



Quantitative Food Webs Indicate Modest Increases in the Transfer of Allochthonous and Autochthonous C to Macroinvertebrates Following a Large Wood Addition to a Temperate Headwater Stream

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Headwaters suffer from reduced leaf and wood inputs and retention capacity from historical land actions like watershed logging and agriculture. When in-stream wood is reduced, stream retention capacity declines and subsequent changes in streamwater flow-paths and patterns of deposition alter decomposition and primary production that influence secondary invertebrate production via modified habitat and resources. Wood additions are commonly used as stream restoration tools for habitat improvements that can restore or strengthen food web connections; however, changes in carbon (C) flow through food webs are rarely measured because of time and expense. We quantified allochthonous and autochthonous C flow through aquatic macroinvertebrate communities 1 year before and 2 years after an experimental addition of large wood, compared to macroinvertebrates in an upstream control, in a temperate headwater stream. We predicted wood additions increase macroinvertebrate consumption and assimilation of allochthonous and autochthonous C through retention of leaves and altered flow-paths that expose more gravel and cobble for periphyton colonization. Macroinvertebrate allochthonous C assimilation tended to increase in years with greater organic matter retention and autochthonous C increased with more exposed gravel and cobble across seasons and between reaches. While the effect of wood addition on C flow through the macroinvertebrate community was minimal, it increased by ~20% relative to the control from an increase in production and C assimilation of common mayfly and caddisfly scrapers, *Baetis* and *Glossosoma*. Because the amount of organic matter retained and coarse substrate exposed corresponded with C form and amount consumed, restoration of large wood has the potential to increase organic matter C trophic transfer.

Keywords: headwaters, large woody debris, restoration, stable isotopes, organic matter

INTRODUCTION

Freshwater ecosystems cover only a small fraction of Earth's surface but receive at least 1.9 Pg carbon yr^{-1} from terrestrial ecosystems. At least 50% of these carbon (C) inputs are stored in or emitted from freshwaters (Cole et al., 2007). Consequently, cross-ecosystem terrestrial-aquatic exchanges are essential for understanding global C cycling (Cole et al., 2007). Stream food webs are strongly influenced by leaf-litter and wood from adjacent riparian areas. Changes in riparian detrital quality and quantity dictate aquatic biological community structure, organismal growth, and organismal lifecycle completion rates, described as organismal performance (Wallace et al., 1997b). Collectively, this community performance governs aquatic ecosystem functions like secondary production and decomposition (Webster et al., 1997). In turn, in-stream secondary production and decomposition govern the capacity of small streams to transfer and transport C that subsidizes downstream and riparian communities.

Human actions in forested watersheds can change the timing or amount of allochthonous and autochthonous C that affect the organisms available to consume and transfer C (Cummins et al., 1989). For example, greater retention of allochthonous material in streams stimulate growth of fungi and bacteria, and organic matter consumption by macroinvertebrates (Richardson, 1991; Negishi and Richardson, 2003; Tiegs et al., 2008), increasing the relative amount of allochthonous versus autochthonous C transferred through the stream food web (Rosemond et al., 1993). However, increases in water velocity can increase exposure of large substrates subsequently covered in sand and stimulate periphyton growth (Kail, 2002), increasing the relative contribution of autochthonous C to secondary consumers like macroinvertebrates (McNeely et al., 2007). Large wood results in both organic matter retention and substrate sorting via modified flow paths. Therefore, adding wood to streams can change available habitat and food resources for aquatic biota. The concurrent increase in autochthonous and allochthonous C in low-production, forested streams could lead to an overall increase in C contributions to secondary macroinvertebrate production, resulting in greater trophic transfer and more trophic linkages.

Attempts to restore headwater streams through a bottom-up organic matter addition (e.g., addition of large wood) provide stability and greater resource availability that could also alter the relative terrestrial- and instream-derived C eaten and assimilated by aquatic heterotrophs (e.g., Rosi-Marshall and Wallace, 2002). Forested headwater streams have historically been considered to be strongly influenced by the volume and timing of terrestrially derived allochthonous material delivered to the stream (Polis and Strong, 1996; Wallace et al., 1997b; Moore et al., 2004). However, algae can also be seasonally important in many temperate streams (Finlay, 2001; Hall et al., 2001). The alternating increase of algae in winter when the canopy is more open and leaf litter inputs retained in autumn may act to stabilize the food web when resources may otherwise be scarce (Power et al., 1988; Polis and Strong, 1996; Moore

et al., 2004; Guo et al., 2016). We are unaware of any studies that have quantified allochthonous C (i.e., terrestrially derived) versus autochthonous C (algal-derived) following in-stream wood addition.

Ecosystem processes that restorations aim to affect include rates of nutrient uptake (Sudduth et al., 2011), organic matter retention, and decomposition (Lepori et al., 2005; Frainer et al., 2017). These functional metrics are especially useful in restoration studies because they reveal how changes in physical structure may influence the rate, and pathway of energy or elements flowing through an ecosystem. Stable isotopes can be used to identify the source and amount of C assimilated by a target community and are increasingly used in restorations (for example, Fry, 2002; Kennedy et al., 2005; Lepori et al., 2006). For example, Lepori et al. (2006) added boulders to several streams in Sweden that increased detrital retention, but did not result in consumers $\delta^{13}\text{C}$ more similar to the retained detritus.

Still, stable isotopes may identify food source assimilated and can be used in combination with measures of community structure and secondary production to develop quantitative food webs (e.g., Rosi-Marshall and Wallace, 2002). Quantitative food webs can reveal consumer-level controls on ecological processes and illustrate changes in trophic structure (Rosi-Marshall and Wallace, 2002), community assemblage, and nutrient flow (Cross et al., 2007) following ecosystem restoration. Tracking pathways of energy flow through the food web integrates changes in food resource assimilation, community structure, survival, and production (Benke and Wallace, 2011). Carbon flow measurements are commonly used to test ecological theory (e.g., Cross et al., 2007), but have not been used to assess restorations.

We quantified allochthonous and autochthonous C assimilation in macroinvertebrates before and after experimental wood addition in a Michigan headwater stream using natural abundance of C isotopes for macroinvertebrates and their food resources. Then we combined estimates of C assimilation with secondary production (Entrekin et al., 2009) to calculate trophic basis of production (Benke and Wallace, 1997). Finally, by using assumed assimilation efficiencies, we were able to back-calculate the amount of allochthonous and autochthonous C consumed (expressed as a rate) and compare that C flow with the amount available. Our previous work in this stream showed that total macroinvertebrate secondary production was low before wood addition, but increased by $\sim 25\%$ 2 years after wood addition, resulting in a statistically significant increase in invertebrate biomass and greater secondary invertebrate production (Entrekin et al., 2009). Here, we sought to quantify how assimilation, trophic basis of production and C allochthonous and autochthonous C flowing through the macroinvertebrate food web changed after wood was added. We predicted wood addition would increase consumption and assimilation of allochthonous and autochthonous C by macroinvertebrates through an increased retention of leaf litter, and the exposure of sand-covered large inorganic substrates for periphyton colonization.

STUDY SITE

State Creek is a 1st-order stream draining 3.9 km² in the Ottawa National Forest in the Ontonagon River basin in the Upper Peninsula of Michigan, United States (46° 28'N, 89° 1'W). We began sampling monthly in May 2003, 1 year before wood addition (Y0) and continued for 1 (Y1) and 2 (Y2) years after wood addition in both the wood-added (treatment) and the upstream control reach. Treatment and control reaches were separated by a 50 m distance to promote some independence between sites, while reducing differences associated with longitudinal changes. We measured no difference in the amount of in-stream wood between the control and treatment reaches prior to the wood addition (Entrekin et al., 2007). The stream section we studied had 90% canopy cover during spring and summer and 75% canopy cover in autumn and winter, an average bank-full width of 2.4 m in the control reach and 2.5 meters in the treatment reach, an average water depth at base flow of 13 cm in the control reach and 12 cm in the treatment reach, and an average discharge in the control reach of 64 and 67 L s⁻¹ in the treatment reach (for more details see Entrekin et al., 2007; Hoellein et al., 2009). The stream flows through a managed, second-growth forest with intact, but young, riparian vegetation in a catchment of 95% deciduous forest that was last logged in 1967. Riparian trees include *Populus tremuloides* Michx. (trembling aspen), *Acer rubrum* L. (red maple), *Acer saccharum* Marsh. (sugar maple), *Betula papyrifera* Marsh. (paper birch), *Tsuga canadensis* L. (hemlock), *Pinus alba* L. (white pine), with a thick understory of *Alnus serrulata* Alt. (tag alder). The study stream had low in-stream large wood density (13 pieces/100 m stream length) and low storage of coarse benthic organic matter [annual average of 146 ± 70 (SE) g AFDM m⁻²] before wood addition (Cordova et al., 2007; Entrekin et al., 2007) from a history of region-wide logging and shale mining (Webster et al., 2008).

In May 2004, we added 25 logs (each 2.5 m long × 0.5-m diameter) of big tooth aspen *P. grandidentata* Michx., purchased from a nearby tree farm, haphazardly to a 100-m stream reach, while maintaining a 100 m upstream control reach. After 2 years in the stream, 15 of the 25 logs moved. Most of the added logs moved less than two meters with one moving the farthest at 18 m (G. Lamberti unpublished data). None of the added wood moved out of the study reaches. We did measure an increase in the amount of organic matter retained and more exposed cobble from a localized increase in water velocity caused by the added logs (Entrekin et al., 2008).

MATERIALS AND METHODS

Habitat Characteristics

Standing crops of coarse (CBOM) and fine (FBOM) benthic organic matter were measured from five 804-cm² benthic cores sampled in each reach on each sampling date; CBOM was separated from FBOM using a 1-mm sieve. After CBOM was removed from the corer, a FBOM slurry was made by stirring the sediment in the core and then subsampled using a

160 mL specimen container. Subsamples were stored on ice until processing. In the laboratory, samples for CBOM were dried at 60°C, sorted by organic matter type (leaves, moss, and wood), and weighed. A subsample of each organic matter type was then combusted at 550°C, and reweighed to determine ash-free dry mass (AFDM; Benfield 2006). For FBOM, subsamples were filtered onto glass fiber filters (GF/F), dried at 60°C, weighed, combusted at 550°C, and reweighed for AFDM.

On each sampling date, we also measured discharge and velocity from the dilution of a conservative tracer during concurrent measurements of whole-stream nutrient uptake rates (Hoellein et al., 2007). Water temperature was recorded at the bottom of each stream reach hourly from May 2003–May 2006 using HOBO® data loggers (Onset Computer Corporation, Bourne, MA, United States). We also surveyed benthic habitat using transects spaced every 5 m (perpendicular to flow) in both reaches in May and August of 2003 (before wood addition) and in May, July, and November 2004–2006 (after wood addition). Sediments were categorized using the Wentworth scale (Minshall and Rugenski, 2006). Inorganic sediments were classified as boulders, gravel and cobble, and sand, while organic substrates were moss, CBOM (>1 mm), silt, small wood (<10 cm), and large wood (>10 cm). Measurements were recorded every 20 cm across the channel. We calculated percent cover for each substrate at each transect-scale, and as the mean of each category across all transects combined (i.e., reach-scale).

Measuring δ¹³C Natural Abundance Signature

We measured δ¹³C values for the most productive taxa that collectively represented ~90% of the total macroinvertebrate community production (Entrekin et al., 2009). Macroinvertebrate abundance, community composition, and secondary production were measured from monthly (none collected in January) Hess (32 cm diameter, 250 μm-mesh) samples (5 per stream reach) in the treatment and control reaches for the 5-year study period. For secondary production, size-frequency histograms were developed for each taxon and corrected using cohort production intervals. For rare taxa, we used either production to biomass ratios we developed or published values (Entrekin et al., 2009). We then used a sub-set of those individuals for stable isotope analysis. Samples were preserved in 6–8% formalin, which we note may result in a 1–1.65‰ systematic depletion of δ¹³C across taxa (Sarakinis et al., 2002; Bicknell et al., 2011). However, a comparison of δ¹³C differences between frozen and formalin-preserved samples for four dominant taxa in one season showed no consistent change (S. Entrekin, unpublished data). Despite some inorganic C, we did not acidify samples because inorganic C was low and acidifying can lead to fractionation (Schlacher and Connolly, 2014). To measure C stable isotopes of macroinvertebrates, we selected late instar taxa from March (late winter), May (spring), June or July (summer), and November (autumn) for the three study years to represent possible seasonal changes in taxa. Thus, our macroinvertebrate sampling incorporated seasonal variation in macroinvertebrate diet as well as any potential changes due to the wood addition.

For each stable isotope measurement, the number of individuals representing a single taxon varied based on an individual's mass (i.e., 2–20 individuals). When possible, we used individuals from at least three different replicate Hess cores that were taken haphazardly along each 100-m reach to incorporate reach-scale variation and expressed as averages with standard error (see **Supplementary Appendix 1**). Finally, we measured the $\delta^{13}\text{C}$ signature of macroinvertebrate food resources including conditioned leaves (i.e., leaf litter colonized by bacteria and fungi) and algae from grab samples in the selected months. Conditioned leaves were collected along each stream reach from State Creek in spring, summer, autumn, and winter and frozen until analysis. Periphyton samples were also collected seasonally by scraping multiple rocks throughout the stream reach.

Macroinvertebrate taxa and food resources were dried at 60°C, ground to a fine powder, and analyzed on a Finnigan Delta Plus Stable Isotope Mass Spectrometer. Stable isotope values were expressed in δ notation as the difference in parts per thousand (‰) from a standard (PeeDee Belemnite), using the calculation: $\delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) * 1000$; where R is $^{13}\text{C}/^{12}\text{C}$. Our attempts at silica separation (Hamilton et al., 2005) were unsuccessful in isolating low amounts of algae (mostly diatoms) from periphyton biofilm scrapings. Therefore, we used the $\delta^{13}\text{C}$ value of a known grazer, the caddisfly *Glossosoma* sp., as the presumed proxy for periphyton, as has been done in other studies (e.g., Finlay, 2001). *Glossosoma* gut contents were examined each season to verify the presence of algae; however, small amounts of allochthonous C may have been assimilated that would result in an underestimate of the contribution of autochthonous C (to the invertebrate community diet).

Our first goal was to partition allochthonous (i.e., leaf litter) from autochthonous (i.e., in-stream algae) C assimilation by macroinvertebrates as indicated by $\delta^{13}\text{C}$ signatures using a two-source mixing model rather than using gut content analysis. Therefore, no other food resources were used in the mixing model because leaf litter and epilithic algae (or periphyton) represented the end members (allochthonous versus autochthonous food resources) in this food web. We used the model IsoSource and IsoError from the Environmental Protection Agency¹, which has the advantage of accounting for replicate sample variation (Phillips and Gregg, 2001). The $\delta^{13}\text{C}$ average of conditioned leaf litter was $-29.98 \pm 0.01\text{‰}$, and $\delta^{13}\text{C}$ of algal signature of *Glossosoma* sp. was $36.94 \pm 0.2\text{‰}$ (upper confidence interval = 1 and lower confidence interval = 0.97) across our sample period, indicating separation of the two C sources. $\delta^{13}\text{C}$ signatures were not corrected for C fractionation rates as they are likely low and unpredictable (McCutchan et al., 2003).

Relative Allochthonous and Autochthonous C Assimilation and Their Contribution to Production

Then, we used a modified method to quantify the trophic basis of production described by Benke and Wallace (1997, 2011) where the proportional contribution of allochthonous and

autochthonous C to assimilation for each taxon, as indicated by stable isotopes, was multiplied by the total production of that taxon to determine the contribution to secondary invertebrate production or the trophic basis of production. The contribution of allochthonous and autochthonous C to production was then calculated for each dominant macroinvertebrate taxa and summarized for each functional feeding group (FFG). The advantage of this method was that assumptions about assimilation efficiency were not needed because natural abundance of isotopes reflect assimilation. However, we were not able to estimate total amount of resources consumed. Therefore, to quantify how much autochthonous and allochthonous food was consumed (i.e., in units of AFDM per m^{-2} time^{-1}), we divided secondary production by published assimilation efficiencies for detritus and algae and then multiplied that by published net production efficiency (Benke and Wallace, 1997; Hall and Meyer, 1998; Rosi-Marshall and Wallace, 2002) to get autochthonous and allochthonous C consumption (Benke and Wallace, 1997):

Algal or detrital C consumption = (secondary production/assimilation efficiency) * net production efficiency where secondary production is expressed as mg dry mass per m^{-2} yr^{-1} , assimilation efficiency is a proportion (detritus = 0.1 or algae = 0.3), and net production efficiency is a proportion (NPE = 0.5).

Statistical Analyses

We used a Before-After-Control-Impact Analysis of Variance (BACI-ANOVA) (Stewart-Oaten et al., 1986; Underwood, 1992) with a Tukey *post hoc* when significance at $\alpha = 0.05$ was met to test for changes in $\delta^{13}\text{C}$ and the contribution of allochthonous versus autochthonous C for the macroinvertebrate community, functional feeding groups, and for individual taxa. Pearson product moment correlations were used to explore the relationship among $\delta^{13}\text{C}$ values and environmental parameters that may contribute to variation across stream reaches through time (e.g., organic matter standing crops and % substrate cover). All data were tested for conformance to homogeneity of variance using Levene's test and assumptions of ANOVA using Kolmogorov-Smirnov tests, and transformed when assumptions of normality were not met. All analyses were performed with SAS software (v. 8.02, SAS Institute Inc., Cary, NC, United States).

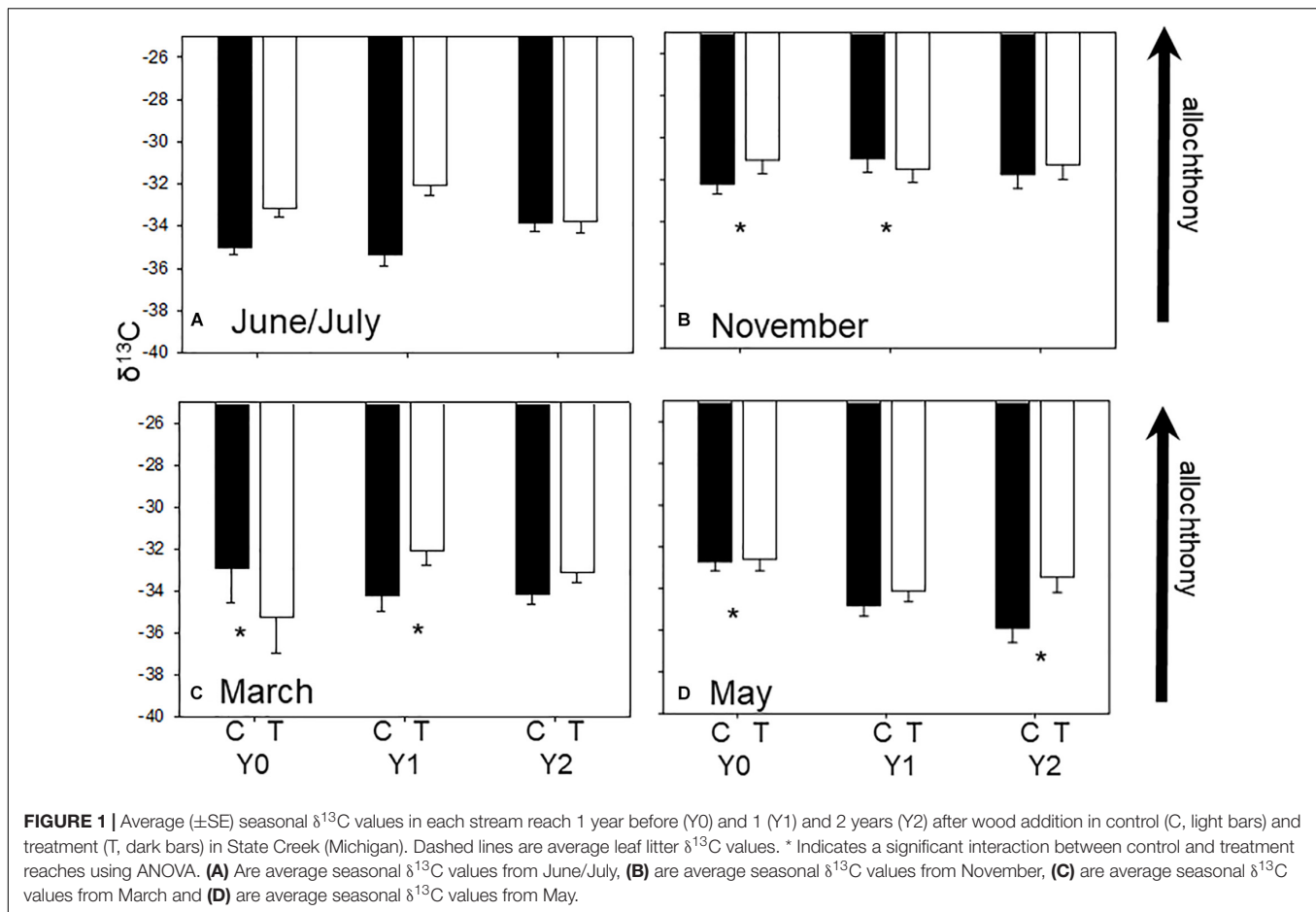
The amount of C consumed, assimilated and supporting secondary production (i.e., flux or flow) were not replicated measurements; therefore, we did not conduct statistics, but we present and describe the effect size as the difference between the treatment and control reaches before and after wood addition.

RESULTS

Changes in $\delta^{13}\text{C}$ Signatures After Wood Addition

$\delta^{13}\text{C}$ ranged from $\sim -25\text{‰}$ to -37‰ across all macroinvertebrate taxa (**Supplementary Appendix 1**). We were surprised that overall average macroinvertebrate $\delta^{13}\text{C}$ remained mostly unchanged following wood addition (**Figure 1**). Before wood

¹http://www.epa.gov/wed/pages/models/stableIsotopes/isotopes/isoerror1_04.htm



addition, the mean (± 1 standard error) $\delta^{13}\text{C}$ signature across all macroinvertebrate taxa differed by $\sim 1\text{‰}$ between the control and treatment reaches (**Figure 1** and **Table 1**). The pattern remained after wood addition, and there was no difference in community-level $\delta^{13}\text{C}$ between the treatment and control reach in Y1 ($F_{3,12} = 0.42$, $P = 0.74$) or Y2 ($F_{3,12} = 0.23$, $P = 0.87$) (**Table 1**). Similarly, there was no difference in functional feeding group and taxa-specific $\delta^{13}\text{C}$ between reaches after wood addition in Y1 or Y2 (**Table 1**).

Seasonal Changes in $\delta^{13}\text{C}$ Signatures

Macroinvertebrate $\delta^{13}\text{C}$ signatures reflected seasonal variation in resources as expected in a temperate headwater stream with signatures closer to leaf-litter in autumn (**Supplementary Appendix 1** and **Figure 1B**) and farther from leaf-litter in March (**Figure 1C**) when canopy is more open. Community mean $\delta^{13}\text{C}$ values were different among seasons ($F_{3,8} = 13.54$, $P < 0.001$) with greater $\delta^{13}\text{C}$ value of -31.79‰ (i.e., closer to leaf litter signature) in autumn (November) and a lower $\sim -33.5\text{‰}$ $\delta^{13}\text{C}$ in winter, spring, and summer (i.e., farther from leaf litter signature) (**Figure 1**, Tukey's test $P < 0.001$).

The $\delta^{13}\text{C}$ signatures of many individual taxa responded to wood addition differently depending upon taxonomic identity and season, although not necessarily in a predictable manner

(**Supplementary Appendix 1** and **Table 1**). For example, *Tipula*, an obligate shredder, tended to have $\delta^{13}\text{C}$ closer to leaf litter in the treatment reach relative to the control in Y2 compared to their difference in Y0. A dominant filterer, *Simulium*, had lower $\delta^{13}\text{C}$ in the treatment reach in May of Y2 compared to the difference between the control and treatment reach in Y0 (**Supplementary Appendix 1**, $F_{3,4} = 77.77$, $p < 0.001$). In addition, the predatory stonefly, *Isogenoides*, had greater $\delta^{13}\text{C}$ in the treatment reach in autumn of Y1 compared to the difference between the control and treatment reach in Y0 (**Table 1**, $F_{3,5} = 11.7$, $p = 0.01$). *Baetis*, among the most productive facultative scraper and gatherers, had greater $\delta^{13}\text{C}$ in the treatment reach in Y2 winter compared to the difference between the control and treatment reach in Y0 (**Supplementary Appendix 1**, $F_{3,7} = 18.6$, $p = 0.001$).

Variation in Macroinvertebrate Functional Feeding Group C Assimilation Related to Substrate Composition and Organic Matter Standing Stocks

We correlated allochthonous C assimilation of average community and functional groups with substrate type to identify environmental factors influencing variation across stream reaches and season (**Figure 2**). The overall % allochthonous C assimilation at the community level was not explained

TABLE 1 | Mean annual $\delta^{13}\text{C}$ values for each taxon.

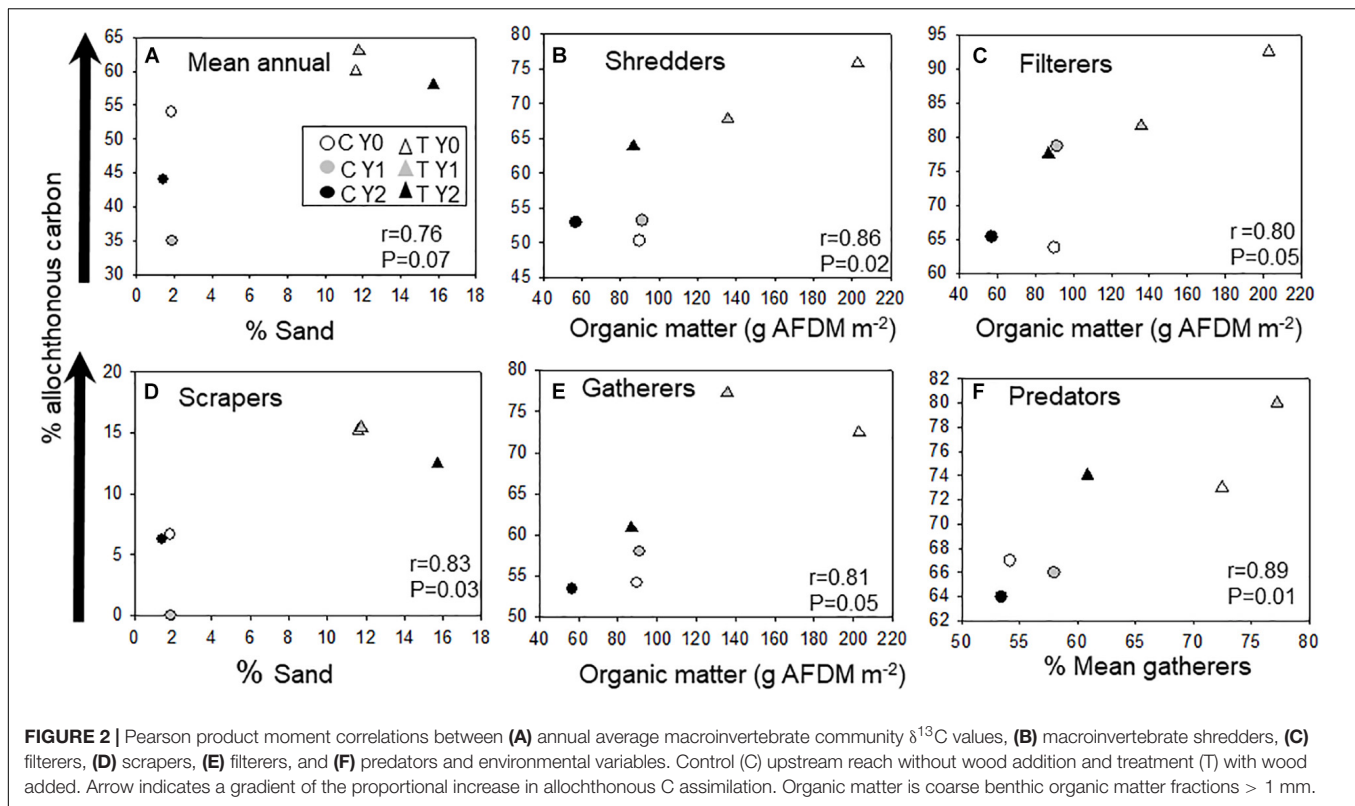
	Y0			Y1			Y2		
	C	T	D	C	T	D	C	T	D
Shredders									
<i>Amphinemura</i> sp.	-34.70	-33.90	0.80	-36.70	-34.50	2.20	-34.90	-34.40	0.50
Capniidae	-31.10	-31.30	-0.20	-28.60	-30.30	-1.70	-33.20	-31.80	1.40
<i>Nemoura</i> sp.	-37.80	-33.20	4.60	-38.20	-34.10	4.10	-34.10	-34.60	-0.50
* <i>Tipula</i> sp.	-29.00	-26.70	2.30	-28.20	-28.90	-0.70	-29.30	-29.10	0.20
Mean	-33.15	-31.28	1.88	-32.93	-31.95	0.98	-32.88	-32.48	0.40
Gatherers									
Diamesinae	-36.80	-34.30	2.50	-34.80		34.80	-35.30	-33.60	1.70
<i>Ephemerella</i> spp.	-33.00	-31.60	1.40	-34.80	-31.10	3.70	-35.40	-31.90	3.50
<i>Eukiefferiella</i> spp.	-36.40	-34.00	2.40	-38.40	-36.30	2.10	-36.20	-35.80	0.40
<i>Hesperoconopa</i> sp.	-31.30	-30.40	0.90	-32.40	-30.80	1.60	-32.50	-31.80	0.70
<i>Parametriocnemus</i> sp.	-30.20	-30.10	0.10	-31.40	-29.10	2.30	-29.00	-29.50	-0.50
<i>Tanytarsus</i> spp.	-32.50	-30.10	2.40	-31.60	-29.60	2.00	-34.40	-33.30	1.10
Oligochaeta	-29.80	-30.90	-1.10	-30.10	-29.90	0.20	-28.40	-29.10	-0.70
Mean	-32.86	-31.63	1.23	-33.36	-31.13	2.22	-33.03	-32.14	0.89
Scrapers									
* <i>Baetis</i> spp.	-36.00	-35.50	0.50	-37.80	-34.90	2.90	-35.90	-34.70	1.20
<i>Gossosoma</i> sp.	-36.70	-36.00	0.70	-37.60	-36.70	0.90	-37.70	-37.40	0.30
<i>Neophylax</i> sp.	-38.20	-35.70	2.50	-38.40	-35.60	2.80	-38.40	-38.00	0.40
<i>Rhithrogena</i> sp.	-30.40	-31.30	-0.90		-35.10			-34.30	
Mean	-35.33	-34.63	0.70	-37.93	-35.58	2.36	-37.33	-36.10	1.23
Filterers									
<i>Dolophilodes</i> sp.	-31.90	-30.40	1.50	-29.40	-30.60	-1.20	-32.10		
<i>Parapsyche</i> sp.	-31.40	-29.80	1.60	-31.90	-30.80	1.10	-33.20	-30.70	2.50
<i>Prosimulium</i> sp.	-32.50	-33.50	-1.00	-32.60	-32.00	0.60	-31.70	-33.30	-1.60
* <i>Simulium</i> spp.	-33.70	-32.20	1.50	-31.00	-31.60	-0.60	-30.90	-30.50	0.40
Mean	-32.38	-31.48	0.90	-31.23	-31.25	-0.02	-31.98	-31.50	0.48
Predator									
Ceratopogonidae	-32.60	-33.10	-0.50	-31.40	-30.90	0.50	-31.30	-31.90	-0.60
<i>Dicranota</i> sp.	-31.30	-32.20	-0.90	-32.00	-31.00	1.00	-30.70	-31.70	-1.00
* <i>Hexatoma</i> spp.	-30.80	-29.70	1.10	-31.50	-29.70	1.80	-32.80	-28.10	4.70
<i>Isogenoides</i> sp.	-32.90	-31.90	1.00	-32.60	-31.90	0.70	-33.90	-33.70	0.20
<i>Rhyacophila</i> sp.	-32.70	-31.60	1.10	-31.00	-32.60	-1.60	-33.40	-31.20	2.20
Mean	-32.06	-31.70	0.36	-31.70	-31.22	0.48	-32.42	-31.32	1.10
Community mean	-33.15	-32.14	1.01	-33.43	-32.23	1.20	-33.53	-32.71	0.82

*Indicates a significant change in mean macroinvertebrate $\delta^{13}\text{C}$ value after wood addition. D is the difference in treatment (T) and control (C). B is before (Y0) and A is after (Y1 and Y2) wood addition. Functional groups are in bold.

by any one variable, although variation between stream reaches was related to the proportion of sand making up the stream bottom (Figure 2A). The relative amount of allochthonous C assimilated by macroinvertebrate FFGs across season and reaches was mostly correlated to differences in available coarse substrate and organic matter standing crop. The overall proportion of allochthonous C assimilation by shredders, filterers, and gatherers was positively related to the mean annual coarse organic matter standing crop (Figures 2B,C,E). In contrast, scrapers, that feed mostly on inorganic substrates, ranged from less than 1 to 15% allochthonous C assimilation. The proportion of terrestrially derived C assimilated by predators closely tracked gatherer assimilation of allochthonous C (Figure 2F).

All Functional Feeding Groups Consumed Allochthonous and Autochthonous C Sources, but FFGs Consumed Different Amounts of the C Sources

Allochthonous and autochthonous-based C contributed roughly equal parts to macroinvertebrate production in the control and treatment reaches, but with little change following wood addition. Annual contribution of allochthonous- C ranged from 35 to 44% in the control reach and 41–56% in the treatment reach over the 3-year study (Figure 3A). The origin of C assimilated by FFGs differed among groups as expected. Autochthonous-based C contributed the most to scraper production, ranging from 70 to 100%. However, allochthonous-based C contribution decreased in scrapers in Y1 relative to the difference between the control and treatment in Y0 (Figure 3B). For shredders,



contribution of allochthonous C ranged from 50 to 90%, varying among years, reach, and taxa (Figure 3C and Supplementary Appendix 1). Contribution of allochthonous C to gatherer production increased in Y1 in the treatment reach relative to the difference between the control and treatment in Y0, ranging from 58 to 66% in the control reach and 72 to 88% in the treatment reach (Figure 3D). Allochthonous C contribution to filterer production increased in both reaches in Y1 and declined back to pre-wood addition values in Y2 (60–90%; Figure 3E). Allochthonous C contribution to predator production ranged from 62 to 77% and changed very little following the addition of wood (Figure 3F).

Actual Allochthonous-Based and Autochthonous-Based C Contributing to Macroinvertebrate Production

In the control reach, allochthonous and autochthonous production declined each year of the study. In contrast, allochthonous and autochthonous -based production in the treatment reach was identical in Y0 and Y2 but lower in Y1 (Table 2 and Figure 4A). All FFG secondary production declined in Y1, while scraper and shredder production increased beyond Y0 values. Autochthonous-based scraper production was lowest in the treatment reach in Y1 and greatest in the treatment reach in Y2 (Figure 4B). Changes in scraper production in Y2 of the treatment reach were driven by an increase in mayfly *Baetis* production and C assimilation changed from mostly autochthonous to more allochthonous (Table 2). Allochthonous

based shredder production was also lowest in Y1 and greatest in the treatment reach in Y2 from an increase in *Tipula* production (Figure 4C). Other FFGs did not show evidence of a treatment effect. Gatherer production was consistent across years in the control reach, but declined in the treatment reach, while filterer production declined across years in both reaches (Figure 4E). Finally, overall predator production and the ratio of allochthonous and autochthonous-based C contributions to production changed little across dates and reaches (Figure 4F).

Consumption of Allochthonous and Autochthonous- C

We calculated the amount of allochthonous and autochthonous C consumed by macroinvertebrates from published assimilation efficiencies to document changes in the amount of each resource flowing through the macroinvertebrate food web. Because leaf litter has a lower assimilation efficiency (0.1) than algae (0.3), macroinvertebrates must consume more allochthonous -based C to support their energetic demands (Benke and Wallace, 1997). Therefore, autochthonous-based C contributed a larger proportion to production, even though autochthonous consumption was less than allochthonous (Figure 5A). All functional feeding groups consumed both C sources. However, scrapers consumed relatively more autochthonous (Figure 5B) and filterers (Figure 5C) consumed more allochthonous C. Shredders (Figure 5D) and gatherers (Figure 5E) consumed mostly allochthonous C and contributed more to overall energy

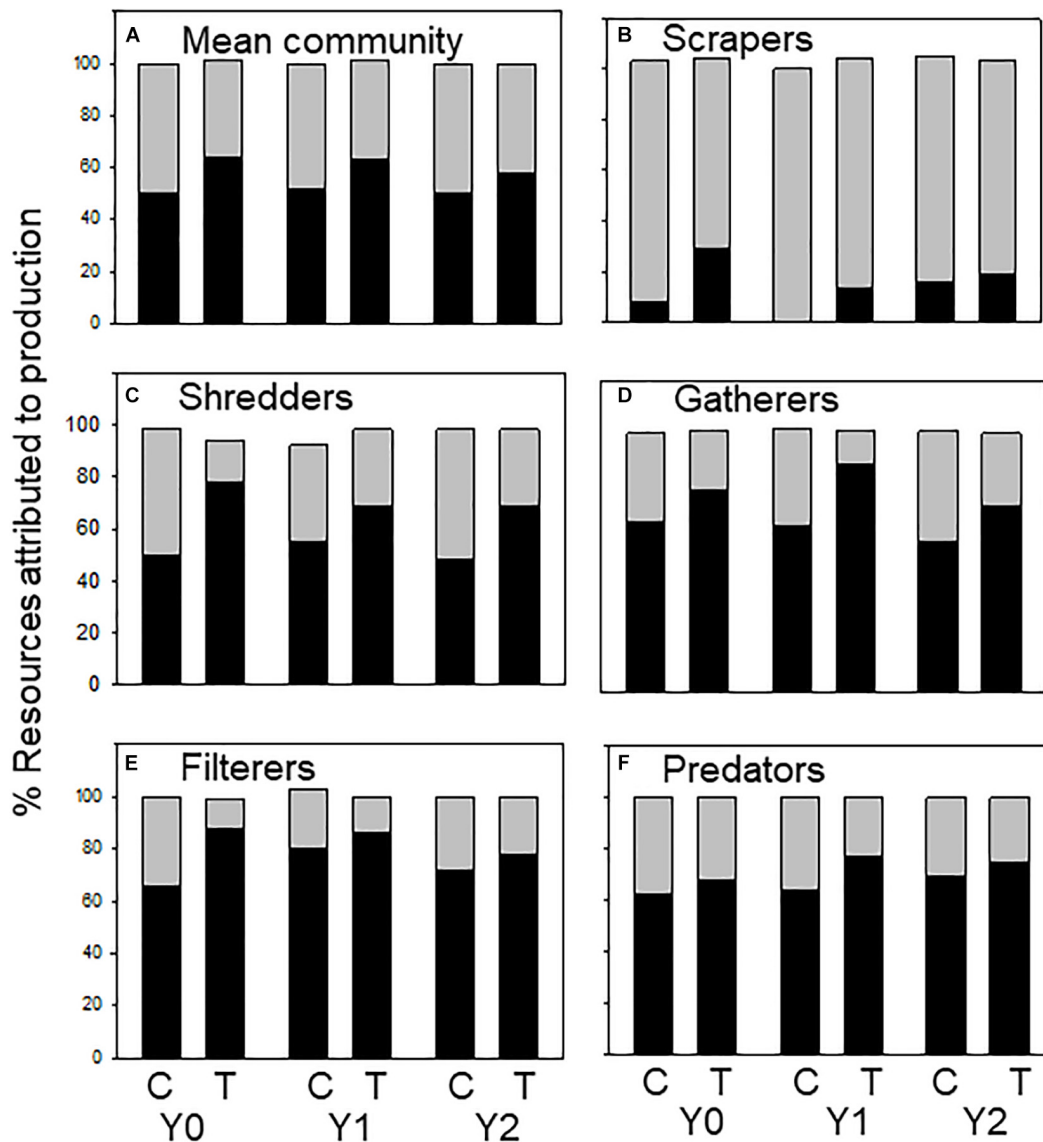


FIGURE 3 | Percent allochthonous (dark bars) and autochthonous (light bars) C contributing to macroinvertebrate secondary production (categorized by functional feeding groups) in an upstream control C and downstream wood-added reach (treatment, T) 1 year before (Y0) and 1 (Y1) and 2 years (Y2) after wood addition. Values may not equal 100% because percentages were averaged across taxa within functional feeding groups (FFGs). (A) % resources attributed to total macroinvertebrate production, (B) % resources attributed to scraper production, (C) % resources attributed to shredder production, (D) % resources attributed to gatherer production, (E) % resources attributed to filterer production, and (F) % resources attributed to predator production.

flow by consuming more material to support relatively greater secondary production (Figure 5).

Energy Flows From Autochthonous and Allochthonous-Based C

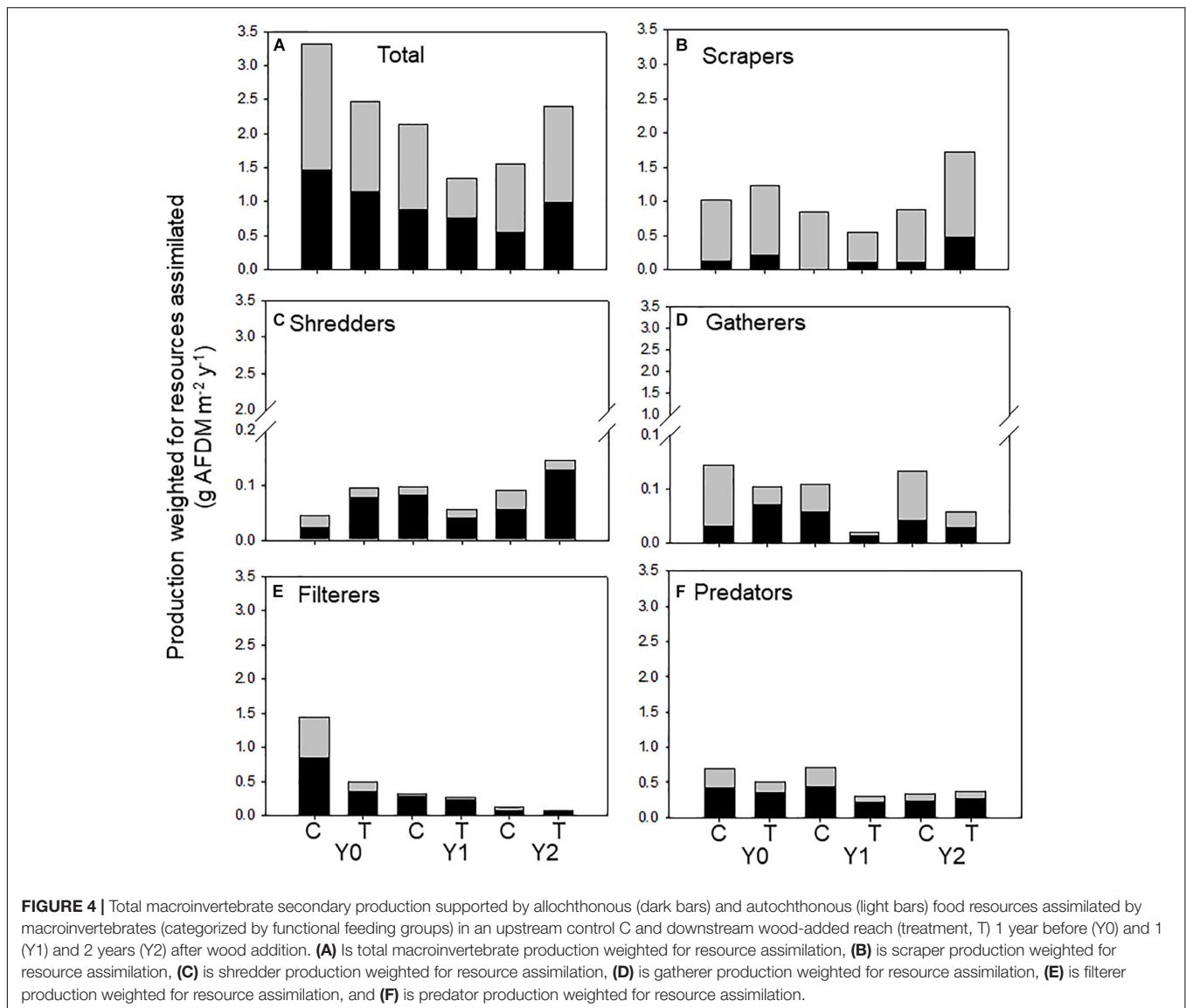
In the control reach, the dominant energy flows remained fairly consistent among Y0, Y1, and Y2 (Table 3 and Figure 6). autochthonous C consumed by scrapers remained fairly evenly distributed between *Baetis* and *Glossosoma* in each year, with a slight increase to *Baetis* in Y2 (Figure 6). Allochthonous

production also remained constant flowing mostly through the filtering black flies, *Simulium*, and to a lesser extent a net-spinning caddisfly, *Parapsyche*, with no changes from Y0 to Y2. In contrast, energy flow in the treatment reach showed much greater variation across years (Figure 6). Scraper basal C flow in Y0 was split between *Baetis* and *Neophylax* in the treatment reach and was predominantly autochthonous C sources. In Y1 and Y2 in the treatment reach, allochthonous C contributed the most to *Baetis* production in Y2 (Figure 6). For shredders, C flow through allochthonous C flow through *Tipula* was greater in Y2 than in Y0 or Y1.

TABLE 2 | Total allochthonous (alloch) or autochthonous (auto) carbon based production (mg AFDM m⁻² y⁻¹) of each taxon calculated as production multiplied by the proportion of alloch or auto carbon source assimilated (estimated from $\delta^{13}\text{C}$ values).

Taxon	Y0						Y1						Y2					
	C		T		D		C		T		D		C		T		D	
	Alloch	Auto	Alloch	Auto	Alloch	Auto	Alloch	Auto	Alloch	Auto	Alloch	Auto	Alloch	Auto	Alloch	Auto	Alloch	Auto
Shredder																		
<i>Amphinemura</i> sp.	4.7	13.4	2.8	3.7	-1.9	-9.7	0.8	5.2	2.5	4.6	1.8	-0.6	3.5	9.2	2.0	2.4	-1.5	-6.9
Capniidae	11.7	3.8	3.7	0.9	-8.0	-2.9	32.7	0.0	24.5	1.6	-8.2	1.6	18.7	16.2	35.6	11.6	16.9	-4.7
<i>Nemoura</i> sp.	0.0	5.2	53.6	14.6	53.6	9.4	0.0	8.4	7.1	10.0	7.1	1.6	5.0	11.3	2.3	4.4	-2.8	-6.9
<i>Tipula</i> sp.	5.8	0.0	17.0	0.0	11.2	0.0	49.3	0.0	5.7	0.0	-43.6	0.0	27.9	0.0	88.2	0.0	60.3	0.0
Total	22.2	22.4	77.1	19.2	54.9	-3.2	82.8	13.6	39.8	16.1	-43.0	2.5	55.2	36.8	128.1	18.4	72.9	-18.5
Gatherer																		
Diamesinae	3.0	48.4	11.3	16.2	8.3	-32.2	12.8	12.5	0.0	0.0	-12.8	-12.5	3.9	12.5	7.1	6.6	3.2	-6.0
<i>Ephemerella</i> sp.	5.9	5.9	12.2	3.5	6.2	-2.3	24.8	53.1	57.4	23.3	32.5	-29.8	1.7	4.7	7.3	3.3	5.7	-1.4
<i>Hesperoconopa</i> sp.	23.1	5.5	76.9	5.3	53.8	-0.2	8.9	4.9	83.5	12.0	74.6	7.2	25.5	15.0	20.1	11.8	-5.4	-3.2
Oligochaeta	12.7	0.0	3.4	0.4	-9.3	0.4	20.6	2.7	8.5	0.2	-12.0	-2.4	15.6	0.8	17.2	4.6	1.6	3.8
<i>Parametrioconemus</i> sp.	0.4	0.0	0.3	0.0	-0.2	0.0	0.5	0.1	0.3	0.0	-0.2	-0.1	0.4	0.0	0.3	0.0	-0.1	0.0
<i>Tanytarsus</i> sp.	12.2	7.0	23.7	0.3	11.5	-6.7	16.2	8.5	6.9	0.0	-9.3	-8.5	16.4	30.3	7.0	6.4	-9.4	-23.9
Total	57.4	66.8	127.8	25.7	70.4	-41.1	83.8	81.7	156.6	35.5	72.8	-46.2	63.4	63.3	44.6	22.9	-18.8	-40.5
Filterer																		
<i>Dolophilodes</i> sp.	51.1	22.3	23.8	1.7	-27.4	-20.6	0.0	0.0	0.0	0.0	0.0	0.0	4.0	1.9	31.8	3.1	27.8	1.2
<i>Parapsyche</i> sp.	104.1	30.9	332.6	29.7	228.6	-1.1	80.0	26.8	202.3	16.0	122.3	-10.8	58.8	51.9	28.0	3.5	-30.9	-48.3
<i>Prosimulium</i> sp.	461.1	294.0	0.0	98.7	-461.1	-195.3	29.4	15.3	32.1	13.3	2.7	-2.0	5.9	2.0	2.3	2.1	-3.6	0.1
<i>Simulium</i> sp.	224.4	245.2	0.0	0.0	-224.4	-245.2	173.9	0.0	0.0	0.0	-173.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	840.7	592.4	356.4	130.2	-484.3	-462.2	283.3	42.1	234.4	29.3	-48.9	-12.8	68.8	55.7	62.1	8.7	-6.7	-47.0
Scraper																		
<i>Baetis</i> spp.	109.2	698.2	178.7	622.0	69.5	-76.2	0.0	414.8	89.1	292.6	89.1	-122.2	113.7	614.0	467.0	886.7	353.4	272.7
<i>Eukiefferella</i> sp.	0.2	1.6	0.2	0.3	-0.1	-1.3	0.1	4.0	0.3	2.7	0.3	-1.2	0.4	2.8	0.4	1.5	0.0	-1.3
<i>Glossosoma</i> sp.	11.8	194.8	17.6	218.5	5.8	23.7	0.0	419.4	2.8	87.3	2.8	-332.1	3.3	134.0	6.7	327.2	3.3	193.3
<i>Neophylax</i> sp.	0.0	7.6	166.1	27.7	166.1	20.1	0.0	16.6	13.5	54.9	13.5	38.3	0.0	12.4	0.0	26.7	0.0	14.3
Total	121.3	902.1	362.7	868.5	241.4	-33.7	0.1	854.7	105.7	437.5	105.6	-417.2	117.4	763.1	474.1	1242.1	356.7	479.0
Predator																		
Ceratopogonidae	11.4	7.1	23.1	18.2	11.7	11.1	3.6	2.8	3.7	0.4	0.1	-2.4	5.0	1.9	7.4	2.8	2.3	0.9
<i>Dicranota</i> sp.	110.6	29.6	75.9	46.5	-34.7	16.9	38.7	20.4	60.8	13.1	22.1	-7.3	154.4	17.7	119.3	40.7	-35.1	23.0
<i>Hexatoma</i> sp.	3.6	0.5	1.8	-0.1	-1.7	-0.6	3.9	1.1	3.4	0.4	-0.5	-0.7	3.8	2.6	5.2	0.0	1.4	-2.6
<i>Isogenoides</i> sp.	74.5	80.3	51.9	31.0	-22.5	-49.4	233.6	190.1	109.9	35.5	-123.7	-154.5	40.4	33.5	33.4	38.8	-6.9	5.4
<i>Rhyacophila</i> sp.	219.1	151.7	201.7	59.8	-17.5	-91.9	159.1	50.1	42.6	25.7	-116.5	-24.4	35.1	39.9	97.9	27.8	62.9	-12.0
Total	419.2	269.1	354.4	155.4	-64.7	-113.8	438.9	264.4	220.4	75.0	-218.5	-189.4	238.6	95.6	263.2	110.2	24.6	14.6
Community total	1460.6	1852.8	1278.4	1198.9	-182.3	-653.9	888.9	1256.5	756.9	593.5	-131.9	-663.0	543.3	1014.5	972.1	1402.1	428.7	387.7

*Indicates a significant change in mean macroinvertebrate $\delta^{13}\text{C}$ value after wood addition. D is the difference in treatment (T) and control (C). B is before (Y0) and A. is after (Y1 and Y2) wood addition. Functional groups are in bold.



DISCUSSION

Implications for Management and Restoration

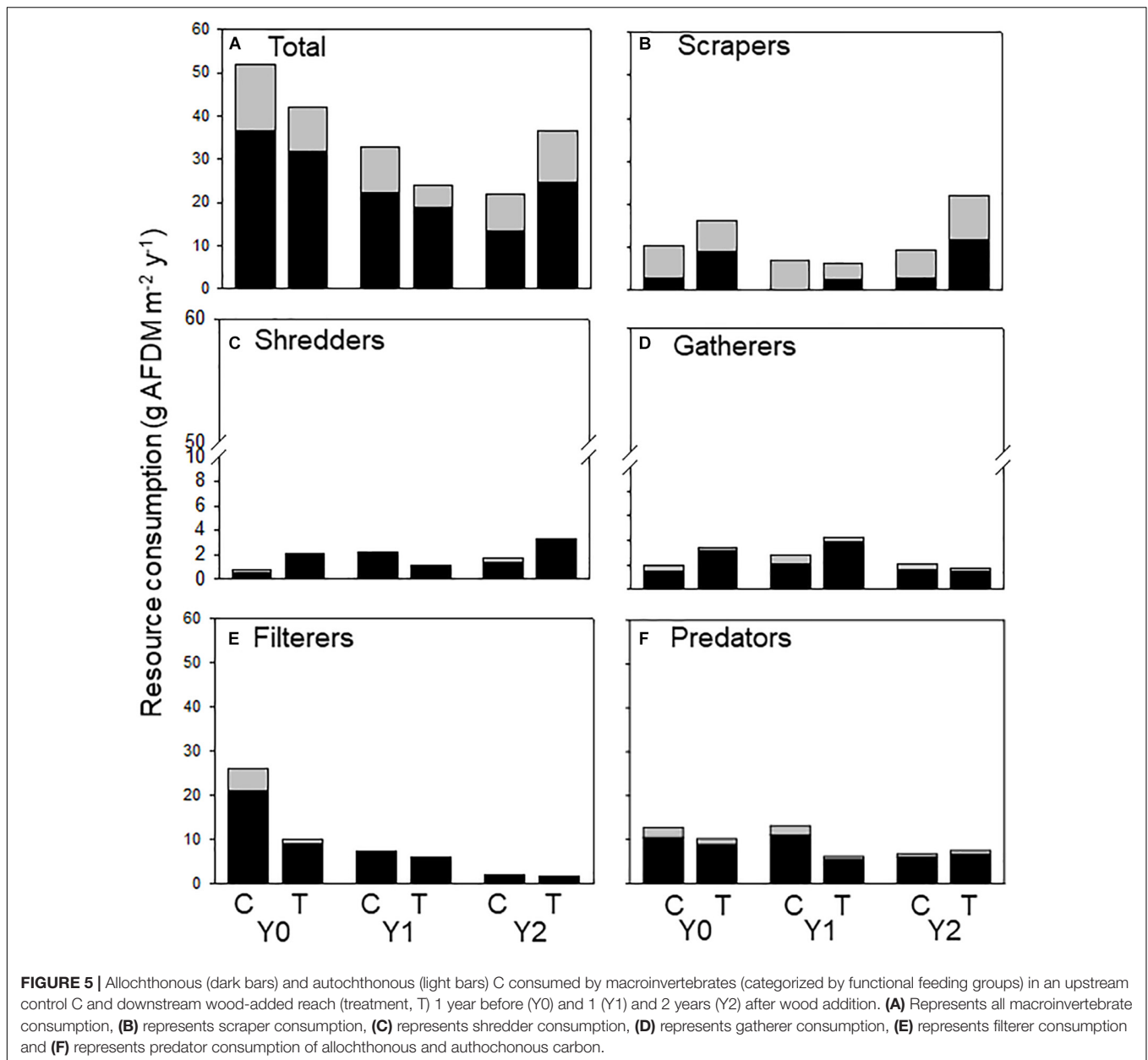
Combining $\delta^{13}\text{C}$ signatures to get allochthonous and autochthonous C assimilation with secondary production provided a unique investigation into the macroinvertebrate trophic response to wood addition (Figure 6). The combined approach of C assimilation and macroinvertebrate secondary production indicated changes in the consumption and flow of allochthonous and autochthonous C through the macroinvertebrate food web. Here we demonstrated that the abundance of organic matter and locally exposed cobble and gravel, which supports periphyton, could drive increased allochthonous and autochthonous C flow to several macroinvertebrate functional feeding groups most notably greater autochthonous C contribution to scrapers and greater

allochthonous C contribution to filterers shredders (Figure 3). Taxon-specific seasonal and annual shifts in C assimilation were documented (Supplementary Appendix 1). Still, we observed changes in total resource consumption from the coupled increase in organic matter standing crop and substrate sorting at a local scale (i.e., around the added logs) following wood addition (Entrekin et al., 2008). Unfortunately, we did not replicate this study, which is a commonly encountered challenge for reach-scale quantitative food web approaches that measure energy flow (e.g., Cross et al., 2007). Despite the limits for generalizations, our results support methods for future evaluations of restoration (see also Vander Zanden et al., 2006) by stressing the need for trophic-based analysis to assess restoration success or failure in terms of resource availability and energy flow. The combined structural and functional approach here points to physical changes from the restoration that increased allochthonous and autochthonous C flow (Figure 5) and increased food web

TABLE 3 | Total amount of allochthonous (alloch) and autochthonous (auto) food sources consumed by each taxon (mg AFDM m⁻² y⁻¹).

Taxon	Y0						Y1						Y2					
	C		T		D		C		T		D		C		T		D	
	Alloch	Auto	Alloch	Auto	Alloch	Auto	Alloch	Auto	Alloch	Auto	Alloch	Auto	Alloch	Auto	Alloch	Auto	Alloch	Auto
Shredder																		
<i>Amphinemura</i> sp.	118	111	71	31	-47	-81	19	43	63	38	44	-5	88	77	50	20	-38	-57
Capniidae	292	32	91	8	-201	-24	818	0	612	13	-205	13	469	135	890	97	422	-39
<i>Nemoura</i> sp.	0	43	1340	122	1340	78	0	70	176	83	176	13	126	95	57	37	-69	-58
<i>Tipula</i> sp.	145	0	425	0	280	0	1233	0	142	0	-1091	0	697	0	2206	0	1509	0
Total	554	187	1927	160	1373	-27	2070	113	994	134	-1076	21	1380	307	3203	153	1824	-154
Gatherer																		
Diamesinae	76	403	283	135	207	-268	321	104	0	0	-321	-104	97	105	177	55	80	-50
<i>Ephemerella</i> sp.	149	49	304	30	156	-19	621	442	1434	194	813	-249	41	39	183	27	141	-12
<i>Hesperoconopa</i> sp.	577	46	1923	44	1346	-2	223	41	2088	100	1865	60	638	125	503	98	-135	-26
Oligochaeta	317	0	85	3	-233	3	514	22	214	2	-300	-20	391	7	431	39	40	32
<i>Paramethocnemus</i> sp.	10	0	6	0	-4	0	11	1	8	0	-4	-1	10	0	7	0	-3	0
<i>Tanytarsus</i> sp.	305	58	593	2	288	-56	406	71	172	0	-234	-71	409	252	175	53	-234	-199
Total	1434	557	3194	214	1760	-342	2095	681	3915	296	1820	-385	1585	528	1475	272	-110	-255
Filterer																		
<i>Dolophilodes</i> sp.	1279	186	595	14	-684	-171	0	0	0	0	0	0	101	16	795	26	694	10
<i>Parapsyche</i> sp.	2601	257	8316	248	5714	-9	2000	223	5058	133	3058	-90	1471	432	700	30	-771	-403
<i>Prosimulium</i> sp.	11528	2450	0	823	-11528	-1627	736	128	803	111	67	-17	148	16	58	17	-90	1
<i>Simulium</i> sp.	5610	2044	0	0	-5610	-2044	4346	0	0	0	-4346	0	0	0	0	0	0	0
Total	21017	4936	8910	1085	-12107	-3852	7082	351	5861	244	-1221	-106	1719	464	1552	73	-167	-392
Scraper																		
<i>Baetis</i> spp.	2731	5818	4469	5183	1738	-635	0	3457	2228	2439	2228	-1018	2841	5117	11676	7389	8834	2272
<i>Eukieffehella</i> sp.	5	13	4	3	-1	-11	2	33	8	23	6	-10	10	23	10	12	0	-11
<i>Glossosoma</i> sp.	296	1623	441	1821	145	198	0	3495	70	727	70	-2768	83	1117	167	2727	84	1611
<i>Neophylax</i> sp.	0	63	4154	231	4154	167	0	138	338	457	338	319	0	103	0	222	0	119
Total	3032	7518	9067	7237	6035	-281	2	7123	2643	3646	2641	-3477	2935	6359	11852	10351	8918	3991
Predator																		
Ceratopogonidae	285	59	577	152	292	93	91	23	93	3	3	-20	126	16	185	23	59	7
<i>Dicranota</i> sp.	2766	246	1898	387	-868	141	968	170	1519	109	552	-61	3860	148	2982	339	-878	192
<i>Hexatoma</i> sp.	89	4	46	-1	-43	-5	97	9	84	3	-13	-6	94	21	129	0	35	-21
<i>Isogenoides</i> sp.	1862	669	1299	258	-564	-411	5840	1584	2749	296	-3092	-1288	1009	279	836	324	-173	45
<i>Rhyacophila</i> sp.	5478	1264	5042	499	-436	-765	3978	417	1066	214	-2912	-204	876	332	2448	232	1572	-100
Total	10479	2243	8861	1295	-1619	-948	10973	2203	5511	625	-5462	-1578	5965	796	6579	918	614	122
Community total	36516	15440	31959	9991	-4557	-5449	22221	10471	18923	4945	-3298	-5525	13584	8454	24661	11766	11078	3312

*Indicates a significant change in mean macroinvertebrate $\delta^{13}\text{C}$ value after wood addition. D is the difference in treatment (T) and control (C). B is before (Y0) and A. is after (Y1 and Y2) wood addition. Functional groups are in bold.



connections predicted from large wood additions in temperate streams (**Figure 6**).

Modest changes in C flow following wood additions were from a combination of greater assimilation of allochthonous and autochthonous C by a few macroinvertebrate taxa. *Baetis* mayflies, a multivoltine taxa, responded with greater secondary production that was fueled by increasing amounts of allochthonous C. *Glossosoma* caddisflies also showed an increase in production fueled by autochthonous C (**Figure 6**). Allochthonous and autochthonous resources were nearly equally important in this forested temperate stream. Typically, allochthonous resources are most important and organic matter standing crop predicts macroinvertebrate secondary production (Wallace et al., 1987, 2015); however, in the main

channel of our stream, autochthony was as well documented. For example, 2 years after the wood additions, ~40–50% of the macroinvertebrate community was supported by autochthonous C (**Figure 3**). In contrast to a manipulation of allochthonous C in an Appalachian stream (Wallace et al., 1997b), autochthonous C was a more important and substantial resource to the food web (**Figures 4, 5**). The difference in our study compared to studies in the Appalachian stream is the absence of a dense understory of rhododendron and a mature forest (Greenwood and Rosemond, 2005; Wallace et al., 2015). In the Appalachian streams, rhododendron and a more mature forest canopy limited light for primary production (Greenwood and Rosemond, 2005). Light was not limiting during seasons of open canopy in our study stream and this was likely the primary reason for

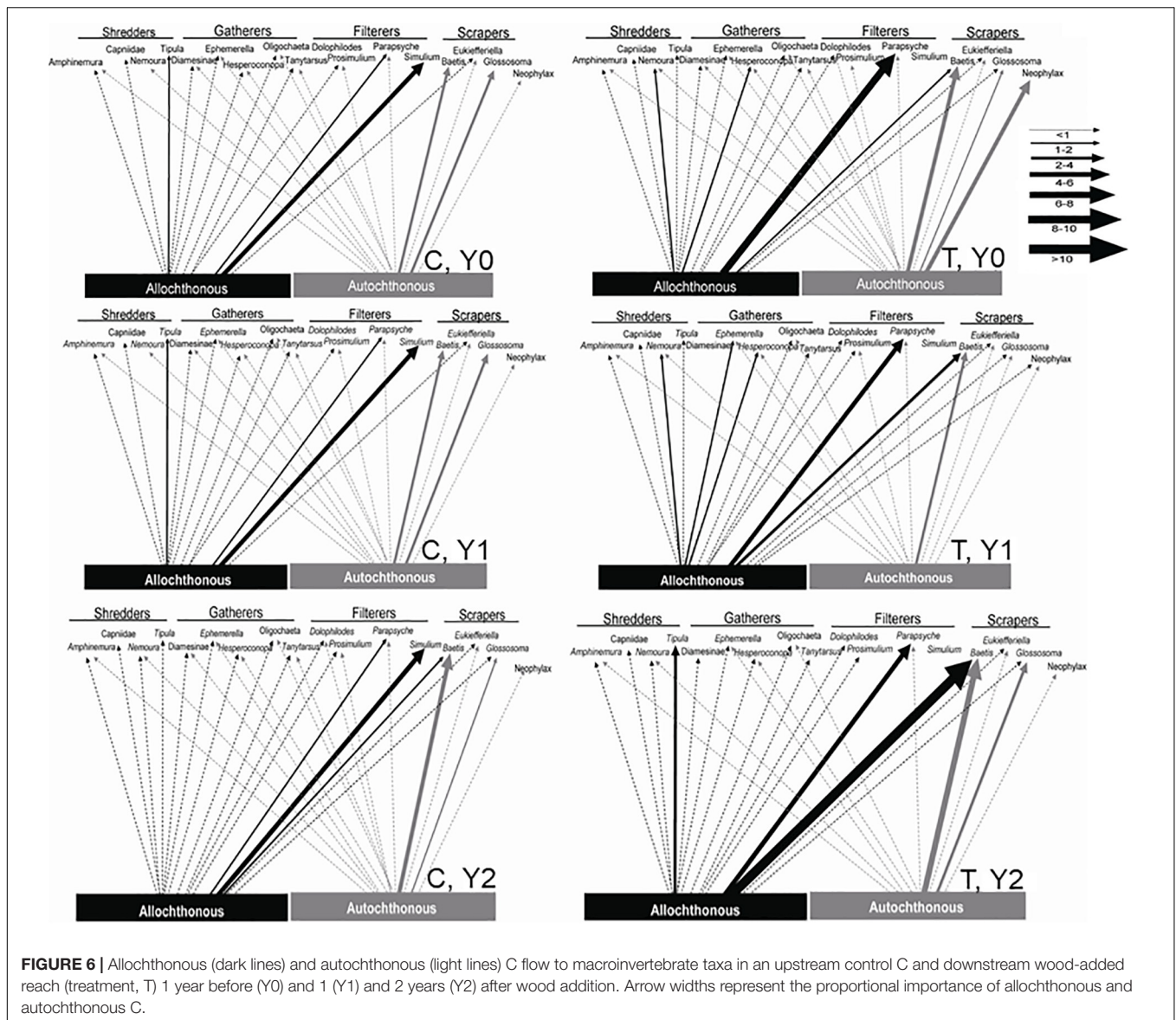


FIGURE 6 | Allochthonous (dark lines) and autochthonous (light lines) C flow to macroinvertebrate taxa in an upstream control C and downstream wood-added reach (treatment, T) 1 year before (Y0) and 1 (Y1) and 2 years (Y2) after wood addition. Arrow widths represent the proportional importance of allochthonous and autochthonous C.

greater autochthonous resource availability in this study stream compared to others conducted in headwater streams draining in-tact forests (Hoellein et al., 2007). Still, allochthonous C was the primary C source contributing to macroinvertebrate secondary production, particularly for shredders (~50–80% contributed to secondary production), gatherers (~60–90%), filterers (~55–90%), and predators (~60–80%) (Figure 3). The overall contribution of allochthonous C to secondary production ranged from 50 to 60% because scraper production was relatively high compared to the other functional groups (Figure 4).

Seasonal Effects of Added Wood on Macroinvertebrate $\delta^{13}\text{C}$

While annual average macroinvertebrate isotopic signatures changed only a little through space and time, taxon-specific signatures did change seasonally (Figure 1). Taxa with

seasonally variable $\delta^{13}\text{C}$ signatures may act as ‘indicators’, useful in evaluating ecosystem restorations aimed at changing or increasing basal resources [sensu 44]. In this study, *Glossosoma*, considered obligate consumers of algae, showed little variability in $\delta^{13}\text{C}$ across months, years, and stream reaches; therefore, their production rather than their $\delta^{13}\text{C}$ signatures may serve as a metric of restoration success (Table 1 and Supplementary Appendix 1). In contrast, facultative feeders such as the shredding stonefly *Nemoura* sp., and *Baetis* spp., showed spatially and temporally variable $\delta^{13}\text{C}$ values, and so their average $\delta^{13}\text{C}$ values at a population level may be more indicative of seasonal changes in basal food resource availability. Using the natural abundance of C stable isotopes for assessing resource acquisition complements assessments based on community-level diversity and tolerance values (e.g., Gratton and Denno, 2006).

Influence of Large Dead Wood on Macroinvertebrate C Flow

Given the forested nature of our study stream and the well-documented role of leaves and wood in governing macroinvertebrate secondary production, we hypothesized that most C in stream macroinvertebrates would bear an allochthonous signature (Wallace et al., 2015). Indeed, we predicted primary production would be limited by the deciduous canopy that was 75% closed in the winter and spring and 90% closed in the summer and autumn (Entrekin et al., 2008). Furthermore, a comparison of organic matter standing crop across studies and years show a strong positive relationship with macroinvertebrate secondary production suggesting reliance on allochthonous C as a primary food resource (e.g., Newbold et al., 1997; Webster et al., 1997; Hall et al., 2001; Chadwick and Hury, 2007; Cross et al., 2007; Entrekin et al., 2007). However, these studies did not measure assimilation and algae can contribute more to production than often assumed from feeding mode analysis and correlations to resource availability (Finlay, 2001; Hall et al., 2001; Marcarelli et al., 2011). Therefore, we were surprised to find that the autochthonous C supported 47–59% of secondary macroinvertebrate production in both reaches (Figure 3).

The few studies that measured the contribution of primary production to macroinvertebrates in temperate headwater streams have based their conclusions on gut content analysis or tracer stable isotopes. For example, Mayer and Likens (1987) used gut contents to conclude that algae were more important than expected for caddisflies in forested headwater streams. More recently, allochthonous and autochthonous C resource contributions have been delineated for entire macroinvertebrate communities in a variety of biomes using stable isotopes with the consensus being algae were more important to the food web than expected (Guo et al., 2016; Brett et al., 2017; Neres-Lima et al., 2017). In fact, macroinvertebrates sampled from headwaters in the Salmon River, where the River Continuum Concept was developed, showed a surprising amount of diatoms in their guts (Rosi-Marshall et al., 2016). Still, macroinvertebrates in the Salmon River headwaters consumed more allochthonous C. In fact, macroinvertebrates also consumed about 80% allochthonous C in this study. These two perspectives are important to consider: consumption versus assimilation. Both processes are essential aspects of C cycling; however, each indicate different C fates. Carbon assimilation compared with available resources can be used to quantify trophic transfer efficiency, while assimilation subtracted from consumption quantifies egestion that is a measure of C recycling (i.e., carbon available in a different form to a range of organisms). Therefore, autochthonous C trophic transfer was relatively high compared to resource availability. Yet, allochthonous C consumption was relatively high and critical for C recycling. Lambert and Steinman (1997) reviewed studies from 30 streams, mostly in the Northern Hemisphere, that indicated periphyton was an energetically important food resource in a range of stream sizes, biomes, and geographic areas. Furthermore, Finlay (2001) compiled macroinvertebrate $\delta^{13}\text{C}$ values from 70

streams worldwide that indicated functional groups other than scrapers and shredders were assimilating significant amounts of epilithic algae, particularly in streams with catchments greater than 10 km². Our results, confirming the importance of primary producers to macroinvertebrate production reflect that (1) algae are assimilated more efficiently than leaf litter because of higher nutritive value (e.g., lower C:N:P content) (Berg and Hellenthal, 1992), (2) algae have higher turnover rates that increase availability to macroinvertebrate consumers (Lamberti et al., 1989), and (3) algae are available year round even in streams with nearly closed deciduous canopy (Hamilton et al., 2004).

When we compared the amount of algal C available (calculated as gross primary production; GPP) to the total amount of algae consumed, we found that macroinvertebrates were consuming 4–36% of the total GPP [or about 8–72% net primary production (NPP)]. This autochthonous contribution of C to the macroinvertebrates is greater than we anticipated and greater than in tropical forested streams (Neres-Lima et al., 2017; Rosas et al., 2020). The contribution of autochthonous C was likely relatively high in this stream and perhaps others because: (1) filtering taxa were likely ingesting recycled autochthonous C in the form of epilithic scrapers and sloughed particles (e.g., Strayer, 1988; Benke and Wallace, 1997), (2) scrapers were likely increasing algal turnover rates through efficient grazing, while keeping biomass low (e.g., Lambert et al., 1995), and (3), estimation errors associated with both whole-reach estimates of GPP and NPP (e.g., Hall and Tank, 2003) and using published estimates of C assimilation by macroinvertebrates using measurements of production could underestimate C consumption (e.g., Morin et al., 1987). Our results highlight assumptions that must be made and the difficulty in estimating the contribution of autochthonous and allochthonous resources in systems with consumers ingesting a complex mixture of both types simultaneously.

When we compare the amount of leaf litter consumed by macroinvertebrate community compared to the amount available, consumption values ranged from 13 to 40% of available. Organic matter evidently was not limiting macroinvertebrate population growth on an annual scale, although it might have been limited in some seasons, as has been shown in other studies (e.g., Richardson, 1991; Dobson and Hildrew, 1992; Marks, 2019). The addition of large wood increased the percentage of GPP and coarse benthic organic matter consumed by 25 and 10%, respectively, for the macroinvertebrate community in the treatment reach relative to the control reach. Other studies have found that changes in microhabitat, such as increased leaf litter accumulations, may change the invertebrate community structure and increase production (e.g., Wallace et al., 1997a; Kobayashi and Kagaya, 2004). In our study, increased patches of inorganic coarse substrate and deposited leaf litter and fine sediment associated with the added wood seemed to have increased production by the shorter-lived taxa, such as *Baetis*, resulting in increased community-level secondary production (Entrekin et al., 2009). Longer-term increases

in organic matter retention are predicted to increase the relative importance of allochthonous organic matter to the food web and sustain greater production and trophic transfer in the future.

DATA AVAILABILITY STATEMENT

All macroinvertebrate datasets generated for this study are included in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

SE co-designed the experiments, analyzed the data, and was the lead manuscript writer. ER, JT, GL, and TH co-designed the experiments and provided critical input and edits to the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2020.00114/full#supplementary-material>

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