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Increased Light Availability Reduces the Importance of Bacterial Carbon in Headwater Stream Food Webs

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Abstract

Many ecosystems rely on subsidies of carbon and nutrients from surrounding environments. In headwater streams that are heavily shaded by riparian forests, allochthonous inputs from terrestrial systems often comprise a major part of the organic matter budget. Bacteria play a key role in organic matter cycling in streams, but there is limited evidence about how much bacterial carbon is actually assimilated by invertebrate and fish consumers, and how bacterial carbon assimilation varies among streams. We conducted stable isotope tracer additions of ^{13}C-acetate, that is assimilated only by bacteria, and ^{15}N-ammonium, that is assimilated by both bacteria and algae, in two small, shaded streams in the Adirondack region of New York State, USA. Our goal was to determine whether there is an important trophic link between bacteria and macroconsumers, and whether the link changes when the light environment is experimentally altered. In 2009, we evaluated bacterial carbon use in both streams with natural canopy cover using 10-day dual-isotope tracer releases. The canopy was then thinned in one stream to increase light availability and primary production and tracer experiments were repeated in 2010. As part of the tracer experiments, we developed a respiration assay to measure the δ^{13}C content of live bacteria, which provided critical information for determining how much of the carbon assimilated by invertebrate consumers is from bacterial sources. Some invertebrate taxa, including scraper mayflies (*Heptagenia* spp.) that feed largely on biofilms assimilated over 70% of their carbon from bacterial sources, whereas shredder caddisflies (*Pycnopsyche* spp.) that feed on decomposing leaves assimilated less than 1% of their carbon from bacteria. Increased light availability led to strong declines in the magnitude of bacterial carbon fluxes to different consumers (varying from −17 to −91% decrease across invertebrate taxa), suggesting that bacterial energy assimilation differs not only among consumer taxa but also within the same consumer taxa in streams with different ecological contexts. Our results demonstrate that fluxes of bacterial carbon to higher trophic levels in streams can be substantial, that is over 70% for some taxa, but that invertebrate taxa vary considerably in their reliance on bacterial carbon, and that local variation in carbon sources controls how much bacterial carbon invertebrates use.

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Introduction

Studies of carbon flow to macroconsumers have largely focused on the autotrophic pathways (Moore and others 2004); however, many ecosystems receive subsidies of detrital carbon and there is a growing interest in quantifying the contribution of these subsidies to macroconsumer production. Stream food webs are strongly influenced by transfers of particulate and dissolved organic matter and nutrients from adjacent terrestrial environments (Hynes 1975; Vannote and others 1980) and reciprocal flows from riparian forests and headwater streams often have a strong influence on food web dynamics and energy flow to consumers (for example Wallace and others 1999; Nakano and Murakami 2001; Baxter and others 2005; Bartels and others 2012). Although research investigating the importance of heterotrophic food web pathways in forested streams has highlighted the need for emphasis on detrital food web pathways (for example Webster and others 1999; Hall and others 2000; Tank and others 2010), relatively little is known about the importance of food web links between heterotrophic bacteria and invertebrate consumers, and how they vary in different environmental conditions.

Heterotrophic bacteria play an important role in the decomposition and assimilation of allochthonous carbon in freshwater systems, through both the uptake of dissolved organic carbon (DOC) and breakdown of particulate organic matter such as leaf litter (Webster and Benfield 1986; Suberkropp and Chauvet 1995; Webster and others 1999; Wiegner and others 2005). In many planktonic systems, the transfer of allochthonous energy from microbes to higher trophic levels is not a substantial flux because most of the bacterial energy is dissipated in trophic transfers and little reaches higher trophic levels, referred to as the ''microbial loop'' (for example Azam and others 1983; Ducklow and others 1986; Pace and others 1990; Hairston and Hairston 1993; Fenchel 2008). In contrast, larger macroinvertebrate consumers in streams often ingest bulk biofilm or bulk detritus that includes the associated microbial community, so stream metazoan consumers may assimilate a high proportion of their carbon from heterotrophic microbes (Cummins 1974; Meyer 1994). Despite this recognition, quantifying fluxes from bacteria to higher trophic levels in stream food webs remains a challenge, and few studies have quantified the importance of bacterial carbon in natural streams. Past studies confirm that heterotrophic bacteria can be a significant source of carbon to metazoan consumers (Hall and others 2000), but additional study is needed to generalize about the role of bacteria in stream food webs and how it varies among systems (Findlay 2010).

Several studies have shown that single species of stream invertebrates, usually in the scraper or filterer functional feeding groups, can assimilate high proportions of bacteria relative to other food sources (for example Rounick and Winterborn 1983; Edwards and Meyer 1987, 1990). Other species, usually shredders, assimilate very little, perhaps because while shredders feed directly on decomposing organic matter that includes fungi and bacteria, they do not preferentially assimilate bacterial carbon over much more abundant leaf carbon (Findlay and others 1986). A few notable studies have investigated bacterial energy fluxes in whole-stream systems (Hall and Meyer 1998; Simon and others 2003). Hall and Meyer (1998) found that many invertebrate taxa assimilate high proportions of carbon from heterotrophic bacteria, but the results were difficult to interpret because methodological challenges led to some implausible results (for example $>100\%$ bacterial contributions to some diets). Simon and others (2003) also measured high contributions of heterotrophic bacterial carbon to higher trophic levels, but the study was conducted in cave streams where bacterial carbon was expected to be important.

The role of bacteria in supporting food webs may also be related to autotrophic production because of evidence that bacterial production and primary production can be closely related to some systems (Haack and McFeters 1982; Cole and others 1988; Findlay and others 1993). Specifically, some prior studies show that increased primary production was accompanied by a parallel increase in bacterial production (for example Cole and others 1988; Findlay and others 1993), whereas others concluded that algal and bacterial production were effectively uncoupled (for example Findlay and others 1991; Findlay and others 1998), suggesting that the relationship between algal and bacterial production may differ among systems or in different environmental contexts. In addition, in streams, there are high fluxes of algal exudate carbon to bacteria in biofilms, possibly supporting a concurrent increase in algal and bacterial production (Haack and McFeters 1982). In other ecosystems, there was no correlation between algal and bacterial biomasses, suggesting that they are unre-

lated and regulated independently (Sobczak and Burton 1996). The relationship between algae and bacteria in some systems suggests that autotrophic production, which is often limited by light in headwater ecosystems (for example Hill and others 1995), may also have a strong effect on the role of bacteria in food webs. Under open canopies, algal standing stocks and production increase (Hill and others 1995; Hill and Dimick 2002), and comparative and experimental studies have found that increased light availability alters stream ecosystems at multiple trophic levels (for example Haack and others 1988; Sabater and others 2000; Ambrose and others 2004). However, the equivocal nature of the relationship between algal and bacterial production suggests a need for further investigation, and the influence of light and autotrophic production on trophic linkages between bacteria and consumers has not been explored.

Isotope tracer injections are one way to effectively trace material flows through food webs. Many researchers, most notably the Lotic Intersite Nitrogen Experiment (LINX), conducted N tracer additions in streams across a range of biomes (for example Mulholland and others 2000; Peterson and others 2001; Mulholland and others 2008). Because all microbes (that is bacteria, fungi, and algae) assimilate dissolved inorganic nitrogen (for example ammonium, nitrate), these experiments have allowed investigators to trace the movement of N from the water column to basal resources (that is microbes in biofilms, fine organic sediment, and leaf litter) and higher trophic levels in the food web. Carbon isotope tracers have been used in a few stream studies and provide a powerful method for examining linkages between bacteria and consumers (for example Hall and others 2000; Hotchkiss and Hall 2015). Bacteria and algae are closely associated in the biofilm of stream sediments, so it is difficult or impossible to separate them physically. However, labile organic compounds are selectively taken up by bacteria (Wright and Hobbie 1966), whereas photosynthesizing algae fixes CO_2. Hence, the addition of isotopically labeled DOC or DIC makes it possible to differentially track the flow of carbon from algae and bacteria to other compartments of the food web.

In this study, we sought to examine the extent to which heterotrophic bacteria are a food resource in stream food webs, and to determine how changes in the light environment may influence the amount of bacterial carbon assimilated by consumers. We conducted a dual-isotope tracer injection, simultaneously enriching streams with ^{13}C-acetate to label heterotrophic bacteria, and ^{15}N-NH_4, which is assimilated by bacteria, fungi, and algae. Our objectives were first, to determine the magnitude of bacterial carbon fluxes to several taxa in various functional feeding groups in headwater streams, and second, to determine whether the role of bacterial carbon differs in streams with dense intact riparian forests compared with streams where riparian cover has been experimentally reduced.

In this experiment, we tested two hypotheses. First, we hypothesized that bacteria would comprise different proportions of the diets of invertebrate functional feeding groups, and that scrapers would assimilate bacterial carbon and shredders would not assimilate bacterial carbon (Findlay and others 1986; Edwards and Meyer 1987; Hall and Meyer 1998). Second, we hypothesized that canopy thinning increases primary production and rates of bacterial production and hence would increase the assimilation of bacteria by invertebrates. However, the alternative hypothesis that increased algal production would lead to decreased bacterial production is also possible if there is increased competition for scarce nutrients in these streams. Understanding the effects of canopy thinning may also provide an important basis for our understanding of how anthropogenic changes in watersheds influence streams. Human activities often change riparian conditions with significant consequences for adjacent stream ecosystems (Allan 2004), and this study begins to explore the implications of those changes for heterotrophic bacterial communities and their role in supporting consumer production in streams.

Methods

Study Site

In July–August 2009 and July–August 2010, we added stable isotope tracers to two small, headwater streams located near Old Forge, NY, USA in the Adirondack Mountains. Both were small, groundwater-fed, headwater streams (discharge approximately 5 l s^{-1}) with low concentrations of dissolved inorganic nitrogen (NH_4 < 2 μg N l^{-1}, NO_3 < 100 μg N l^{-1}) and phosphorus (SRP < 10 μg P l^{-1}), and moderate concentrations of dissolved organic carbon (DOC < 2.5 mg l^{-1}). Each stream drains a second-growth forests dominated by American beech (*Fagus grandifolia*). The reference stream, Combs Brook Tributary, hereafter ''reference stream,'' had a natural canopy cover during both study years. The thinned stream, Blues Brook, hereafter ''canopy-thinned stream,'' was

thinned in September 2009 following the first year of isotope addition and sampling (Supplementary material). The goal of the thinning was to increase light availability, while minimizing the other effects of watershed deforestation, so we only removed trees within 5 m of the stream. Trees were cut down using a chain saw so that they did not fall in the stream bed, and downed trees were removed from the vicinity of the canopy-thinned stream (either downstream or over a ridge).

Benthic macroinvertebrate communities in both streams were dominated, in terms of biomass, by a suite of large-bodied invertebrate genera that spanned multiple functional feeding groups (classified according to Barbour and others 1999). Common taxa included *Heptagenia* spp. (mayfly scraper), *Ephemerella* spp. (mayfly collector-gatherer), *Lanthus* sp. (predatory dragonfly), *Remenus* sp. (predatory stonefly), and *Pycnopsyche* spp. (caddisfly shredder). The only fish species observed during electrofishing surveys in each stream was brook trout (*Salvelinus fontinalis*).

^{13}C-acetate and ^{15}N-ammonium addition

We added ^{13}C labeled acetate (as 99 atom % sodium acetate-2-^{13}C, Sigma–Aldrich) and ^{15}N labeled ammonium (as 98 atom % $^{15}NH_4Cl$, Sigma-Aldrich) to both streams using a continuous drip with an injection rate of 10 ml min^{-1} over a 10-day period. A total of 36 g of labeled sodium acetate was added to each stream over 10 days, which increased the concentration of ^{13}C in streamwater by 1.25 μg l^{-1} and elevated the δ^{13}C of the DOC pool to approximately 100%. A total of 2.1 g of labeled ammonium chloride was added to each stream over 10 days, which elevated δ^{15}N of dissolved ammonium to approximately 15,000%. Mean discharge of each stream ranged from 4.0 to 5.2 l s^{-1} depending on stream and year, and was stable throughout the experimental period. Despite the disparity in enrichment between the C and N tracers, uptake compartments became comparably enriched in both ^{13}C and ^{15}N because acetate is a highly labile compound and is taken up rapidly relative to bulk DOC. The target enrichments were not intended to fertilize the system, and the concentration of ^{15}N added was less than 5% of ambient NH_4 and the concentration of ^{13}C added was less than 1% of ambient DOC. Algal uptake of the ^{13}C tracer should have been minimal relative to bacterial uptake (Wright and Hobbie 1966) and fungal uptake should have been negligible because the half saturation constants for fungal uptake are several orders of magnitude higher than the concentration at which we added acetate to the streams (Newell 1984; Hall and Meyer 1998; Simon and others 2003). We also added sodium chloride as a conservative tracer for the entire 10-day drip, which we used to correct for dilution along the study reach. We measured the conservative tracer indirectly by determining the relationship between NaCl concentrations and conductivity in both streams and measuring stream conductivity during the isotope tracer release. Sodium chloride was added at a concentration of approximately 4.5 mg NaCl per l of stream water, which resulted in a conductivity increase from approximately 25 μS/cm to approximately 35 μS/cm.

Food Web Sampling

We collected samples of food web compartments at three sites: 10, 30, and 60 m downstream from the point of isotope addition. Distances were based on a pilot study in the reference stream, which demonstrated that we would be able to detect both C and N isotope tracer in all food web compartments. We collected samples during the week before isotope addition (to record background isotopic levels and standing stocks of basal resources), on three dates during the 10-day tracer release period, and on three dates during the 10 days after the end of tracer addition. In 2010, we also collected samples approximately 1 month after the tracer addition began. Food web compartments that were collected included: epilithon, surface fine benthic organic matter (FBOM), coarse benthic organic matter (CBOM), four abundant macroinvertebrate taxa representing different functional groups (*Pycnopsyche*—shredder, *Heptagenia*—scraper, *Ephemerella*—collector/gatherer, and *Remenus*—predator), and brook trout. During the isotope release and during the post-release sampling, we carried out non-quantitative sampling of organic matter compartments to avoid disrupting stream sediments.

Because both streams contained many large rocks that could not be removed from the stream, we sampled epilithon with Loeb samplers with a sampling area of 12 cm^2 (Loeb 1981). Briefly, a Loeb sampler is a brush-fitted cylinder with a neoprene gasket that creates a seal around a known area of rock. The brush removes periphyton and the suction is created through a finger-controlled intake that pulls water into the cylinder and suspends periphyton in a slurry. We used the same Loeb sampling technique to quantify standing stocks and to sample biofilms for isotopic analysis. Seven Loeb samples per transect were combined

into a single sample for analysis and were filtered through glass fiber filters (Whatman GFF, 0.7 μM pore size) to analyze chlorophyll *a*, ash-free dry mass, and isotopes. We extracted chlorophyll *a* by incubating filters in film canisters in the dark in 15 mL of 90% buffered ethanol for 18–22 h (Nusch 1980; Jespersen and Christoffersen 1987). Immediately after extraction, we analyzed chlorophyll *a* using an AquaFluor handheld fluorometer (Turner Designs, Sunnyvale, CA).

For quantitative assessment of standing stocks, fine benthic organic matter (FBOM) was sampled by sinking a plastic 13-cm diameter cylinder into an area of soft sediment, measuring the water depth in the cylinder, suspending the surface layer of organic matter into the water, and removing a known quantity of slurry from the bucket. We allowed FBOM to settle, remove excess water, and dry the remaining organic material. For non-quantitative isotope sampling, we suctioned FBOM from the stream bottom with a turkey baster. For quantitative assessment of standing stocks, CBOM was sampled by haphazardly selecting a point on the stream and removing all leaf litter and woody material in a 20-cm wide transect across the entire width of the stream. This protocol for sampling CBOM can lead to results with high variation because leaf aggregations in streams are not evenly distributed spatially, but we could not do more extensive CBOM biomass sampling (that is remove all CBOM from large areas of the streambed) because we needed to avoid major disturbance to the streambed for other sampling efforts. For non-quantitative isotope sampling, we picked 3–5 random spots at the sampling transects and grabbed a small amount of mixed leaf litter. For all organic matter compartments, we sampled at points near to our sampling stations (10, 30, and 60 m downstream from the point of isotope injection). We dried epilithon, FBOM, and CBOM samples at 50°C until they reached a constant mass and recorded all biomasses. After measuring dry mass of epilithon filters, we burned them at 450°C for 6 h to quantify ash-free dry mass.

For non-quantitative isotope sampling, invertebrates were sampled by hand to avoid disturbing the substrate, either by turning over rocks or by sorting through leaf packs in a plastic tray before returning them to the stream. We targeted abundant large-bodied invertebrate groups and kept them alive in 15 mL plastic tubes filled with stream water until they could be returned to the laboratory for identification under a dissecting microscope. We removed the gut tracts from all invertebrates manually before drying their tissue for stable isotope analysis. Brook trout populations were small, so they were only sampled on the last day of the isotope release and 10 days after the isotope release. We used a backpack electrofishing unit to capture brook trout and euthanized them immediately. We removed a sample of muscle tissue from each fish for isotopic analysis. All samples were dried at 50°C, ground, loaded into tin capsules, and analyzed on a Thermo Finnigan isotope ratio mass spectrometer coupled to a Carlo Erba elemental analyzer with a Conflow II open split at the Cornell Isotope Laboratory (COIL).

Ecosystem Response to Canopy Thinning

We monitored other ecosystem attributes to assess responses to canopy thinning. We recorded light levels every 10 min throughout the summer and fall seasons of 2009 and 2010 with HOBO pendant temperature and light loggers (Onset Computer Corporation, Part UA-002-64) and converted data to units of photosynthetically active radiation (PAR) using a correction factor from Thimijan and Heins (1983). In each stream, light loggers were attached approximately 25 cm above the water level to six stakes of construction rebar spaced evenly along the study reaches. In July 2010, we deployed substrates in each stream to determine how canopy thinning affected algal accrual. We put seven replicate glass crucible covers (19.6 cm^2 area) in three locations: approximately 20 m upstream of the canopy thinning in the canopy-thinned stream, in the thinned canopy area of the canopy-thinned stream, and in the reference stream. We allowed biofilm to accrue on the substrates for 18 days before removing them, placing them in film canisters, and extracting and analyzing chlorophyll using the same methods as epilithon analyses. Measuring algal accrual in addition to standing stock gave us a dynamic rate that would serve as a rough but comparable proxy for primary production, which we did not measure. Since the study streams were unproductive and natural biofilms included a lot of detritus and inorganic matter in addition to algae, accrual data were also useful for measuring recently produced biofilm, that is likely important for consumers (Dodds and others 2014).

We used the ^{15}N tracer injection to measure N uptake during each isotope tracer release. We measured $\delta^{15}N$ of the ammonium tracer in water at each downstream sampling station using a filter pack diffusion method (Holmes and others 1998). Briefly, we collected 0.95 l of filtered stream water in 1.0 l bottles and added 50 g of salt, 5.0 g magnesium oxide, and a 1.0 cm filter (Whatman GFD)

sealed in Teflon tape to each sample. Because stream water ammonium concentrations were low (<2 μg l^{-1}), we also added a 50 μg ammonium spike to each incubation to provide sufficient N on the filter for analysis on a mass spectrometer. After 1 month of incubation at room temperature with occasional shaking (approximately once every 3–4 days), we removed filters and dried them in a desiccator for isotopic analysis. We corrected data to account for the 50 μg ammonium spike and to account for dilution along the reach using the conservative tracer, and created a regression of decline in $^{15}N\text{-}NH_4$ as a function of distance downstream from the point of tracer addition. We used the negative slope of the regression (k) to calculate uptake length and uptake velocity of N (Tank and others 2007). Uptake length (S_w), or the distance it takes a molecule of nitrogen to be immobilized from its dissolved form, is equal to the inverse of k (k^{-1}). Uptake lengths often differ among streams with a different discharge, so we also calculated uptake velocity (V_f):

$$V_f = Qkw^{-1},$$

where Q is stream discharge and w is the average wetted width of the stream (2.6 m for the reference stream and 1.5 m for the canopy-thinned stream).

We compared chlorophyll accrual and N uptake length among streams and canopy thinning treatments using fixed effects linear models in R (R Core Team 2013). For chlorophyll data, we compared algal accrual rates among three groups: the thinned canopy reach of the canopy-thinned stream, an upstream reach of the canopy-thinned stream that still had a natural canopy, and the reference stream; for N uptake, we compared uptake length or uptake velocity rates among four groups: canopy-thinned stream in 2009 (pre-thinning), reference stream in 2009, canopy-thinned stream in 2010 (post-thinning), and reference stream 2010.

Measuring $\delta^{13}C$ of Active Bacteria Using Respiration Incubations

One challenge in conducting bulk collections of food web compartments is that invertebrates often selectively ingest or assimilate live parts of organic matter pools (Dodds and others 2014). Although it is possible in some cases to physically separate the components of epilithon (that is algae, bacteria, and detritus) using centrifugation gradients (Hamilton and others 2001), the process is time-intensive and does not work in many systems nor with all types of organic matter. We developed a respiration chamber method to measure the $\delta^{13}C$ of active bacterial biomass by examining the isotope ratio of respired CO_2 under field incubations. We conducted field incubations during the isotope tracer addition by filling 475 ml glass incubation chambers with 150 ml of stream water containing either coarse organic matter or fine organic matter. It was not possible to incubate rocks with epilithon because all rocks in the study sites were much larger than our chambers. Incubations were sealed and headspace was scrubbed of CO_2 by circulating air from the headspace through a container of soda lime using a vacuum pump. We used a syringe and needle to collect 12 ml samples of headspace air through a rubber septum on the top of the incubation chamber. Samples were collected at 1, 4, and 7 h time periods after the incubation began and stored in evacuated vials for $^{13}CO_2$ analysis at the Cornell Isotope Laboratory. We also killed all live bacteria with $HgCl_2$ in control treatments to establish that enriched CO_2 in the headspace was actually a product of bacterial respiration.

A high proportion of the CO_2 released into the respiration chamber headspace was from inorganic diffusion from the water, so we used a two-ended mixing model to calculate the $\delta^{13}C$ of CO_2 in respiration chambers due to organic respiration and so reflective of the $\delta^{13}C$ of live bacteria. We used the following model, where f_{inorg} and f_{org} are the relative fractions of headspace CO_2 that are derived from inorganic diffusion and respiration, respectively, and always sum to 1, and δ is the $\delta^{13}C$ of CO_2 from either the bulk headspace air (''total''), organic component from respiration (''org''), or inorganic component from diffusion (''inorg'').

$$\delta_{total} = \delta_{org} \times f_{org} + \delta_{inorg} \times f_{inorg}$$

We used this equation to solve for δ_{org}, which we used to calculate $\delta^{13}C$ of bacteria under the assumption that bacteria respire CO_2 with an isotopic ratio that reflects their tissue isotopic ratio. We calculated f_{org} though comparisons of the amount of CO_2 diffused in natural respiration assays versus chambers where all bacteria had been killed with sodium azide, and used f_{org} data to calculate f_{inorg} by difference ($f_{org} + f_{inorg} = 1$). Although we used $HgCl_2$ to kill bacteria during field experiments, we found that $HgCl_2$ increased inorganic diffusion of CO_2 and confounded results, but sodium azide did not. Hence, we used sodium azide in a field trial to determine f_{org}. Estimated values for all parameters and a detailed description of field experiments to obtain them can be found in Supplementary material.

Because fungi also respire and greatly outweigh bacteria on leaf detritus (for example Weyers and Suberkropp 1996; Mille-Lindblom and Tranvik 2003; Das and others 2007), we corrected for dilution due to fungal respiration using a 1:10 ratio of fungal to bacterial respiration for coarse organic matter (leaves) and a 1:1 ratio for fine organic matter (sediment). These ratios were derived as conservative estimates from the literature (for example Findlay and others 2002). We selected conservative values because underestimation of fungal production relative to bacterial production on leaves would lead to underestimation of f_{inorg} because fungal respiration should not be enriched and would dilute bacterial respiration in the same way that inorganic diffusion of CO_2 does. Hence, incorporating more respiration due to fungi would lead to lower bacterial $\delta^{13}C$ estimates, and higher estimates of bacterial carbon assimilation by invertebrates based on the calculations below. We corrected for fungal dilution by changing f_{org} to reflect the fraction, that is bacterial, rather than a combination of bacterial and fungal, hence the relationship between the correction for fungal dilution and δ_{org} is linear and any uncertainty would have a linear effect on our estimates of bacterial carbon.

Calculating Percent Bacterial Carbon Used by Invertebrates

We estimated the proportion of total consumer carbon that was derived from bacteria using a simple mixing model (Hall and Meyer 1998):

$$\text{Fraction bacterial C} = \left(\delta \text{I}_{\text{labeled}} - \delta \text{I}_{\text{background}}\right) / \left(\delta \text{B}_{\text{labeled}} - \delta \text{B}_{\text{background}}\right)$$

where invertebrates are I, bacteria are B, ''labeled'' values represent each compartment at maximum isotope enrichment, and ''background'' values represent the unlabeled background isotopic signature. For bacteria, maximum enrichment typically occurred on the final day of the 10-day release because rapid turnover rates caused bacterial signatures to decline quickly after isotope addition ceased. Invertebrates often continued to become more enriched after the addition ended, presumably because they continued to feed on enriched food sources and have relatively slow turnover rates compared to bacteria. The mixing model to calculate percent bacterial contributions assumes that both invertebrates and bacteria reach equilibrium with their carbon source during the course of the experiment. Violating this assumption means this model underestimates the percent of invertebrate carbon, that is derived from bacteria.

We calculated turnover rates of invertebrates to estimate the degree to which our assumption of equilibrium was violated. We used ^{15}N tracer data in a dynamic compartment model that has been used to analyze data from several dozen ^{15}N tracer studies (for example Dodds and others 2000; Whiles and others 2013; Dodds and others 2014). Briefly, we modeled each compartment separately and used the Solver function in Microsoft Excel to minimize the sum of squares error by altering the uptake and loss rates of ^{15}N from the food source. If a consumer became more enriched than its presumed bulk food source, we also solved for a multiplier that elevated the level of the food source by a consistent ratio. The multiplier was constrained by the amount of ^{15}N in the water column (maximum enrichment of pure algae or bacteria). Detailed methods are described in Dodds and others (2014) and in Supplementary material.

We used N tracer data instead of C tracer data to estimate turnover time because it was easier to detect patterns in the N tracer over a time series in basal resource compartments. For example, in resource compartments with high C:N ratios (for example CBOM), N tracer accumulated during the isotope release and declined following, but C tracer showed much less accumulation and decline over time, most likely due to dilution by the high amounts of ''inert'' C in dead leaf tissue. The lack of appreciable accumulation and decline of C tracer made it impossible to calculate turnover accurately, but N tracer data were appropriate for calculating turnover times.

Results

The canopy thinning was successful in increasing light availability, which had the predicted effect of increased algal accrual in the thinned reach of the canopy-thinned stream (Figure 1, Table 1). The thinned reach had significantly higher chlorophyll accrual rates than the reference stream or the upstream unthinned reach the canopy-thinned stream (Figure 1, fixed effects linear model, $F = 68.6$, df = 2, $p < 0.001$, no significant contrasts except for Thinned Reach, $t = 9.9$, df = 2, $p < 0.001$). Differences in N uptake length were not significant among the study reaches (Figure 2, fixed effects linear model, $F = 2.027$, df = 3, $p = 0.16$), but there was a significant increase in N uptake velocity in the canopy-thinned stream after canopy thinning (Figure 2, fixed effects linear

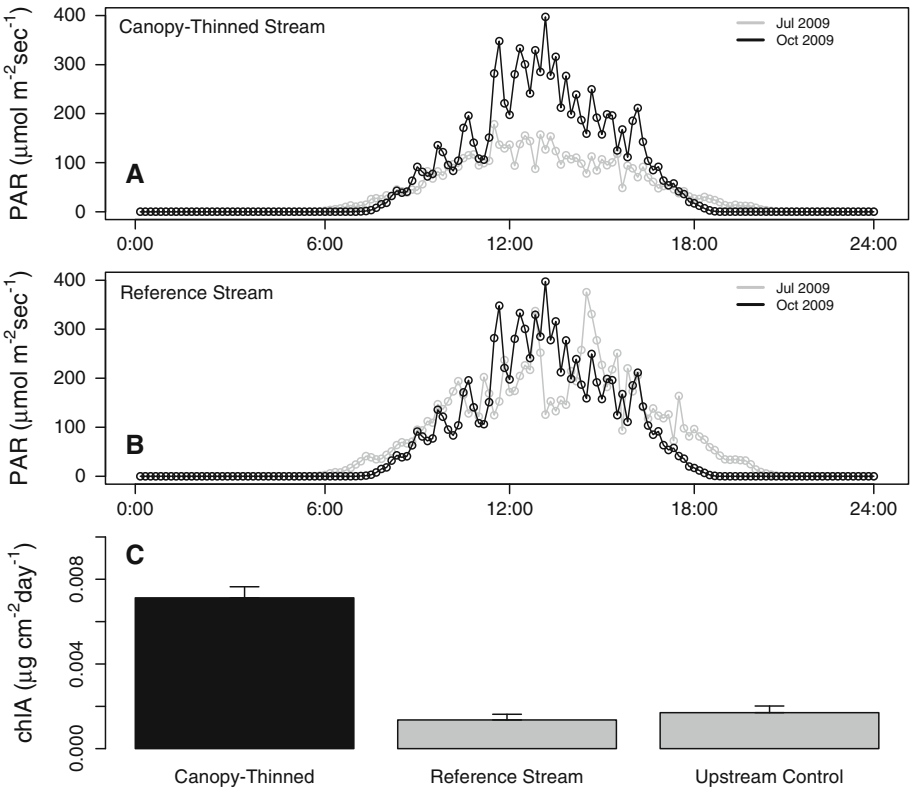

Figure 1. Canopy thinning increased light availability and chlorophyll accrual in the canopy-thinned stream. Light measurements from the canopy-thinned stream (*top panel*) show increase in light from before (July 2009) to after (October 2009) canopy thinning. There was no change in light availability between the same time periods for the reference stream (*Panel B*). Each point on the light curves is the average photosynthetically active radiation (PAR) based on a week of measurements. Chlorophyll data (*Panel C*) show differences in chlorophyll-a accrual between the thinned canopy reach of the canopy-thinned stream (*black bar*) relative to an upstream control reach of the canopy-thinned stream, and the reference stream. Error bars represent one standard error. All chlorophyll-a data are from algal accrual measurements in July 2010.

Table 1. Characteristics of the Two Study Streams in 2009 and 2010

	Stream width (m)	Discharge (l sec^{-1})	Epilithon Chl-a (mg m^{-2})	Epilithon AFDM (g m^{-2})	Leaf DM (g m^{-2})
Reference stream					
Natural canopy—2009	1.7	4.8	4.22 (2.1)	2.03 (1.3)	92.4 (106)
Natural canopy—2010	1.7	4.0	3.94 (1.6)	1.20 (0.49)	19.8 (19)
Canopy-thinned stream					
Natural canopy—2009	1.5	5.2	0.896 (0.71)	2.50 (1.6)	74.5 (62)
Thinned canopy—2010	1.5	4.3	9.14 (3.9)	1.31 (0.35)	23.8 (26)

Biomasses of epilithon and leaves are means with standard deviations in parentheses.

model, $F = 10.3$, df = 3, $p = 0.001$, no significant contrasts except for canopy-thinned stream in 2010, $t = 3.16$, df = 3, $p = 0.01$).

Tracer addition resulted in labeling of all food web compartments with both ^{13}C and ^{15}N tracers above background isotopic values (Figure 3, 4). Most invertebrate groups, especially scraper mayflies (*Heptagenia*), were more enriched than any presumed bulk food sources (that is epilithon, CBOM, and FBOM), but $\delta^{13}C$ of active bacteria measured through the incubation chamber method exceeded the label found in any invertebrate group (Figure 3).

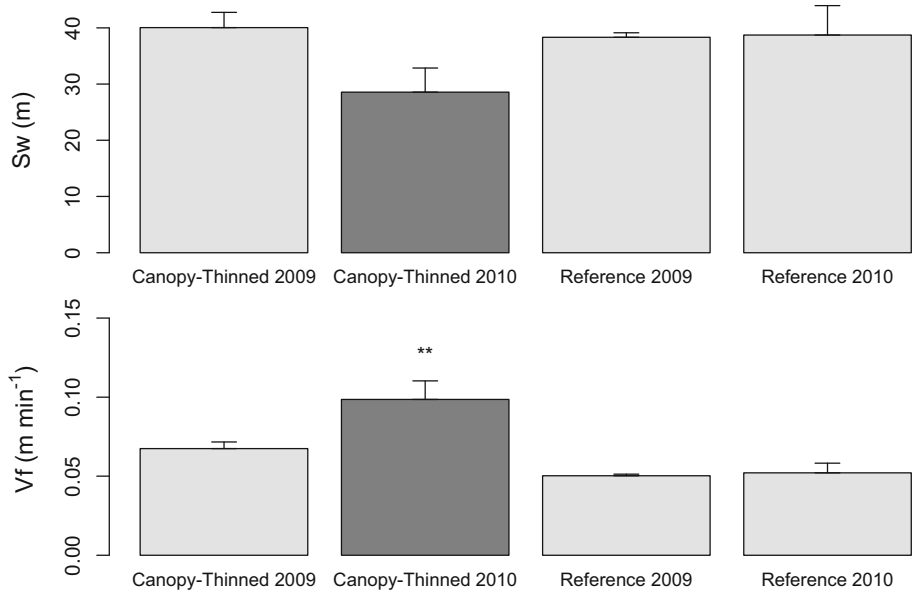

Figure 2. Canopy thinning resulted in decreased N uptake length (S_w) but differences were not significant due to high variance, and significantly increased N uptake velocity (V_f) in the canopy-thinned stream in 2010, which differed from other groups at the $p < 0.01$ level (denoted by double asterisk). *Dark gray bars* show the canopy-thinned stream in 2010 after thinning was complete, and *light gray bars* have natural canopy cover (canopy-thinned stream before thinning, reference stream in both years).

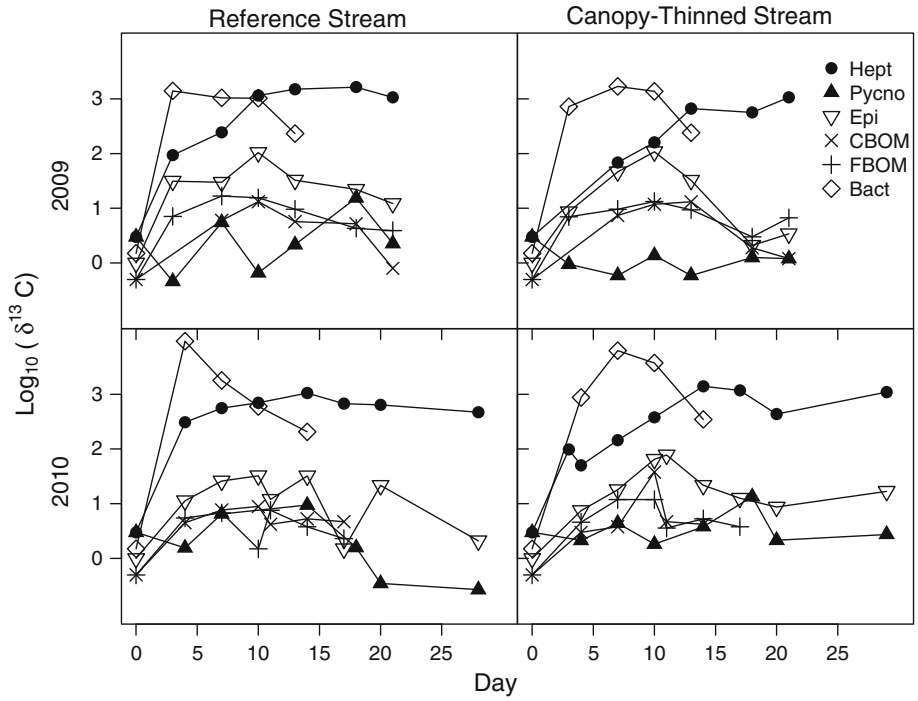

Figure 3. Carbon-13 isotope tracer found in different food web compartments (*Heptagenia* mayflies, *Pycnopsyche* caddisflies, bulk epilithon, CBOM, FBOM, and live bacteria) as a function of days since the start of C-13 addition. *Heptagenia* mayflies are shown because they were the most enriched of all invertebrate taxa, with δ^{13}C values that consistently exceeded the δ^{13}C of bulk basal resource pools. *Pycnopsyche* caddisflies are shown because they were the least enriched of all invertebrate taxa. Other invertebrate taxa were intermediate. Bacteria data are derived from respiration assays and represent the δ^{13}C of actively respiring bacteria. Note that data are graphed on a log scale because of substantial differences between food web compartments.

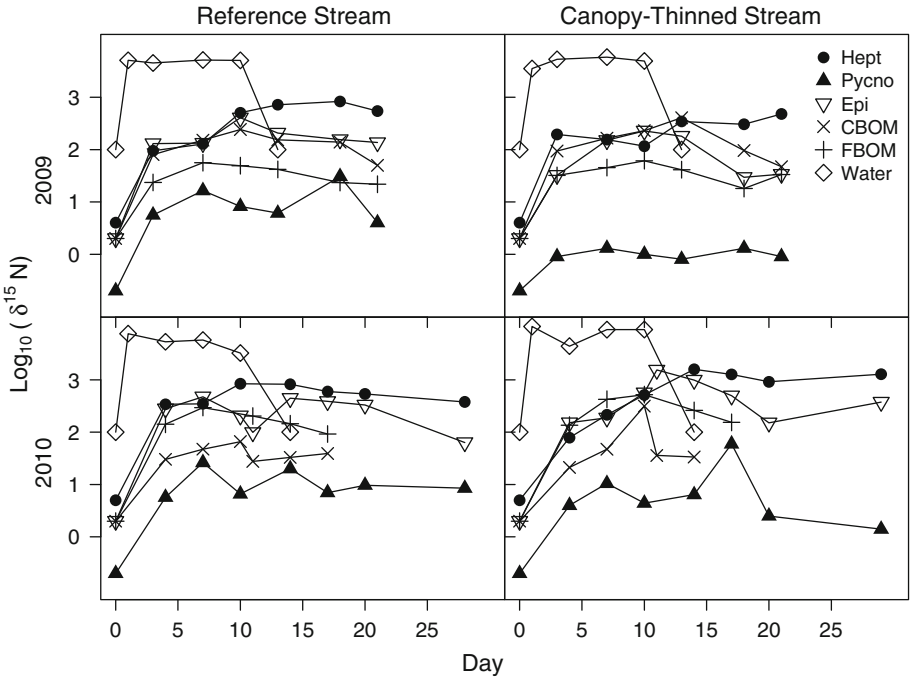

Figure 4. Nitrogen-15 isotope tracer found in different food web compartments (*Heptagenia* mayflies, *Pycnopsyche* caddisflies, bulk epilithon, CBOM, FBOM, and water column ammonium) as a function of days since the start of N-15 addition. *Heptagenia* mayflies are shown because they were the most enriched of all invertebrate taxa, with δ^{15}N values that consistently exceeded the δ^{15}N of bulk basal resource pools. *Pycnopsyche* caddisflies are shown because they were the least enriched of all invertebrate taxa. Other invertebrate taxa were intermediate. Note that data are graphed on a log scale because of substantial differences between food web compartments.

Table 2. Percent Bacterial Carbon Assimilation by Invertebrate Taxon, Functional Feeding Group, Stream, and Year

Taxon	FFG	Reference stream year 1	Reference stream year 2	Reference stream % change	Canopy-thinned stream year 1	Canopy-thinned stream year 2	Thinned stream % change
Heptagenia	Scraper	78%	75%	−4%	65%	44%	−32%
Remenus	Predator	14%	18%	+29%	10%	0.9%	−91%
Ephemerella	Collector	NA	NA	NA	NA	4.0%	NA
Pycnopsyche	Shredder	0.03%	0.09%	+200%	0.06%	0.05%	−17%

Percent change from 2009 to 2010 is noted in the right column. Ephemerella were only abundant enough for collection in the canopy-thinned stream in 2010. We thinned the canopy of the canopy-thinned stream after the 2009 field season.

The highest active bacterial isotope signature was the only measured food source that always exceeded the highest isotope label found in primary consumers.

Bacterial carbon use varied by invertebrate taxon (Table 2), with extremely high (~75%) assimilation of bacterial carbon observed for mayfly scrapers (*Heptagenia* spp.), moderate (10–18%) assimilation of bacterial carbon by predatory stoneflies (*Remenus* spp.), low (4%) assimilation of bacterial carbon by collector-gatherer mayflies (*Ephemerella* spp.), and extremely low (<1%) assimilation of bacterial carbon in shredder caddisflies (*Pycnopsyche* spp.). These differences among taxa were consistent across years and in both natural and canopy-thinned conditions. Brook trout were enriched in both ^{15}N and ^{13}C, but did not follow typical spatial patterns observed in other food web compartments (that is enrichment did not decline from upstream to downstream sampling stations like it did for all other food web compart-

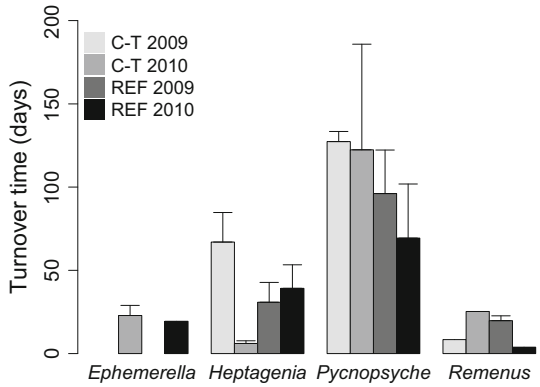

Figure 5. Turnover times, calculated with the dynamic compartment model using ^{15}N tracer data, for invertebrates in two study streams in 2009 and 2010 (*C-T* canopy-thinned stream and *REF* reference stream). *Error bars* represent one standard deviation based on variation among sampling stations within a reach.

ments so we could not determine how much bacterial carbon was assimilated).

Changes in the resource base associated with canopy thinning in the treatment stream reduced the proportion of bacterial carbon use for the three invertebrate taxa that we were able to sample during both years. In contrast, bacterial carbon use in the reference stream remained similar or increased in 2010 for those taxa (Table 2). We were not able to calculate changes in bacterial carbon use by *Ephemerella* spp. because they were not sufficiently abundant for collection in either stream in 2009 or in the reference stream in 2010. Turnover time of invertebrates (calculated using the dynamic compartment model) varied by taxon, stream, and year, ranging from 4 to 120 days (Fig. 5). Turnover times were consistently longest for *Pycnopsyche* compared to other invertebrate taxa and shortest for *Remenus* (Figure 5). Nearly all invertebrate taxa had longer turnover times than the 10-day duration of the isotope tracer release.

Discussion

Comparison of Bacterial Carbon Use Among Functional Feeding Groups of Consumers

Our results indicate that stream invertebrates can derive a high proportion of their carbon from heterotrophic bacteria, but that the proportion of assimilated carbon, that is derived from bacteria, depends on the functional feeding group and light regime. Interestingly, scraper mayflies (*Heptagenia*) assimilated nearly all of their carbon from bacteria (70% or more), while shredder caddisflies (*Pycnopsyche*) assimilated almost no carbon from bacterial sources. These results are consistent with previous studies that found high-bacterial carbon use (50% or more) by filterer black flies and scraper mayflies (Edwards and Meyer 1987; 1990) and extremely low bacterial carbon use (<1%) by shredder invertebrates (Findlay and others 1986). Our estimates of bacterial carbon use are also comparable to estimates from a previous whole-stream ^{13}C tracer addition study by Hall and Meyer (1998), except that the absolute value of our estimates was generally lower. For example, Hall and Meyer found that scraper heptageniid mayflies assimilated very high fractions of bacterial carbon relative to other invertebrate taxa, but methodological issues led to values in excess of 100% that are difficult to compare estimates from our system.

The respiration method we developed to measure $\delta^{13}C$ of bacteria allowed us to identify a highly enriched source that could account for the isotope label of invertebrate consumers. Actively respiring bacteria were several orders of magnitude more enriched than $\delta^{13}C$ of bulk epilithon, FBOM or CBOM, and far exceeded the most enriched invertebrates (scraper mayflies in the genus *Heptagenia*). These results suggest that invertebrates selectively feed on or assimilate carbon from components of bulk resource pools, such as live bacteria. Hall and Meyer (1998) suggested that direct consumption of DOC or consumption of highly enriched exopolymers might account for the high ^{13}C label of consumers, but our respiration results suggest that measuring the isotopic label of live components of biofilms and detrital pools can also account for the label found in consumers.

Identifying a carbon source (that is live bacteria) that was more enriched than consumers greatly improves the plausibility of estimates of bacterial carbon consumed by invertebrates. Previous ^{13}C tracer studies (Hall and Meyer 1998; Simon and others 2003; Parkyn and others 2005) have had difficulty calculating how much carbon invertebrates derive from food sources because bulk food pools are often less enriched than invertebrate primary consumers, making it impossible to identify the source of isotope label found in invertebrates or to estimate how much carbon invertebrates derive from bacterial sources accurately. The issue of ''over-enrichment'' of consumers relative to their food is also commonly observed in ^{15}N tracer studies. Dodds and others (2014) synthesized results from 19 ^{15}N isotope tracer studies in streams across North America, Europe, and the Neotropics, and found that 41 of 90 consumers taxa studied had tissues that were

more enriched than their presumed food sources, likely due to consumers selectively assimilating live elements of ingested foods or selectively grazing on live elements that are not isolated when researchers sample suspected food resources.

Our data are unlikely to meet the assumption that invertebrates reached an equilibrium isotope content, especially for taxa with slow turnover rates. Our estimates of turnover calculated using the dynamic compartment model indicated that turnover time of scraper mayflies (*Heptagenia*) was much faster than shredder caddisflies (*Pycnopsyche*). The average turnover time was 97 days for *Pycnopsyche* and 45 days for *Heptagenia*, but the mixing model to determine bacterial carbon use assumes that invertebrates reach equilibrium during the course of our 10-day tracer release. Hence, the model produces more of an underestimate of bacterial carbon used by *Pycnopsyche* than by *Heptagenia*. The duration of our study was limited to the dry, late-summer season to avoid flood events, but longer release times would improve future studies by allowing shredder invertebrates to reach equilibrium with enriched food sources.

Although we suspect that the extremely low percent bacterial carbon use by *Pycnopsyche* is partially an artifact of the duration of the tracer release, it is consistent with previous laboratory studies (Findlay and others 1986) that found very low ($<1\%$) bacterial carbon assimilation relative to respired C by shredders. These results suggest that *Pycnopsyche* likely consumes bulk detrital material and is efficient at assimilating the fungal or terrestrial carbon it consumes. Studies of various shredder species indicate that *Pycnopsyche* may differ from other shredders (for example tipulid cranefly larvae, *Pteronarcys* stoneflies, and *Gammarus* amphipods) in their preference for microbially conditioned leaf food (Arsuffi and Suberkropp 1989; Motomori and others 2001; Rong and others 1995) and their tendency to aggregate and feed in areas with large accumulations of leaves (Tiegs and others 2008). Hence, our results for *Pycnopsyche* are unlikely to apply to all shredder species. Examination of multiple shredder species in future studies would be useful for determining whether use of bacterial carbon varies across species with different feeding behaviors, food preferences, and morphologies.

Influence of Canopy Cover on Bacterial Carbon Use

The light environment had a strong influence on the percentage of carbon assimilated by consumers that were derived from bacterial sources. Experimental canopy thinning led to increases in light availability and algal accrual that increased the algal resource base available to invertebrate consumers in the canopy-thinned stream. Although we did not measure primary production directly, the approximately 10-fold difference in algal accrual rate provides a dynamic estimate of autotrophic components and suggests that primary production was likely higher in the treatment reach relative to the unthinned reach directly upstream.

In some freshwater systems where autochthonous carbon is an important substrate for bacterial production, increases in light and autotrophic production can lead to concurrent increases in bacterial production (for example Cole and others 1988; Findlay and others 1993), but in other systems, changes in primary production or algal abundance are not linked to bacterial production (for example Findlay and others 1991; Findlay and others 1998). In this study system, increased light led to declines in the proportional use of bacterial carbon by consumers, suggesting that even if an increase in bacterial production had occurred that it was not tracked closely by invertebrate consumers. Either the increase in autochthonous food was large enough to outweigh any increase in bacteria, or consumers preferentially fed on or assimilated the more available algal resource. Although we cannot distinguish among mechanisms, it is clear that the feeding of some macroconsumers and their subsequent growth in detrital-based streams are sensitive to shifts in light that increase the availability of autochthonous resources.

We conducted the canopy thinning experiment in September 2009 and conducted post-manipulation studies during the following summer (2010). The 10-mon treatment period was sufficient to see the effects on algal accrual, nutrient uptake, and bacterial carbon assimilation, and the increases in algal biomass relative to nearby streams were substantial. For example, most nearby small streams with heavy canopy shading had chlorophyll standing stocks of less than 4.0 mg m^{-2}, whereas a mid-order river with an open canopy (Moose River) had chlorophyll standing stocks of 18 mg m^{-2} (Collins and others 2015). Hence, chlorophyll standing stock in the thinned reach of the canopy-thinned stream (9.1 mg m^{-2}) was at least twice as much as other small streams, but only about half as high as standing stock in a river with a completely open canopy. Changes in chlorophyll accrual on artificial substrates indicate that canopy thinning led to accrual rates that were approximately five times higher than an unthinned upstream reach. While the treatment period was long enough to see

the effects of light increases on the ecosystem, a longer treatment period might have demonstrated more complex food web effects. For example, previous studies of nutrient addition in streams found complex effects on predator–prey relationships which developed over a 5-year period, with production of all primary consumers increasing over the first 2 years, but only large-bodied species that were resistant to predation increasing in later years (Davis and others 2010).

Studies of how riparian canopy cover and light influence in-stream processes have implications for how watershed land use change affects stream ecosystem function. Although it has been recognized for decades that deforestation of entire watersheds can have profound influences on streams (for example Likens and others 1970; Findlay and others 1993), our results indicate that stream food webs are also sensitive to riparian tree removal. Results from other systems also indicate that streams are sensitive to changes in light availability, which can have a strong influence on basal resources and consumers in streams. For example, changes in catchment forests and shading can result in increased algal accrual and higher nutrient retention (for example Sabater and others 2000; Ambrose and others 2004), changes in how consumers utilize terrestrial subsidies (England and Rosemond 2004; Giling and others 2009), and altered rates of leaf breakdown rates and palatability of leaves to consumers (Lagrue and others 2011).

Overall, it appears that the percent of carbon derived from bacteria can be substantial for many abundant invertebrate taxa in stream food webs that bacterial carbon use can vary dramatically among consumer species, and that it can be strongly influenced by the light environment. An increase in light availability is one of many changes that may influence in-stream processes when watersheds are deforested, and our results demonstrate that light changes can have substantial effects on food web fluxes, specifically with respect to the type of organic matter, that is assimilated by invertebrate consumers. These results suggest that in highly subsidized streams with natural canopy cover, low light, and low algal accrual rates, the percentage of consumer carbon, that is derived from heterotrophic bacteria, can be very high. However, bacterial carbon becomes a relatively less important energy source for several focal invertebrate taxa in open-canopy streams with higher rates of algal accrual.

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