition of labile carbon to a forested headwater stream would elevate microbial abundance and activity and result in greater resource availability and higher macroinvertebrate growth rates. We expected gatherers, scrapers, and filterers (consumers of epilithon and associated heterotrophic organisms) to show the greatest short-term response to treatment by assimilating more dextrose-derived carbon and having the largest increases in abundance and biomass. Finally, we predicted that the impact of dextrose addition would be lower during autumn than summer due to lower ambient stream temperatures and autumnal increases in leaf-litter standing crop. We anticipated that this natural pulse of leaf organic matter would increase background carbon availability and hence reduce the impact of the experimental labile carbon addition on the heterotrophic community.

Study site

This study was conducted in Jenny Branch (WS 4), a first-order stream that drains a 4-ha catchment at the Coweeta Hydrologic Laboratory in Macon County, North Carolina, U.S.A. Coweeta is a 1,625-ha drainage basin in the Blue Ridge Province of the southern Appalachian Mountains. The steep forested catchment (gradient ~30 cm m^{-1}) is dominated by white and red oaks (*Quercus alba* L. and *Quercus rubra* L.), tulip poplar (*Liriodendron tulipifera* L.), and dogwood (*Cornus florida* L.). A dense understory of *Rhododendron maximum* L. shades the stream year round, limiting primary productivity (Webster et al. 1983). The stream is strongly heterotrophic; litter inputs into a nearby stream total ~500 g ash-free dry mass (AFDM) m^{-2} yr^{-1} (Wallace et al. 1997). The substrate consists of bedrock outcrop or mixed substrate (cobble and pebble mixed with sand). The catchment has not been manipulated since the 1940s, with the exception of a 2-yr period (1962–1964) when the entire Coweeta basin was sprayed with DDT to control elm spanworm (*Ennomos subsignarius* Hubner). Jenny Branch has never been manipulated experimentally. Stream pH in the Coweeta basin ranges from 6.0 to 7.0. (Swank and Waide 1988). The only vertebrates present in the stream were salamanders.

Methods

Experimental design—An 80-m reach of the stream was divided into two 40-m reaches. The upstream portion served as the reference reach and received no treatment. During the summer (8 August 2001–6 October 2001) and autumn (8 November 2001–10 January 2002), a concentrated solution of dextrose (Corn Products International food grade dextrose; 250 g L^{-1}) was added to the treatment reach from a holding tank at the 40-m mark via a peristaltic pump (504S, Watson and Marlow). We chose dextrose as a carbon source because it is a readily available simple sugar that is representative of the carbon compounds commonly found in food processing by-products. Dextrose can also be traced through food webs easily using stable isotope analysis because of its distinctive $\delta^{13}C$ value. The dripper was positioned over a small cascade to ensure full mixing in the water column within a short distance from the site of addition. The rate of dextrose addition to the stream was adjusted every 3 d to keep the downstream DOC concentration at 20 mg L^{-1} above the ambient concentration of ~1–2 mg L^{-1}. In order to reduce microbial growth in the holding tank, the tank was replaced weekly with an acid-washed replacement filled with fresh dextrose solution. Water samples were taken weekly to determine DOC concentrations (5 m above dripper and 5, 20, and 40 m below the dripper).

Stream flow was calculated three times per week by weighing the amount of flow captured in a large plastic bag at a weir immediately downstream of the experimental reach. Weekly water samples were taken 5 m above the dripper in the reference reach and at 5-, 20-, and 40-m downstream from the dextrose addition. All samples were filtered through precombusted Gelman A/E glass fiber filters and stored in precombusted glass bottles at 4°C until analysis. DOC concentration was determined in duplicate using a Shimadzu TOC-5000A total organic carbon analyzer.

Bacterial counts—Twenty-five unglazed ceramic tiles (5.4 × 5.4 cm) were placed in each of the treatment and nontreatment reaches of the stream (five tiles were placed at each of five randomly selected locations throughout each reach) 1 week before each dextrose release began to allow for microbial colonization. One day prior to the start of the addition and on four other biweekly sampling dates during

each study period, five tiles (one from each of the five randomly selected locations) were removed from each reach. Tiles were preserved in the field using 5% formalin solution and were processed within 1 month of collection. Epilithon was scraped from the tiles with a soft toothbrush and suspended material was sonicated for ~15 s. Subsamples were stained with acridine orange, filtered through an irgalan black-stained nucleopore filters (0.22-μm pore size), and bacteria counted by epifluorescence microscopy at ×1,000 magnification (Hobbie et al. 1977). Ten fields with 10–50 bacterial cells were counted per slide (Kirchman et al. 1982). Average bacterial numbers for treatment and reference reaches were compared for each sampling date using 95% confidence intervals.

Leaf pack respiration—Rates of microbial respiration associated with decomposing red maple leaves were estimated from measurements of dissolved oxygen consumption. Leaf bags (1-mm mesh size) were deployed in the treatment ($n = 5$) and reference ($n = 5$) reaches of Jenny Branch at the start of each experimental period. Each bag contained 10 g of air-dried red maple (*Acer rubrum* L.) leaves collected the previous year. These bags remained in the stream for the duration of dextrose addition. At the end of each treatment period (summer experimental period = 53 d; autumn experimental period = 45 d), 10 leaf disks were cut from each leaf bag using a cork borer and placed into glass chambers containing 29 ml of membrane-filtered (0.2-μm) stream water. Changes in dissolved oxygen were monitored every 5 min for 30 min using polarographic O_2 sensors (YSI model 5100 and model 58). All measurements were conducted in darkness at ambient stream temperature. For each stream reach, an additional chamber containing only filtered stream water served as the control. After respiration rates were determined, leaf disks from chambers were dried, weighed, ashed at 500°C, and reweighed to obtain AFDM. Mean respiration rates (mg O_2 g^{-1} AFDM h^{-1}) in the treatment reach with values higher than the 95% confidence intervals of the control were considered to be significantly different.

Chironomid growth rates—Chironomid growth rates were determined for non-Tanypodinae taxa. Growth rates were measured twice during each dextrose addition (August, September, November, and December 2001). Chironomids were obtained by collecting leaf litter from nearby Coweeta streams. Leaf litter was washed through a series of nested sieves with stream water. Chironomid larvae were removed from the sieves, measured, and placed within one of three size classes: 1.5 mm, 1.5–2.5 mm, and 4.5+ mm. Each larva was measured live to the nearest 0.1 mm with an ocular micrometer under a dissecting microscope. Each size class (25–70 chironomids) was then placed in a wedge-shaped growth chamber containing red maple leaves as a substrate (4–6 leaves collected from the reach in which the specimens were to be incubated). The growth chambers were constructed of plastic frames with side panels covered with 63 μm Nitex® mesh. The tops of the chambers extended above the water surface with the base anchored to the streambed. The chambers were oriented directly into the current to reduce drag and clogging of the mesh. Three growth chambers (one containing each size class) were placed in both the treatment (20 m below the dextrose dripper) and reference (10 m above the dripper) reaches of the stream and incubated for 7–15 d. The growth chambers were positioned ~1 m apart in both reaches.

Estimates of average larval biomass before and after incubation were used to calculate growth rates. Biomass (AFDM) was obtained using length–mass regressions derived from animals in nearby Coweeta streams (Benke et al. 1999). All individuals recovered in each size class were measured after incubation. Instantaneous growth rate coefficients (IGRs) (mg mg^{-1} d^{-1}) were calculated using the following equation:

$$\mathrm{IGR} = (\ln W_f - \ln W_i)/t$$

where W_i and W_f are the initial and final larval AFDM, respectively, observed during a period (t) in days (Romanovsky and Polischuk 1982). Huryn and Wallace (1986) describe the methods and growth chambers used to determine daily growth rates in greater detail. Chironomid growth rates in the dextrose addition and reference reaches were compared by analysis of covariance (ANCOVA) using the slope of the lines generated by regressing growth against initial size of non-Tanypodinae chironomids.

Stable-isotope analysis—Stable-isotope analysis was used to assess any shifts in the fraction of carbon assimilated from natural sources toward the isotope ratio of the added labile carbon source. Dextrose is derived from C_4 plants (corn) and has a $\delta^{13}C$ (~−10‰) easily distinguished from typical leaf-derived carbon at Coweeta (−28‰ to −25‰) and periphyton of small forest streams ($\delta^{13}C$ of ~−30‰) (Smith and Epstein 1971; Hall and Meyer 1998; Findlay and Sinsabaugh 1999).

At the conclusion of the summer and autumn dextrose additions, benthic samples of coarse particulate matter (CPOM) were collected by hand (from random locations within each reach), dried at 50°C, and finely ground in a Spex® ball mill. Fine particulate organic matter (FPOM) was elutriated from inorganic sediments, sieved through a 1-mm sieve, and processed in the same manner as CPOM. Epilithon was scraped from submerged rocks in the stream, collected on Gelman A/E glass-fiber filters, and dried. Microbial growth on bedrock habitats in the treatment reach was collected, dried, and processed in the same manner as CPOM.

Invertebrates were collected from representative habitats (from random locations in bedrock outcrops and benthic habitats) in the treatment and reference reaches of Jenny Branch at the end of each addition (6 October 2001 and 10 January 2002). Benthic samples were elutriated, poured over 1-mm and 250-μm nested sieves, and animals picked from debris. Invertebrates were all larvae or nymphs. Specimens were transported to the laboratory on ice and frozen. Later, invertebrates were thawed, cleaned of any attached detritus, and their gut contents removed by dissection. Guts of chironomids were not removed because of their small size. All salamanders analyzed were larval and were processed in the same manner as invertebrates. After removing the guts, all animals were dried at 50°C. Three replicates (individuals or ground composite subsamples; ~1 mg) were analyzed for

each consumer taxon in each reach and season. Samples were weighed using a microbalance and combusted in a Carlo Erba NA 1500 CHN analyzer coupled to a Finnigan Delta C mass spectrometer as a continuous flow system. $\delta^{13}C$ was calculated according to the following equation:

$$\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1{,}000$$

where R is the ratio of $^{13}C:{}^{12}C$. Samples enriched with dextrose will have a higher $\delta^{13}C$ relative to the unenriched samples. The relative importance of the added dextrose to the diet of each taxon was calculated using a linear mixing model (Phillips and Gregg 2001). We used the $\delta^{13}C$ of dextrose (−10.6‰) and the ^{13}C of consumers in the reference reach as end members in mixing model calculations. We were unable to collect enough material for two taxa (*Paraleptophlebia* and Chironomidae) from the reference reach for analysis at the end of the summer addition. For these two taxa, we used the reference $\delta^{13}C$ values obtained at the end of the autumn addition (mean difference between summer and autumn $\delta^{13}C$ for reference taxa sampled after both additions was +0.25‰). Some invertebrates collected at the end of the autumn addition may also have assimilated dextrose from the summer addition. We comment on the potential for this carryover in dextrose label in the Discussion.

Benthic sampling—Random benthic samples were collected biweekly during summer and autumn for a total of eight collection dates. Two methods were used for benthic sampling. Bedrock habitats (outcrops) were sampled by scraping and brushing moss and associated particles from 15- × 15-cm areas into a 250-μm mesh bag that was held flush to the rock surface. Mixed substrate habitats (gravel, sand) were sampled to a depth of 10 cm with a 400-cm^2 corer (Lugthart and Wallace 1992).

Organic matter in samples, including invertebrates and salamanders, was elutriated from the inorganic substrate, passed through nested 1-mm and 250-μm sieves, and preserved in a 6–8% formalin solution containing Phloxine B dye to facilitate sorting. Animals were removed from the CPOM on the 1-mm sieve by hand picking under 15× magnification and preserved in 6–8% formalin. Material on the 250-μm sieve was subsampled (1/8–1/64 of the whole sample) using a sample splitter (Waters 1969) following Lugthart and Wallace (1992). Invertebrates in the samples were then removed by hand, counted, identified to genus, and measured (total body length) under a dissecting microscope (×15) with a graduated stage. Larval chironomids were identified as being either Tanypodinae or non-Tanypodinae. Noninsect invertebrates were generally identified to order. Following invertebrate removal, CPOM and FPOM in the samples were processed, weighed, ashed, and reweighed to obtain AFDM estimates of benthic organic matter storage (Lugthart and Wallace 1992).

Biomass (AFDM) for all insect and noninsect taxa was obtained using length–mass regressions (Benke et al. 1999). Taxa were assigned to functional feeding groups according to Merritt and Cummins (1996). Mean abundance and biomass for specific taxa, functional feeding groups, and the total invertebrate community were estimated separately for mixed substrate and bedrock habitats. Invertebrate abundance and biomass in the treatment and reference reaches were analyzed by ANCOVA to compare the slopes of lines generated by regressing abundance or biomass versus time for each treatment period. Organic matter standing crops (g AFDM m^{-2}) for mixed substrate and bedrock habitats during the summer and autumn dextrose additions were compared using two-way ANOVA.

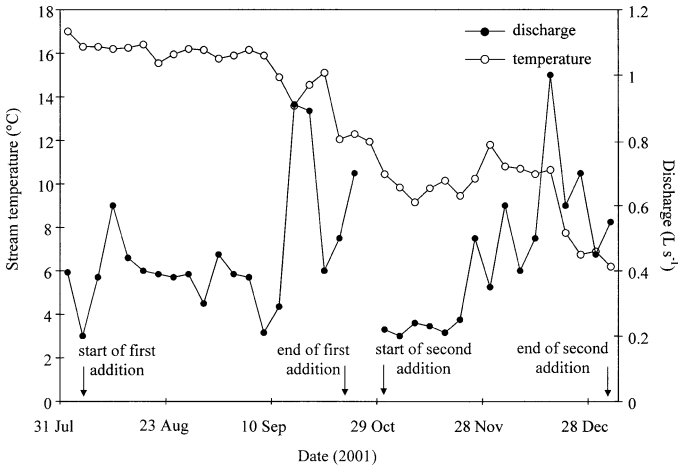

Fig. 1. Discharge and temperature data for Jenny Branch from 31 July 2001 to 31 December 2001.

Results

Physical and chemical characteristics—During the summer dextrose addition, stream flow ranged from 0.2 to 0.9 L s^{-1}, and water temperatures ranged between 11.6°C and 17°C (Fig. 1). Stream flow ranged from 0.2 to 1.0 L s^{-1} and temperatures ranged between 6.2°C and 11.7°C during the autumn addition. DOC concentrations in the reference reach averaged 0.91 ± 0.14 mg L^{-1} (mean ± 1 SE, n = 12; range 0.46–2.27 mg C L^{-1}) throughout the experiment (Fig. 2). Average concentration of DOC in the treatment reach (combined from all sampling stations) during the summer addition was 5.21 ± 1.80 mg L^{-1} (mean ± 1 SE, n = 6; range 0.42–21.54 mg L^{-1}) (Fig. 2). Until day 48 of the summer dextrose addition, DOC concentration in the treatment reach was not markedly higher than that of the reference reach, indicating very rapid uptake and dilution by storm flows (Figs. 1 and 2). During the summer addition, mean concentrations of DOC decreased from the site of DOC addition (Fig. 2). During the autumn addition, DOC concentrations in the reference reach averaged 0.89 ± 0.18 mg L^{-1} (mean ± 1 SE, n = 12; range 0.41–1.59 mg L^{-1}). Mean DOC concentration in the treatment reach during the autumn addition was 10.90 ± 1.97 mg L^{-1} (mean ± 1 SE, n = 6; range 0.44–25.46 mg L^{-1}). DOC concentrations in the treatment reach were greatly elevated above ambient concentrations in autumn. DOC concentration in the treatment reach was highest on day 18 (26 November 2001) at 5 m from the dextrose addition site and then decreased on each subsequent sampling date (Fig. 2).

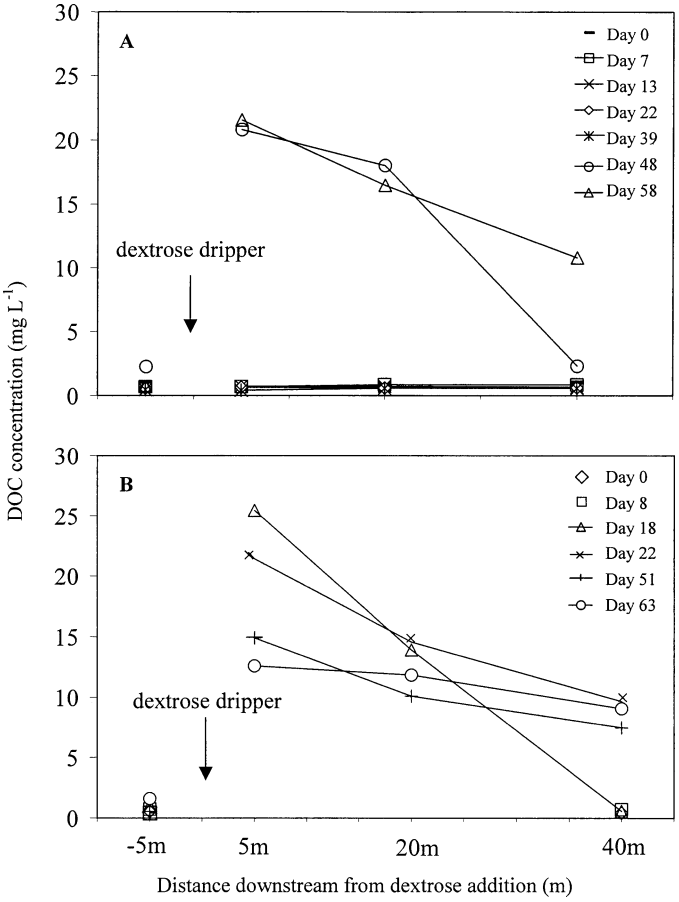

Fig. 2. Water column DOC concentrations on (A) seven sampling dates during the summer dextrose addition (August 2001–October 2001) and (B) six sampling dates during the autumn dextrose addition (November 2001–January 2002).

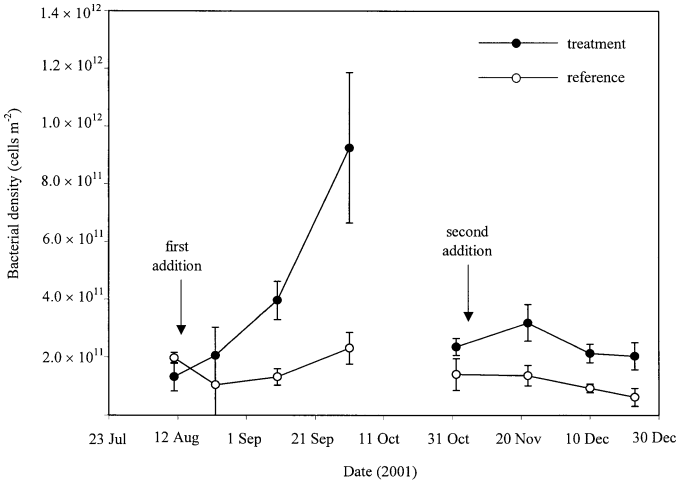

Fig. 3. Average bacterial densities on ceramic tiles in the treatment and reference reaches of Jenny Branch during both dextrose additions. Error bars are 95% confidence intervals based on duplicate samples.

Organic matter—Total standing crop of CPOM in the mixed substrate habitat of Jenny Branch was significantly lower during the summer experimental period (mean = 285 ± 63 g AFDM m^{-2}) than in the autumn (mean 745 ± 230 g AFDM m^{-2}). There was no significant difference in total CPOM or total FPOM between the reference and in the treatment reaches in either season. Total FPOM in mixed substrate or bedrock habitats did not differ between seasons (mean summer = 576 ± 54 g AFDM m^{-2}; mean autumn = 602 ± 56 g AFDM m^{-2} [data not shown]).

Microbial response—Tiles incubated for 59 d during the summer addition showed large increases in bacterial density in the treatment reach compared with the reference reach (Fig. 3). There was no clear pattern in bacterial density in relation to distance downstream from the dextrose addition (R^2 = 0.17, p = 0.75, R^2 = 0.38, p = 0.207). Average bacterial densities in the treatment reach during the summer study period were nearly three times higher (4.15 ± 1.79 × 10^{11} cells m^{-2} [mean ± 1 SE]) than in the reference reach (1.66 ± 0.29 × 10^{11} cells m^{-2}). Bacterial densities increased in the treatment reach from 1.32 ± 0.25 × 10^{11} cells m^{-2} to 9.26 ± 1.30 × 10^{11} cells m^{-2} over the course of the addition. By the third sampling date (10 September 2001), treatment densities were above 95% confidence limits of the reference reach mean. During the autumn study period (beginning on 2 November 2001), bacterial densities were slightly higher in the treatment reach before the addition started and were on average lower than those during the summer. Bacterial densities in the treatment reach did not increase over time as in the summer addition. However, average bacterial densities in the treatment reach (2.42 ± 0.23 × 10^{11} cells m^{-2}) were double those of the reference reach (1.08 ± 0.11 × 10^{11} cells m^{-2}) (Fig. 3). Treatment-reach bacterial densities were significantly higher than those of the reference reach on all sampling dates during both study periods, despite a constant decrease in average daily water temperatures (Fig. 1).

As early as 1 week into each study period, the response of the benthic microbial community was visible to the naked eye as growth on tiles. As the experiments continued, thick (~5-mm) microbial mats developed, particularly in areas of higher velocity flow on bedrock outcrops. Microscopic examination of microbial mats revealed an abundance of nonsheathed bacteria and sheathed bacteria of the genus *Sphaerotilus* (Mulder and Deinema 1981). The aquatic hyphomycete *Lemonniera pseudofloscula* dominated the fungal community (Gulis and Suberkropp pers. comm.).

During the summer dextrose addition, mean respiration rates of leaf disks taken from litter bags in the treatment reach (0.17 mg O_2 h^{-1} g^{-1} AFDM) were more than triple those of disks from litter bags in the reference reach (average rate 0.05 mg O_2 h^{-1} g^{-1} AFDM) (Fig. 4). Respiration rates during the autumn study period were, on average, lower than those during the summer experiment ($p > 0.05$), and there was no significant difference between leaf disk respiration rates in the treatment and reference reach.

Incorporation of labile carbon into the food web—The added dextrose had a ^{13}C value of −10.6‰. FPOM collected from the treatment reach shifted in $\delta^{13}C$ toward the $\delta^{13}C$ of the added dextrose (−27.3‰ in the reference reach to

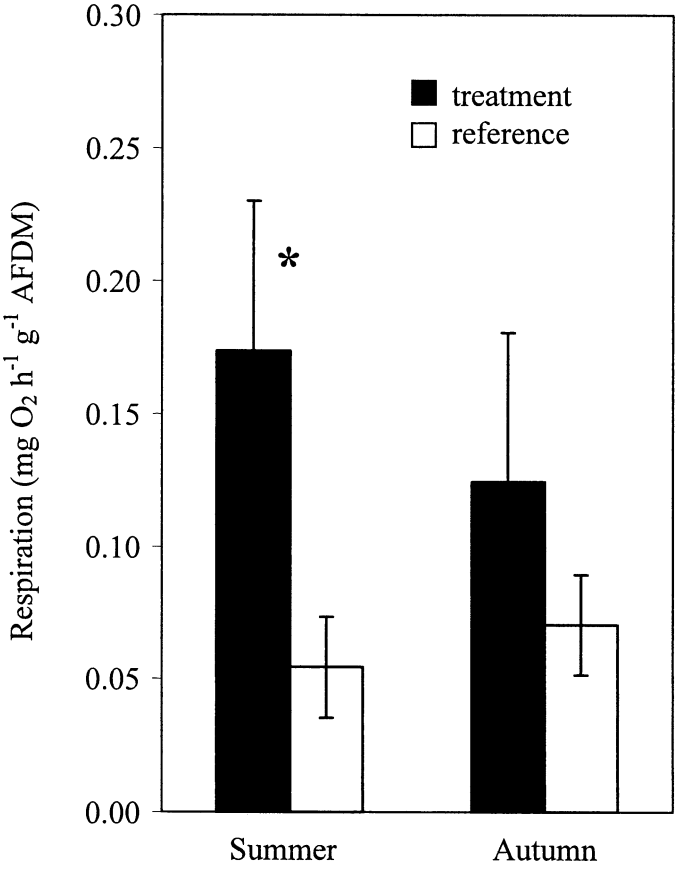

Fig. 4. Leaf disk respiration rates for disks incubated in the treatment and reference reaches of Jenny Branch during the summer (August 2001–October 2001) and autumn dextrose additions (November 2001–January 2002). Error bars represent 95% confidence intervals. * indicates a significant difference at $p < 0.05$.

−26.5‰ in the treatment reach during the summer experimental period) (Table 1). The δ^{13}C value of epilithon shifted from −27.2‰ in the reference to −25.7‰ in the treatment during the summer. However, these differences were small and may have been due either to the dextrose addition or to natural variation in δ^{13}C values. Average CPOM δ^{13}C values in the treatment reach were not found to have shifted during either addition. The microbial mat, containing a mixture of bacteria (*Sphaerotilus*) and the aquatic hyphomycete *Lemonniera pseudofloscula,* was highly enriched with a δ^{13}C value of ~−12‰ during both additions.

All invertebrate taxa collected within the treatment reach during both dextrose additions were ^{13}C enriched relative to those in the reference reach, reflecting a shift toward the δ^{13}C of the added dextrose (Table 1). Using the linear mixing model, standard errors for source contributions of dextrose to macroinvertebrate carbon were all <2% of the mean. Several taxa in the treatment reach were found to have >50% reliance on the added dextrose carbon during one or both additions, including the mayflies *Paraleptophlebia* sp. and *Stenonema* sp., the stonefly *Leuctra* spp., and the caddisfly *Wormaldia* spp. (Table 1). Non-Tanypodinae chironomids were the most strongly labeled during the summer treatment (−14.2‰) and obtained the largest proportion of their carbon from dextrose (78%; Table 1). *Paraleptophlebia* sp. and *Stenonema* sp. were the most enriched organisms during the autumn addition (−15.5‰ and −15.9‰, respectively) and obtained 68% of their carbon from the added dextrose. We were unable to collect Chironomidae and *Paraleptophlebia* sp. in the reference reach for analysis during the summer addition. However, we are confident that their δ^{13}C values in the treatment reach were high enough to rule out the possibility that these values could occur due to natural variation. For other invertebrates, the average difference in the reference reach δ^{13}C values between summer and autumn was only +0.25‰, so, assuming these two taxa followed the same pattern, using autumn δ^{13}C values would slightly underestimate contribution of dextrose C.

A large amount of variation in reliance on dextrose-derived carbon was observed within functional groups. While collector–gatherers and scrapers in the treatment reach (non-Tanypodinae chironomids, *Paraleptophlebia* and *Stenonema*) had δ^{13}C values greatly shifted toward that of the added dextrose (−14.2‰, −16.0‰, and −15.8‰, respectively), oligochaetes in the treatment reach had δ^{13}C values (−22.6‰) only slightly higher than those found for most organisms in the reference reach during the summer addition (Table 1). The scraper mayfly *Stenonema* was highly labeled during both additions. The average δ^{13}C of *Stenonema* shifted from −26.2‰ to −15.8‰ during the two additions.

Both gatherers (non-Tanypodinae chironomids and *Paraleptophlebia*) in the treatment reach sampled during autumn had δ^{13}C values shifted toward that of dextrose (Table 1). All shredder taxa in the treatment reach were ^{13}C enriched during both addition periods. Reference reach shredder δ^{13}C ranged from −27.6‰ to −25.1‰, while values in the treatment reach ranged from −23.2‰ to −16.8‰. *Leuctra* had the highest δ^{13}C value in the treatment reach during both additions.

During the summer addition, the filtering caddisflies *Parapsyche* and *Diplectrona* both had enriched δ^{13}C values in the treatment reach (Table 1). Shifts in average δ^{13}C values were −25.7‰ to −21.2‰ for *Parapsyche* and −25.3‰ to −21.0‰ for *Diplectrona. Wormaldia* was the most enriched filterer (−16.9‰) at the end of the autumn addition. As in the summer treatment period, the filterers *Parapsyche* and *Diplectrona* were both ^{13}C enriched during the autumn addition. Average δ^{13}C for *Parapsyche* was −20.6‰ while that of *Diplectrona* was −19.5‰.

All predators, with the exception of the salamander *Desmognathus,* were ^{13}C enriched in the treatment reach during the summer addition (Table 1). Mean predator δ^{13}C ranged from −24.2‰ to −23.6‰ in the reference reach. In the treatment reach, their δ^{13}C ranged from −23.2‰ to −21.4‰. The predatory dipteran *Pedicia* was the most ^{13}C enriched, shifting from −23.8‰ to −21.4‰. Predator δ^{13}C during the autumn addition was between −18.2‰ for *Hexatoma* and −23.0‰ for the salamander *Eurycea.* Reference ^{13}C values ranged from −23.2‰ to −24.0‰. As in the summer addition, predators were the least labeled of all the functional feeding groups and, for some taxa, differences in δ^{13}C between reaches were quite small.

Table 1. Two-source linear mixing model results for taxa collected from the treatment and reference reaches of Jenny Branch at the end of the summer (Aug–Oct 2001) and autumn (Nov 2001–Jan 2002) dextrose addition periods. Bold type indicates taxa with >50% reliance on dextrose carbon in the treatment reach. — indicates no data. *, calculated using autumn reference $\delta^{13}C$.

		Summer			Autumn		
		$\delta^{13}C$ (‰)		Contribution of dextrose,	$\delta^{13}C$ (‰)		Contribution of dextrose,
Functional group	Taxon	Reference	Treatment	%	Reference	Treatment	%
Collector–gatherers	Oligochaeta	—	−22.6	—	—	—	—
	Paraleptophlebia	—	−16.0	**65***	−25.9	−15.5	**68**
	Chironomidae	—	−14.2	**78***	−27.0	−18.8	**50**
Scraper	*Stenonema*	−25.9	−15.8	**66**	−26.6	−15.9	**68**
Shredders	*Leuctra*	−24.6	−18.1	46	−25.3	−16.8	**58**
	Talloperla	−25.1	−19.0	42	−26.5	−21.1	34
	Fattigia	−26.6	−18.8	49	−26.0	—	—
	Pycnopsyche	—	—	—	−27.6	−23.2	26
	Psilotreta	—	−23.4	—	—	—	—
	Tipula	−26.4	−22.4	25	−26.6	−20.4	39
Filterers	*Diplectrona*	−25.3	−21.0	29	−25.0	−19.5	38
	Parapsyche	−25.7	−21.2	30	−25.2	−20.6	32
	Wormaldia	—	—	—	−25.7	−17.0	**57**
Predators	*Cordulegaster*	−24.2	−22.5	13	−24.2	−20.1	30
	Lanthus	−23.6	−21.6	16	−23.6	−21.9	13
	Beloneuria	−24.0	−22.8	9	−24.5	−19.7	35
	Hexatoma	−23.9	−21.9	15	−24.5	−18.2	45
	Pedicia	−23.8	−21.4	18	—	—	—
	Desmognathus	−23.7	−23.3	3	−23.0	−20.7	19
	Eurycia	−23.2	—	—	−23.2	−23.0	2
	Sweltsa	—	—	—	−24.2	−19.0	38
	Isoperla	—	—	—	−25.1	−18.9	43
	Rhyacophila	—	—	—	−24.4	−20.9	25
Basal resources	Dextrose	—	−10.6	—	—	−10.6	—
	Lemonniera mat	—	−12.3	—	—	−11.4	—
	FBOM	−27.3	−26.5	—	−27.3	−27.7	—
	CPOM	−28.1	−28.9	—	−28.5	−28.1	—
	Epilithon	−27.2	−25.7	—	−25.4	−25.3	—
	Moss	−30.0	−27.6	—	−27.9	−31.4	—

Invertebrate abundance and biomass—Total invertebrate abundance in bedrock habitats did not significantly increase over the course of the summer dextrose addition. Invertebrate biomass did show a small but significant (ANCOVA $F_{3,31} = 4.38$, $p = 0.049$) increase in bedrock habitats in the treatment reach (Fig. 5). At the end of the autumn dextrose addition, total invertebrate abundance in bedrock habitats of the treatment reach was 5× higher than in the reference reach but there was no change in biomass. There were no significant differences in total invertebrate abundance or biomass between the two reaches during either dextrose addition period in mixed-substrate habitats.

Functional group response—Response to the dextrose addition varied among functional feeding groups. Total collector–gatherer abundance increased in the bedrock habitats of the treatment reach during the summer addition and was 3× higher than in the reference (ANCOVA $F_{3,31} = 10.57$, $p = 0.004$). Collector–gatherer biomass in the treatment reach was 5× higher than in the reference (ANCOVA $F_{3,31} = 19.49$, $p = 0.0003$). No other functional group's total abundance or biomass differed significantly between the treatment and reference reaches during the summer or the autumn addition period in bedrock habitats.

In mixed-substrate habitats, there was no difference in any functional feeding group's abundance or biomass during the summer dextrose addition. During the autumn addition, total scraper biomass significantly increased in the treatment reach and was 3× higher than the reference reach (ANCOVA $F_{3,31} = 4.40$, $p = 0.045$). No other functional group showed a significant response to treatment in mixed substrate habitats.

In bedrock habitats, individual taxa within the collector–gatherer functional feeding group showed the greatest response to treatment during the summer addition. Average non-Tanypodinae chironomid densities increased by a factor of three in the treatment reach (ANCOVA $F_{3,31} = 10.34$, $p = 0.004$). Average nematode densities and biomass in the treatment reach were 8× higher than in the reference reach (ANCOVA $F_{3,31} = 5.53$, $p = 0.029$ and ANCOVA $F_{3,31} = 5.77$, $p = 0.026$). *Serratella* (Ephemeroptera) abundance increased 17-fold and had 5× greater biomass (ANCOVA $F_{3,31}$

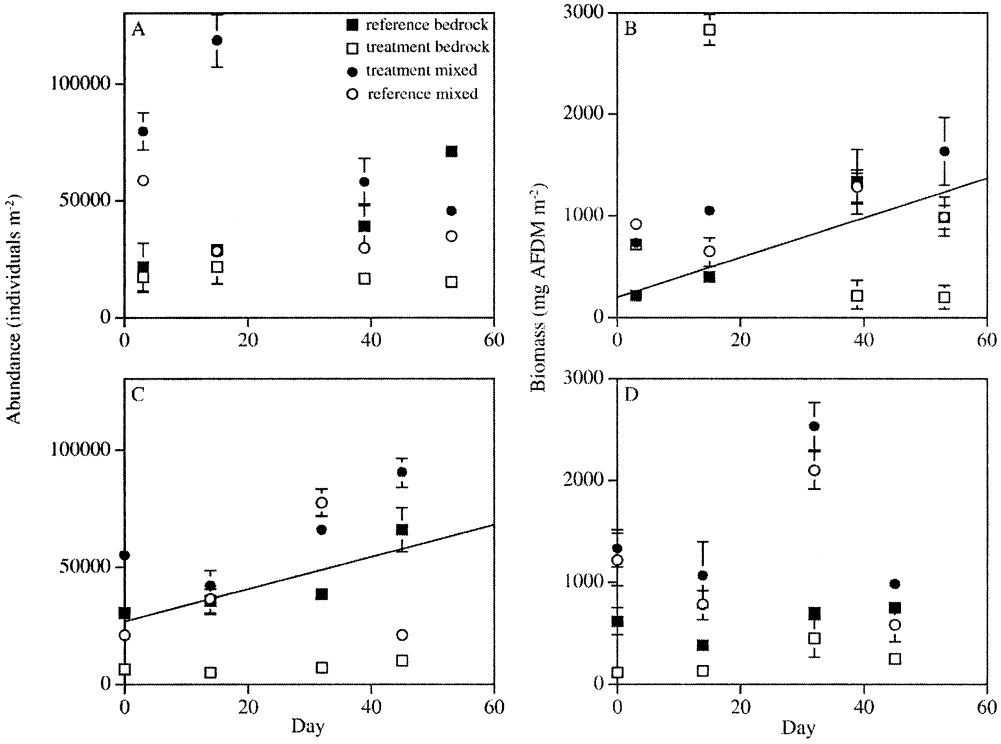

Fig. 5. Mean macroinvertebrate abundance and biomass (±1 SE) during the (A, B) summer and (C, D) autumn DOC additions to Jenny Branch. Significant increases during the additions (i.e., positive slopes) are shown as solid lines for summer biomass (B) and autumn abundance (C) in bedrock habitats.

= 12.95, $p = 0.002$ and ANCOVA $F_{3,31} = 4.73$, $p = 0.042$) in the treatment reach. Copepod densities increased dramatically in bedrock habitats during both additions, but this increase was not statistically significant (mean copepod density during both additions: reference reach = 525 m^{-2}, treatment reach = 4905 m^{-2}). Non-Tanypodinae chironomids (6× higher) and Ceratopogonidae (Diptera) (400× higher) both increased in abundance during the autumn experimental period in bedrock habitats (ANCOVA $F_{3,31} = 6.86$, $p = 0.017$ and ANCOVA $F_{3,31} = 5.73$, $p = 0.027$, respectively).

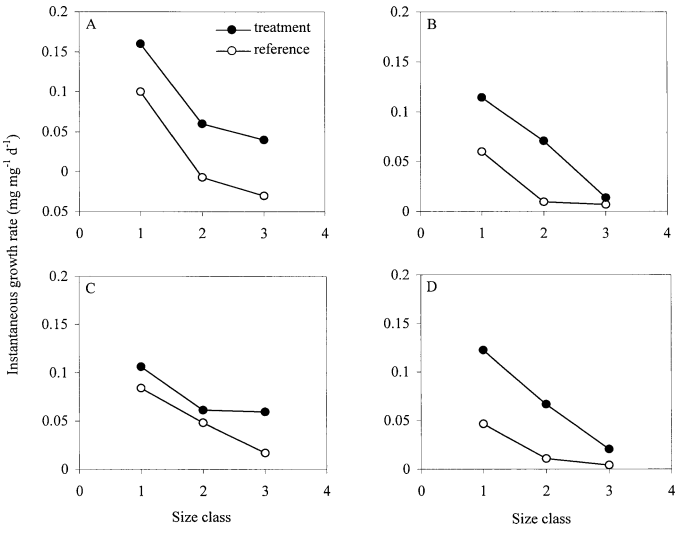

Fig. 6. Instantaneous growth rates for Chironomidae incubated in Jenny Branch during (A) August, (B) September, (C) November, and (D) December 2001. Size class 1 = chironomids with initial length ≤1.5 mm, size class 2 = 1.5–2.5 mm, and size class 3 = 4.5 mm.

In the mixed-substrate habitat during the summer dextrose addition, abundances of *Lepidostoma* (Trichoptera), *Tipula* (Diptera), and non-Tanypodinae chironomids in the treatment reach were twice those of the reference reach. Only *Lepidostoma* increased significantly in biomass (2× higher in the treatment reach) during the summer addition (data not shown). During the autumn addition in the mixed-substrate habitats of the treatment reach, non-Tanypodinae chironomid abundance increased significantly and was twice that of the reference reach. No other taxon showed a significant response to the treatment.

Chironomid growth—Chironomid growth rates were significantly higher in the treatment than in the reference reach (ANCOVA $F_{1,20} = 26.01$, $p < 0.0001$; Fig. 6). Average growth rates in the treatment reach were >2× higher than in the reference reach. Growth rates of chironomids were not significantly related to temperature in the reference reach ($R^2 = 0.001$, $p = 0.92$) or treatment reach ($R^2 = 0.007$, $p = 0.80$). Smaller size classes in both the treatment and reference reaches had consistently higher growth rates than the larger size classes (Fig. 6). Average lengths of larvae in all size classes increased. Negative growth rates in the reference

reach in August were an artifact of limited recovery of individuals. The recovery of individuals from the growth chambers ranged from 11% to 84% of the organisms originally introduced to the growth chambers. There were no significant differences in mortality rates between the two reaches.

Discussion

Addition of a labile form of DOC resulted in a large response by the Jenny Branch community. Increased microbial growth resulted in higher leaf-disk respiration rates on leaves during the summer dextrose release. Chironomids grew faster in the treatment reach during both additions. Assimilation of dextrose, undoubtedly mediated via microbial uptake, increases in invertebrate abundance and biomass, and the alteration of $\delta^{13}C$ values of consumers all suggest that the added carbon was an important food resource during both additions, despite a high ambient standing crop of leaf litter in the autumn. This was particularly apparent for consumers of epilithon and FPOM.

DOC removal from water column—Sediments and sediment-bound organisms rapidly removed added DOC from the water column in the experimental reach. DOC was removed from the water column throughout the summer and autumn addition periods, indicating that Jenny Branch, like other streams to which DOC has been added, has a great capacity for processing added labile DOC (McDowell 1985; Meyer et al. 1988; Hedin 1989). The lack of elevation of DOC concentration above ambient levels until day 48 of the summer dextrose addition is further evidence of the large capacity for uptake of labile carbon by Jenny Branch. Conservative tracer experiments conducted in headwater streams of the Appalachian region have shown extensive and rapid penetration of the sediments by infiltrating water (Munn and Meyer 1988); prolonged contact between the water and sediments may have increased the rate at which DOC was removed from the water column during the experimental periods. The rate at which surface waters enter sediments has been linked to the rate at which DOC may be removed from the water column (Meyer 1990). Published rates of uptake of added labile DOC in North Carolina streams that are geomorphically similar to Jenny Branch are considerably higher than rates found in streams in other areas of North America (Meyer 1990). Additionally, several studies have indicated that higher concentrations of DOC are linked to higher rates of removal of DOC from the water column (McDowell 1985; Meyer et al. 1988). It seems plausible, therefore, that DOC-removal rates in Jenny Branch were elevated due to increased loading of labile DOC. Additionally, DOC sampling on day 39 (17 September 2001) occurred during a large storm event (days 38–40; *see Fig. 1*), which would have diluted DOC concentrations in the treatment reach.

Effects of dextrose addition on the microbial community—Natural carbon inputs to Coweeta streams are likely to be much more refractory than the dextrose we added to Jenny Branch. The experimental addition of a labile form of carbon represented a large and sudden increase of a biologically available energy source. Rapid uptake of DOC by sediments is likely due to uptake by microbes, which are responsible for the majority of DOC removal from the water column (Dahm 1981). The addition of labile carbon led to higher bacterial densities during both dextrose addition periods, reflecting the importance of sediment-bound organisms in determining quantity and quality of DOC present in a stream through selective consumption of specific fractions of DOC (Findlay et al. 1993; Volk et al. 1997). In a lowland stream in Germany, monosaccharides (such as dextrose) were retained more effectively by the sediments than other sugars and bacterial production was strongly correlated with DOC retention within sediments (Fischer et al. 2002).

Metabolic activity of bacteria is often regulated by the concentration and composition of DOC present in a system (Kaplan and Bott 1989; Baker et al. 1999). Higher respiration rates observed during the summer addition were the result of increased microbial activity in the treatment reach. Temperature influences heterotrophic respiration; therefore, lower ambient stream temperature most likely led to lower densities of bacteria and decreased leaf-pack respiration rates during the autumn dextrose addition (Sinsabaugh 1997). Increases in substrate surface area associated with autumnal leaf input also may have influenced microbial respiration rates and bacterial densities (Tank and Webster 1998).

The addition of labile carbon to streams has resulted in growth of the slime bacterium *Sphaerotilus natans* (Warren et al. 1964; Hedin 1989). Microscopic examination of the microbial growth present in Jenny Branch showed that it was a complex of bacterial cells and the fungus *Lemonniera pseudofloscula.* While microbial growth in other studies was mainly associated with sediments, growth in Jenny Branch was concentrated in high-velocity bedrock habitats, possibly due to increased diffusion of DOC into epilithon caused by faster delivery rates and decreased depth of the boundary layer (Borchardt 1996). Bacterial densities were not significantly higher in the treatment reach until the third sampling date. However, the first signs of microbial growth within the treatment reach were observed within 1 week of the dextrose addition and copious growth was evident on bedrock outcrops by day 23 of the summer addition (Wilcox pers. obs.). Extracellular polymers have been found to retain DOC in biofilms, where it can be stored and utilized by microbes (Freeman and Lock 1995; Fiebig 1997). Dense microbial growth in bedrock habitats suggests high uptake of added DOC by fungi and bacteria located in the epilithon. Although epilithic biofilms often represent a small proportion of total biomass, they can be a significant source of energy to higher trophic levels in stream ecosystems (Lock et al. 1984).

Assimilation and use of dextrose-derived carbon by invertebrates—Assimilation of dextrose by all functional feeding groups suggests that the added carbon was an important basal resource for the food web during both dextrose additions. We originally predicted that the impact of dextrose addition would be lower during autumn than summer due to higher CPOM availability. However, other studies have indicated that epilithon and FPOM may efficiently transfer

Table 2. Percent (%) of invertebrate carbon derived from bacteria (WS 53) and dextrose (Jenny Branch) for 11 taxa in functional feeding groups during Jul. and Dec. 1994 in WS 53 (Hall and Meyer 1998) and during October 2001 and January 2002 in Jenny Branch (WS 4; this study). Entries with — indicate taxa for which no data were available.

Functional feeding group	Insect order	Taxon	WS 53 Jul 94	WS 53 Dec 94	Jenny Branch Oct 01	Jenny Branch Jan 02
Shredders	Plecoptera	*Leuctra*	77	>100	46	58
		Tallaperla	38	31	42	34
	Trichoptera	*Fattigia*	—	15	49	—
		Pycnopsyche	—	>100	—	26
	Diptera	*Tipula*	19	10	25	39
Gatherers	Ephemeroptera	*Paraleptophlebia*	—	>100	65	68
	Diptera	*Chironomidae*	34	—	78	50
Filterers	Trichoptera	*Diplectrona*	—	53	29	38
		Parapsyche	—	52	30	32
		Wormaldia	>100	>100	—	58
Scraper	Ephemeroptera	*Stenonema*	—	>100	66	68

DOM to higher trophic levels despite large standing stocks of CPOM (McCutchan and Lewis 2002; Simon et al. 2003).

The number of trophic transfers between bacterial production and macroinvertebrates can be numerous (Allan 1995). Dextrose-derived carbon may have been assimilated by macroinvertebrates through several pathways, including direct consumption of bacterial cells by filterers (e.g., Simuliidae), selective grazing of epilithon, consumption of particle and leaf-associated bacteria and fungi, and on to predators via the metazoan food web. Likely primary consumers of microbial production resulting from the addition of dextrose include protists and micrometazoans. In particular, ciliates and flagellates often exert significant grazing pressure on benthic bacteria (Bott and Kaplan 1990). These protists increased in density during the autumn dextrose addition (Norman unpubl. data). Increases in numbers of protists represented a possible increase in food resources for higher trophic levels. Meiofauna, including copepods, oligochaetes, and nematodes, grow rapidly and may be consumed in great numbers by larger invertebrate consumers. Consumption of meiofauna by macroinvertebrates represents an alternative means for dextrose carbon assimilation.

In general, collector–gatherers derived the highest portion of their carbon from added dextrose (average contribution from dextrose carbon was 65%). Relatively small body size, high growth rates, and the ability to feed on fine particles (<1 mm) likely led to the high contribution of dextrose carbon to the $\delta^{13}C$ of this trophic group. *Stenonema* (Ephemeroptera) are scrapers whose diet includes detritus and diatoms. *Stenonema* were highly enriched and had $\delta^{13}C$ values considerably higher than FPOM or epilithon. This suggests that *Stenonema* were selectively feeding on highly enriched particles within the epilithon or even on the fungal/bacterial growth containing *Lemonniera* itself. Invertebrates are able to consume DOC adsorbed onto epilithon directly, thus bypassing the bacterial intermediate (Hershey et al 1996; Wotton 1996). Consumption of epilithon containing *Lemonniera* and direct consumption of dextrose-derived DOC may have allowed *Stenonema* to remain highly labeled despite lower bacterial growth present in Jenny Branch during the autumn addition.

Other functional groups were not as strongly enriched in ^{13}C as a result of the dextrose addition. Broad differences in the relative contribution of dextrose carbon seen within the shredder and predator functional groups can perhaps be traced to differential consumption of bacterial cells and exopolymers, prey type consumed, or to differences in growth rates. Hall and Meyer (1998) found that many of the same shredder taxa that were highly labeled in our study (*Leuctra* and *Tallaperla*) derived a larger portion of their carbon from bacteria than other taxa in the same functional group (Table 2). All shredders sampled in their study derived a portion of their carbon from the added ^{13}C-acetate. *Leuctra,* although classified as a shredder (Merritt and Cummins 1996), has been found to be primarily a collector in early instars and relies heavily on FPOM and organic matter, particularly in bedrock habitats (Dobson and Hildrew 1992). Higher quality of FPOM in bedrock habitats in the treatment reach of Jenny Branch during the additions most probably contributed to the high labeling observed for *Leuctra*. Bacterial carbon may consist of a large amount of bacterial exopolymers (Hall and Meyer 1998), which may play an important role in supporting invertebrate production (Couch et al. 1996). Our values of the fraction of carbon derived from added dextrose and, by proxy, microbial growth, for the same species are similar to the percentages found by Hall and Meyer (1998; *see Table 2*), who suggested that exopolymers may represent a more important carbon source than the bacterial cells themselves.

Some predators obtained >40% of their carbon from dextrose during the autumn addition (*Isoperla* and *Hexatoma*), suggesting that, despite a relatively short experimental period, dextrose-derived carbon was reaching higher trophic levels. *Isoperla* and *Hexatoma* were presumably more enriched than other predators because they feed mostly on Chironomidae (Merritt and Cummins 1996), which were highly labeled in the treatment reach. These taxa are univoltine and faster growing. Other predators (e.g., *Cordulegaster, Lan-*

thus, Beloneuria) are considered to be generalists and are all semivoltine or merovoltine and slower growing.

Contrary to our expectations, filterers were not as highly labeled as collector–gatherers. The exception was *Wormaldia* (δ^{13}C of −16.98‰ during autumn addition). Filter feeders often do not fit neatly into functional feeding groups. Although *Parapsyche, Diplectrona,* and *Wormaldia* have roughly the same body size and cohort production interval (~300 d), the three taxa feed on very different foods. The capture net built by *Wormaldia* has a smaller mesh size (<1 μm) than either *Parapsyche* or *Diplectrona,* allowing it to filter bacteria-sized particles from the water column (Wallace and Malas 1976). The ability to filter bacteria-sized particles contributed to the high enrichment of *Wormaldia* during Hall and Meyer's (1998) ^{13}C-acetate addition. In contrast, gut analyses by Benke and Wallace (1980) have shown that *Parapsyche* ingests a higher proportion of animal matter than *Diplectrona* and animal matter is most significant for growth and production. The type of food captured by each taxon may explain the higher labeling of *Wormaldia* and possibly the small differences observed in δ^{13}C between *Parapsyche* and *Diplectrona* at the end of the autumn addition.

The degree to which an organism was labeled by the δ^{13}C of dextrose in our experiment was a function of its turnover of body carbon as well as its diet. Our additions were likely too short for δ^{13}C values of all taxa to equilibrate fully with their dextrose-enriched diet (Hamilton et al. 2004). Fast-growing chironomids with high turnover rates (~8 d at 15°C at Coweeta; Huryn and Wallace 1986) were highly labeled, having been exposed to the dextrose-derived carbon for a large proportion of their life cycle. In contrast, slower growing, larger bodied taxa may not have turned over enough of their carbon during the additions to reach a new isotopic equilibrium. In support of this, some invertebrates sampled in a nearby Coweeta stream were found not to have reached isotopic equilibrium with their food at the end of a 3-week ^{13}C addition (Hall and Meyer 1998). Although our additions were longer (8 weeks), we may have underestimated the contribution of dextrose-derived carbon to the trophic support of some taxa. However, some slow-developing taxa assimilated a relatively large proportion of dextrose carbon, particularly in the autumn addition. Invertebrates with cohort production intervals greater than 60 d may have incorporated dextrose-derived carbon during both addition periods and therefore be disproportionately labeled. Carryover of label from the summer dextrose addition would have been most likely in relatively slow-growing taxa that were present as individuals during both summer and autumn and that relied heavily on dextrose-derived carbon during the summer period. Several taxa (*Leuctra, Paraleptophlebia,* and *Wormaldia*) meet these criteria and may have contained some label from the summer addition.

Effect of dextrose addition on invertebrate abundance and biomass—Higher trophic levels in Jenny Branch directly benefited from the microbial biomass produced by DOC uptake. Increased microbial activity in the treatment led to greater food resource availability for chironomid larvae. Huryn (1990) found that the substrate in growth chambers had no influence on growth and that larvae fed predominately on fine organic material entering the incubation chambers. It is likely, therefore, that the high microbial content of FPOM in the treatment reach led to higher growth rates. Consequently, increasing the amount of high-quality organic matter enhanced invertebrate production in Jenny Branch.

A greater number of taxa increased in density or biomass during the summer dextrose addition than in the autumn addition. While collector–gatherers (Nematoda, *Serratella,* and Chironomidae) increased in abundance during the summer addition, scraper and filterer densities did not increase, contrary to our hypothesis. There were no significant changes in filterer biomass during either addition. Chironomids, which are known to use bacterial biomass in sediments and are typically tolerant of organic enrichment (Rounick and Winterbourn 1983), were the only taxon to respond to the labile carbon, with increases in abundance during both dextrose additions in both habitats sampled. Increases in Chironomidae abundance are typical of results seen in other experimental additions of organic matter to stream ecosystems. Warren and others (1964) found that the addition of sucrose to a stream resulted in increases in Chironomidae (Diptera) larvae. Similarly, addition of manure to streams in California dramatically increased Chironomidae densities (del Rosario et al. 2002). Observed increases of invertebrate biomass were most probably due to the increased availability of microbes as food for collector–gatherer and shredder invertebrates, while carnivorous invertebrates benefited indirectly. During this study, bedrock outcrops were covered with microbial growth and enriched FPOM. Although FPOM quantity did not increase significantly during either addition, the quality of organic matter present in bedrock habitats was presumably greater in the treatment reach. This increase in FPOM and epilithon quality may have allowed some taxa to increase in density and biomass during a relatively short period.

In this study, more taxa in bedrock habitats responded to the addition of labile carbon than did those of mixed substrates. Substrate-specific responses of stream invertebrates to experimental treatment have been noted previously (e.g., Gurtz and Wallace 1984; Wallace et al. 1999). Moss associated with bedrock habitats traps particles and aids in biological stability. Additionally, high flow rates in bedrock habitats contribute to high delivery rates of DOC. Dominant bedrock groups (collector–gatherers and filterers) are less dependent on CPOM and rely heavily on organic matter and FPOM trapped within moss (Wallace et al. 1999).

The fact that nearly all organisms we sampled assimilated dextrose but fewer taxa exhibited significant changes in density or biomass suggests that length of addition and sampling effort may not have been sufficient to gauge accurately the effects of enrichment on invertebrate production in Jenny Branch. In a longer term study of the effects of low concentrations of particulates from paper mill effluent on the macroinvertebrate community, alterations in seston quality and substrate composition led to decreased invertebrate diversity, particularly for collector–filterers and scraper taxa (Mayack and Waterhouse 1983). Additionally, the design of our experiment required us to employ methods of statistical analysis that are conservative. The patchy nature of benthic habitats and organisms associated with them led to difficul-

ties in uncovering patterns in changes of macroinvertebrate densities during the dextrose addition. For example, copepods were sometimes found to have increased dramatically in bedrock habitats in the treatment reach, but this difference was obscured because copepods did not consistently increase over the course of the experiment.

Our study revealed no significant relationship between changes in invertebrate abundance or biomass versus their $\delta^{13}C$ values in the treatment reach (linear regression, $p > 0.05$). Invertebrates sampled for stable isotope analysis were pooled from mixed-substrate habitats and bedrock outcrops. A clearer relationship between benthic invertebrate production and $\delta^{13}C$ probably would have emerged had samples from each habitat been collected and analyzed separately. The results of this study point to the need for further research on microhabitat effects on stable isotope signatures (see Finlay et al. 2002).

This study is among the first to examine the effects of a labile carbon addition on higher trophic levels in a stream ecosystem. In this experiment, added labile carbon was taken up by microbes and was an important food resource for macroinvertebrates during both experimental periods. All invertebrates relied to some degree on the dextrose-derived carbon, even when large quantities of CPOM were present in the system, demonstrating the strong linkage between DOC, bacteria, and macroinvertebrates in heterotrophic stream food webs. The effect of dextrose addition on aquatic invertebrates in Jenny Branch depended on their functional feeding group, microhabitat, diet, and turnover time. Increases in the amount of organic matter present in streams can be the result of natural processes, but frequently are caused or accelerated by human activities (Jones 2001). Although not always in as labile a form as dextrose, anthropogenic carbon inputs might lead to changes in community structure that could impact overall stream ecosystem health. The addition of labile carbon to aquatic ecosystems via organic pollution is likely to affect the quantity and quality of basal resources, resulting in effects that can be seen at all trophic levels.

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