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Stream Macroinvertebrates and Carbon Cycling in Tangled Food Webs

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Abstract

The annual global loss of organic carbon from terrestrial ecosystems into rivers is similar to the organic carbon stored in soils each year. Dissolved organic matter (DOM) flows through the food web to macroinvertebrates, but little is known about the effect of DOM increase on stream food webs and how much macroinvertebrates may contribute to the regulation of carbon fluxes in rivers. Using a before and after control impact (BACI) experimental design, we increased by 12% (+ 0.52 mg C L^{-1}) the concentration of DOM in a stream for three weeks by adding sucrose, with a distinctive $\delta^{13}C$ signature, to simulate a pulse of natural DOM supply from soils. We partitioned the diet of macroinvertebrates from carbon sources according to the green pathway (autotrophs) and detrital pathways (bacteria and terrestrial organic matter). Our flow food web approach based on C fluxes, with bacteria as a key node, showed the dominant contribution of the detrital pathways for macroinvertebrates in the reference stream. DOM addition induced changes in the diets of individual taxa, but did not have any strong effects on the relative overall contribution of the detrital pathways *versus* the green pathway. Autotrophic uptake of CO_2 respired by bacteria was much larger than bacterial C flux to invertebrates (that is, the classic microbial loop) and allowed a significant fraction of natural allochthonous organic carbon to make its way to macroinvertebrates via autotrophs fixing CO_2 respired by bacteria. Overall macroinvertebrates did not regulate directly to any great extent the flux of stream DOM towards downstream ecosystems.

Key words: macroinvertebrates; stream food web; resource use efficiency; microbial loop; allochthony; autochthony.

Highlights

- Carbon from bacteria supports autotrophs more than macroinvertebrates

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Author contributions The project was conceived and designed by BOLD, NF and BT. BOLD carried out the experiment. NF, BM and JLK identified the macroinvertebrates. BT was responsible for the stable isotope ratio analyses. BOLD analysed the data and drafted the manuscript. All co-authors provided feedbacks on the manuscript through four rounds of comments. Final version of the manuscript was read and approved by all co-authors.

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- Tangled food web twists the allochthony debate
- Macroinvertebrates do not regulate directly flux of dissolved organic matter

Introduction

The annual global loss of organic carbon from terrestrial ecosystems into rivers is similar to the organic carbon stored in soils each year (Dawson 2013). The delivery of this organic matter to rivers is currently increasing in many parts of the world due to changes in climate, atmospheric pollution, land use and urbanisation (Freeman and others 2004; Monteith and others 2007; Noacco and others 2017). The fate of this organic matter in rivers remains difficult to characterise, however, because of the different forms (for example, leaf litter and dissolved organic matter) and varying quality of organic matter, as well as spatial heterogeneity and temporal dynamics (Raymond and others 2016; Bernhardt and others 2018; Drake and others 2018). There is a pressing need to better understand the interactions between flow regime, biota and ecosystem processes (Demars 2019; Palmer and Ruhi 2019).

Coarse particulate organic matter (including leaf litter and wood) supports macroinvertebrate secondary production via decomposers in small, forested streams (Wallace and others 1997, 2015; Hall and others 2000, 2001; Friberg and others 2002). Labile dissolved organic matter (DOM) has been traced through the food web into macroinvertebrates via the bacterial pathway (Meyer 1994; Hall 1995; Hall and Meyer 1998; Parkyn and others 2005). DOM addition including leaf leachate can rapidly increase bacterial production (Cummins and others 1972; Fischer and others 2002), spread through the microbial food web (Wiegner and others 2015) and increase secondary production of macroinvertebrates and fish (Warren and others 1964; Wilcox and others 2005). The productivity of closed canopy temperate streams can also increase the productivity of the connected riparian food webs (Nakano and Murakami 2001; Baxter and others 2005).

Although the food web detrital pathway dominates in closed canopy streams, increased light increases the proportion of carbon assimilation originating from primary producers by macroinvertebrates (Finlay 2001; Collins and others 2016). Autotrophic carbon compounds are of higher food quality, being richer in energy and essential compounds (Marcarelli and others 2011; Brett and others 2017; Ruess and Müller-Navarra 2019). The extent to which the bacterial (or decomposer) pathway for secondary production may be stimulated by a small increase in DOM supply in open canopy streams remains unknown, because of potential priming or facilitation effects (Danger and others 2013; Hotchkiss and others 2014; Guo and others 2016b; Grieve and Lau 2018), reciprocal carbon exchange between autotrophs and bacteria (Demars and others 2020) and differential trophic transfer efficiencies of essential biomolecules (Gladyshev and others 2011; Guo and others 2016a).

The role of allochthony in aquatic food web has been actively debated (for example, Thorp and Delong 2002; Cole and others 2011; Brett and others 2017; Tanentzap and others 2017). In this debate, the green web (grazing of autotrophs) is considered largely independent of the brown web (consumption of detritus and decomposers)—for example, Thorp and Delong (2002). Yet, strong interactions often exist between autotrophic and heterotrophic productions, at least in nutrient poor aquatic ecosystems (Haack and McFeters 1982; Cole and others 1988; Carr and others 2005; Scott and others 2008; Demars and others 2020). Macroinvertebrates could therefore assimilate a large part of autochthonous carbon via bacteria, that is, via the detrital pathway (Meyer 1994). Allochthonous carbon may also be used by the autotrophs via the uptake of CO_2 respired by bacteria (Demars and others 2020), but it is not known how much may be assimilated by macroinvertebrates. If we want to disentangle the *origin* of organic carbon (allochthony *versus* autochthony) from the *quality* of carbon assimilated by macroinvertebrates (green web *versus* brown web), then three primary sources of carbon for macroinvertebrates must be partitioned: heterotrophs, autotrophs and terrestrial organic matter. Although bulk analysis of periphyton has been used in many studies, periphyton is the product of these three primary sources and is unlikely to help solving the role of the microbial loop in the allochthony debate. One way to partition more effectively the carbon sources assimilated (directly and indirectly) by macroinvertebrates is to use compound-specific stable isotope ratios rather than bulk tissue analyses (for example, Nielsen and others 2018; Liew and others 2019).

The lability of organic matter is less linked to the molecular structure (aromaticity) of the organic matter than to changes in environmental conditions, both biotic and abiotic (Schmidt and others 2011; Dungait and others 2012). Thus, rapid turnover of allochthonous organic matter in aquatic ecosystems can occur once it leaves the soil matrix (for example, Marin-Spiotta and others 2014;

Drake and others 2015; Demars 2019). Here we traced a small addition of labile organic carbon (sucrose, $C_{12}H_{22}O_{11}$) with a distinctive $\delta^{13}C$ through the base of the food web and macroinvertebrates in a blackwater stream. The time scale of the experiment (three weeks) corresponded to a pulse of soil derived DOM during a period of hydrological connectivity (wet soils) driving stream heterotrophic respiration in the studied streams (Demars 2019). The lability of the organic matter added corresponded to a large fraction of the natural dissolved organic matter because 36 ± 18% of the annual carbon inputs from the land (excluding peak flows) were respired away by benthic microbial metabolism within about an hour of water transit time (Demars 2019) and sucrose addition produced similar effects on bacterial respiration as observed during hydrological pulses (Demars and others 2020). We hypothesised that macroinvertebrates from small open canopy blackwater streams would mostly assimilate C derived directly from autotrophs, and a shift towards the bacterial C pathway as bacterial production increased more (+ 89%) than net primary production (+ 12%) following sucrose addition (Demars and others 2020). We further traced allochthonous organic carbon through the green and brown webs. Finally, we related macroinvertebrate secondary production to DOM supply and bacterial DOM demand (heterotrophic respiration and production) to estimate the role macroinvertebrates play in carbon cycling in first order streams.

Methods

Study Area

We studied two heather moorland catchments with soils rich in organic carbon, within the Glensaugh research station of the James Hutton Institute in north-east Scotland (Long 2° 33′ W, Lat 57° 55′ N). The streams were about 0.8–1.0 m wide in the studied sections and their channels significantly undercut the banks by 30–46% of stream width. Brown trout (*Salmo trutta fario*, Salmonidae) were present in both streams. The management of the land included regular heather burning (10–12% of surface area yearly target) for hill farming: mixed grazing of sheep and cattle. The reference stream (Birnie Burn, ECN site) showed substantial increase in annual flow-weighted mean concentrations of stream water DOC (+ 0.28 mg C L^{-1} $year^{-1}$ during 1994–2007, Stutter and others 2011). The reference stream (catchment area 0.76 km^2) was paired with an adjacent stream (Cairn Burn, catchment area 0.9 km^2) in 2005 to carry out whole ecosystem experiments and trace the fate of the dissolved organic matter (Fig. 1; Demars 2019; Demars and others 2020).

Terrestrial DOC: Main Source of Organic Carbon

DOC was the dominant form of organic carbon (98% of organic carbon concentration in the water) under stable flows with average concentrations of 9.3 ± 1.7 mg C L^{-1} in the two studied streams (Stutter and others 2013; Demars 2019). DOC was of terrestrial origin (Stutter and others 2013). The pool of particulate organic carbon in the sediment was very small (Demars 2019), and coarse particulate organic matter (CPOM) was less than 10 g C m^{-2} (determined from Surber sampling of invertebrates, see below). With such extremely low standing mass of CPOM, it is therefore likely that fungi played a negligible role in the studied streams (see Venarsky and others 2018) and thus were not considered in this study.

Dissolved CO_2 and the Carbonate Equilibrium

This study was carried out during a period of stable flows (discharge 3 to 30 L s^{-1}) where the pH averaged 7.1 (range 6.8 to 7.3) and HCO_3 concentrations averaged 0.35 (range 0.2 to 0.5) mmol HCO_3 L^{-1}. HCO_3 concentrations represented about 80% of observed dissolved inorganic carbon concentrations. However, the streams were far from equilibrium with excess partial pressure of CO_2 in the range 1.8 to 5.6 times atmospheric pressure, despite very high gas exchange coefficient at the water–air interface, as noted in Demars (2019). Under the open system conditions with steady state concentrations (under constant light), the *instantaneous* rate of CO_2 hydration, that is, formation of HCO_3^- and H^+ from CO_2 and H_2O, was equivalent to 0.6% of dissolved CO_2 flux and 0.5% of CO_2 evasion rate at the stream reach scale—with calculations following Stumm and Morgan (1981). Bacterial respiration rates (CO_2 production) were derived from O_2 metabolism (assuming 1:1 molar O_2:CO_2 ratio, see Demars 2019). Moreover, photosynthesisers in CO_2 rich and low alkalinity systems use dissolved CO_2 (Maberly and Madsen 2002). In our system HCO_3 came from groundwater and biotic fluxes of dissolved inorganic C were thus represented with CO_2 fluxes. In other systems with low reaeration rates and high pH (abundant

Figure 1. Landscape view of the Birnie Burn (reference stream) and sucrose addition using a 60-L Mariotte bottle in the Cairn burn (treatment stream), Glensaugh research station, north-east Scotland, UK.

OH^-), the conversion of CO_2 to HCO_3 by hydroxylation is important to consider (Emerson 1975).

DOC Addition

A carboy was refilled every two days with 6 kg of sucrose (granulated pure cane sugar, Tate & Lyle PLC, London, UK) dissolved in over 60 L of stream water filtered through muslin square in a large funnel. The carboy was set as a Mariotte bottle to ensure a constant addition of sucrose for 22 days (23 August–14 September) raising the background DOC concentration below the mixing zone by + 0.52 mg C L^{-1} at the top of the treatment reach (28 m downstream the injection point), equivalent to + 12% of ambient average DOC concentration (Fig. 1, Demars and others 2020). This small addition of sucrose did not promote the growth of fungi or sheathed filamentous bacteria (Demars and others 2020).

Identification of Carbon Sources

Natural Sources

DOC was retrieved from the stream water by cation exchange resin (Stutter and others 2013). Autotrophs (filamentous green algae and bryophytes), and allochthonous organic carbon (CPOM), were collected by hand along both studied reaches before and after sucrose addition. Although aquatic bryophytes covered by filamentous algae were not thought to contribute much to primary production at the time of the experiment (Demars and others 2020), they may contribute to macroinvertebrate diet (Jones 1949; Dangles 2002; Parker and Huryn 2006; Carroll and others 2016).

Additional tiles were deployed, three weeks prior to the start of sucrose addition in the reference and treatment reach, to identify periphyton autotrophs and bacteria from $\delta^{13}C$ compound-specific phospholipid fatty acids (PLFAs) from the literature and the change in $\delta^{13}C$ of individual PLFAs due to sucrose addition in the treatment (Demars and others 2020). We assumed a fractionation factor of − 3‰ for the $\delta^{13}C$ of fatty acids relative to bulk tissue samples to make the fatty acid $\delta^{13}C$ comparable with bulk analyses of the other sources and mixtures (including macroinvertebrates)—Demars and others (2020). Biofilm growth was slow and the tiles had to be collected at the end of the experiment to have enough material for the analyses, so the bacterial source could only be compared between the reference and the treatment at the end of the experiment. All samples were freeze-dried and milled prior to analyses for C, N, $\delta^{13}C$ and $\delta^{15}N$. For further details, see Demars and others (2020).

Sucrose

The proportion of carbon derived from added sucrose (F_S) in resources and macroinvertebrates was calculated from their $\delta^{13}C$ in the reference (R) and treatment (T) reaches, before (subscript B) and after (subscript A) sucrose addition as follows:

$$F_s = \frac{T_A - (T_B + (R_A - R_B))}{-12 - (T_B + (R_A - R_B))}$$

with − 12 representing the carbon stable isotope ratio of sucrose ($\delta^{13}C = -12‰$). All uncertainties were added in quadrature using standard deviation δx for sums, and relative uncertainties $\delta x/x$ for the division. The standard error of the mean was calculated as $sem = \delta x/\sqrt{n}$ with n average number of samples in R_B, R_A, T_B, T_A. We also calculated F_S for macroinvertebrate taxa without $\delta^{13}C$ data prior to sucrose addition as follows: $F_S = (T_A - R_A)/(-12 - R_A)$. The observed $\delta^{13}C$ of the macroinvertebrates in the treatment reach following sucrose addition (T_B, Cairn impact) were corrected prior to F_S calculations (see below, Sect. 2.6.). The values for $\delta^{13}C$ and F_S were given in Supplementary Information (see Table S1).

Analytical Methods

The total carbon and total nitrogen concentrations and the $\delta^{13}C$ and $\delta^{15}N$ natural abundance isotope ratios of the macroinvertebrate samples were determined using a Flash EA 1112 Series Elemental Analyser connected via a Conflo III to a Delta^{Plus} XP isotope ratio mass spectrometer (all Thermo Finnigan, Bremen, Germany)—see Demars and others (2020).

Macroinvertebrates

Macroinvertebrate densities were estimated from twelve to thirteen Surber samples (20 × 20 cm, mesh size 200 µm) collected randomly along the reaches of the reference and treatment streams, before and after sucrose addition (total 51 samples). The samples were stored in 70% alcohol, sorted and identified. Macroinvertebrates for C, N and stable isotope studies were collected by kick sampling and hand net. The animals were quickly sorted into Petri dishes to avoid contact between predators and prey, identified live within a day, placed in Eppendorf tubes and freeze-dried. Subsamples of all macroinvertebrate taxa were kept in 70% alcohol for reference and comparison with the Surber samples. We removed portable caddisfly cases prior to freezing and assumed enough time had elapsed for gut evacuation. The average individual biomass of macroinvertebrate taxa was assessed by weighing the freeze-dried mass of all individuals divided by the number of animals within an Eppendorf tube. Whole macroinvertebrates were then crushed before insertion into a tin capsule for C, N, $\delta^{13}C$ and $\delta^{15}N$ analyses. The $\delta^{13}C$ values used in the calculations of diet source partitioning before, after, control, impact are given in Table S1.

Nearly eighty macroinvertebrate taxa were identified from 7049 individuals recovered from the 51 Surber samples. Of these we retained 28 taxa for the estimation of density, representing over 97% of the individuals found, after omitting some rare taxa (notably those present in only one stream or one period of sampling) and grouping some taxa into genus or families (to correspond with the level of identification of the sampling for stable isotopes). We determined the individual freeze-dried mass, C, N, $\delta^{13}C$ and $\delta^{15}N$ on bulk samples for 18 of these taxa representing 97% of the individuals of the 28 taxa, on which all our calculations were based (Table 1). We only included the ten most representative taxa in many of our figures, still covering about 94% of the individuals.

Corrections for Stable Isotopic Ratio at Equilibrium with the New Diet

Bacteria and algae were likely to reach the new stable isotope ratio equilibrium within a week (Hall and Meyer 1998; Collins and others 2016), and three weeks of sucrose addition were thought to be sufficient for invertebrates, albeit not fully for all consumer species (especially predators), with carbon turnover ranging from about 10–35 days (Le Cren and Lowe-McConnell 1980; Hall and Meyer 1998; Collins and others 2016 and references therein). The change in isotopic ratio value (here $\delta^{13}C$ in ‰) from the time sucrose was added to the stream (diet shift) can be estimated with an exponential decay model of tissue isotopic ratio over time (Hobson and Clark 1992; Vander Zanden and others 2015):

$$\delta_t = \delta_n + (\delta_0 - \delta_n)exp(-\lambda t)$$

with $\delta_t$isotopic value (‰) of the organism at time t, δ_0 initial isotopic value (‰) at equilibrium with the old diet, δ_n isotopic value (‰) after equilibration with the new diet, λ tissue isotopic turnover rate (day^{-1}), and t time since the diet switch (days).

The unknown stable isotope ratio at equilibrium δ_n was derived by rearranging the above equation as follows:

$$\delta_n = \frac{\delta_t - \delta_0 exp(-\lambda t)}{1 - exp(-\lambda t)}$$

The proportion of $\delta^{13}C$ isotopic tissue turnover (τ) was calculated as $\tau = \delta_t/\delta_n$ and indicated how close to equilibrium the isotopic ratio $\delta^{13}C$ was in individual invertebrate taxa (Table S1). This model was

Table 1. Macroinvertebrates

Order	Family	Species	FFG	Before density ind m^{-2}	After density ind m^{-2}	Control density ind m^{-2}	Impact density ind m^{-2}	After dry mass mg ind^{-1}	Impact dry mass mg ind^{-1}
Plecoptera	Nemouridae	*Protonemura meyeri*	Shr	31 ± 26	81 ± 50	179 ± 127	227 ± 170	0.27	0.13
	Leuctridae	*Leuctra*[a]	Shr/Scr/Col	173 ± 104	892 ± 557	85 ± 41	138 ± 43	0.23	0.29
	Perlodidae	Perlodidae[b]	Pre/Shr	2 ± 3	38 ± 21	0 ± 0	31 ± 13	0.75	0.05
Ephemeroptera	Baetidae	Baetidae (*Baetis rhodani*)	Scr/Col	358 ± 159	906 ± 446	183 ± 142	194 ± 81	0.39	0.63
Trichoptera	Limnephilidae	Limnephilidae[c]	Shr	0 ± 0	38 ± 12	0 ± 0	19 ± 9	0.08	0.40
	Beraeidae	*Beraea maurus*	Shr/Col	6 ± 5	4 ± 3	4 ± 5	8 ± 5	0.50	0.25
	Odontoceridae	*Odontocerum albicorne*	Shr/Pre	15 ± 7	23 ± 13	12 ± 8	21 ± 10	3.43	0.20
	Polycentropodidae	*Plectrocnemia conspersa*	Pre/Fil	4 ± 3	0 ± 0	4 ± 3	4 ± 3	3.07	1.60
	Rhyacophilidae	*Rhyacophila*[d]	Pre	0 ± 0	12 ± 5	23 ± 15	58 ± 25	2.73	2.95
Coleoptera	Dytiscidae	*Oreodytes sanmarkii* (ad)	Shr/Pre	4 ± 3	10 ± 8	17 ± 11	33 ± 17	1.35	1.03
	Elmidae	*Elmis aenea* (lv)	Scr/Shr	296 ± 194	462 ± 174	440 ± 173	1210 ± 442	0.36	0.40
	Elmidae	*Elmis aenea* (ad)	Scr/Shr	56 ± 62	108 ± 60	179 ± 110	400 ± 317	0.27	0.35
	Scirtidae	*Elodes* sp. (lv)	Scr/Shr	15 ± 13	42 ± 21	27 ± 15	40 ± 36	0.64	1.13
Diptera	Chironomidae	Chironomidae[e]	mixed	106 ± 44	885 ± 284	600 ± 252	3302 ± 968	0.17	0.37
	Pediciidae	*Dicranota* sp.	Pre	31 ± 15	35 ± 18	44 ± 29	42 ± 18	0.48	1.67
	Simuliidae	Simuliidae	Fil	163 ± 110	231 ± 144	88 ± 47	104 ± 73	0.43	ND
Oligochaeta	Oligochaeta	Oligochaeta	Col	25 ± 11	40 ± 17	19 ± 20	48 ± 26	10.20	5.75
Hydracarina	Hydracarina	Hydracarina	Pre	8 ± 8	13 ± 6	12 ± 8	4 ± 3	0.17	ND

Functional Feeding Groups (FFG, Di Sabatino and others 2000; Tachet and others 2002), Density (± sem) and Biomass of the Before and After Control Impact (BACI) Design in the Reference Stream Birnie Burn (Before, After) and Treatment Stream Cairn Burn (Control, Impact).
Col = collector, absorber, Shr = shredder, Scr = scraper, Fil = filter-feeder, Pre = predator, piercer, parasite. lv = larvae, ad = adult. ND = not determined.
[a]*L. hippopus, L. moselyi, L. fusca, L. inermis, L. nigra.*
[b]*Isoperla sp., Diura bicaudata.*
[c]*Allogamus auricolis, Potamophylax sp.*
[d]*R. munda, R. oblitera, R. dorsalis.*
[e]*Mostly Orthocladinae.*

most appropriate for the Simuliidae, able to directly filter out colloids (Wotton 2009) on which sucrose may be adsorbed, but did not account for any time lag for lower trophic levels to reach equilibrium, so the estimates may still be conservative for other macroinvertebrates, the more so with the number of trophic levels (for example, invertebrate predators *Rhyacophila* and *Plectrocnemia*). For this reason, we computed two stable isotope ratios at equilibrium with the new diet δ_n with t = 21 days and t = 14 days. These two estimates produced a bracket of likely δ^{13}C values for primary consumers.

The tissue isotopic turnover rate λ (day^{-1}) was derived from the isotopic half-life study of Vander Zanden and others (2015) general equation for invertebrates using fresh individual body mass, M (g)—see Method S1, Table S1:

$$\lambda = \frac{ln(2)}{25.8M^{0.23}}$$

We converted our macroinvertebrate individual freeze-dried mass into fresh mass assuming freeze-dried mass was 20% of fresh mass (Waters 1977 cited in Wetzel 2001, p. 718).

Data Analyses for Carbon Sources and Pathways

Most food web studies now use δ^{13}C and δ^{15}N to identify the links (that is, diet). Here the BACI experimental design allowed the calculation of the proportion of sucrose (F_s) in all parts of the food web. *Fs* was used as a tracer, in addition to δ^{13}C, to determine the sources of carbon for bacteria and algae in the treatment reach after 21 days of sucrose addition (Demars and others 2020). Thus, the carbon pathways were identified with carbon tracers. End member mixing analyses were used to determine the proportion of C sources and their uncertainties in individual macroinvertebrate taxa. Autotrophic C was the average of filamentous green algae, periphyton autotrophs and bryophytes. The bryophytes were however introduced as a separate source from the other autotrophs in the treatment after sucrose addition (Cairn impact) because both δ^{13}C and its fraction of sucrose differed substantially from the other autotrophs (Demars and others 2020). No trophic enrichment factors (TEF) or isotopic fractionation was applied between sources and macroinvertebrates (*cf* McCutchan and others 2003 reporting an average 0.3 ± 0.1‰ fractionation for δ^{13}C in bulk tissue of consumers).

We checked the mixture data (macroinvertebrate taxa) were within the range of the sources and provided the numerical solutions using a Bayesian approach (MixSIAR 3.1.9. in R version 3.5.0, Stock and Semmens 2016; R Core Team 2018). MixSIAR used the Dirichlet distribution to specify the priors on source proportions. We used MixSIAR default minimally informative priors, assuming every possible set of proportions had equal probability (generalist diet)—see Stock and others (2018). The numerical solutions converged rapidly according to the Markov Chain Monte Carlo convergence diagnostics (Gelman-Rubin and Geweke).

Production of Macroinvertebrates

Secondary production was estimated from the samples collected at the end of the treatment period from the observed standing biomass (mg C m^{-2}) of individual taxa and macroinvertebrate daily growth rate (day^{-1}). The standing biomass was determined from the density (individuals m^{-2}) and average individual biomass (mg C individual^{-1}). The daily growth rate (G, day^{-1}) was determined from:

$$G = a\exp(bT)$$

with T average water temperature (10.5 °C), a and b taxon-specific constants derived from a global compilation of published data (Golubkov 2000; Gladyshev and others 2016, Table S1—similarly to Morin and Dumont 1994). Our approach was a pragmatic way to convert macroinvertebrate biomass estimates into secondary production units, knowing both are tightly related (Figure S1; Eklöf and others 2017; Patrick and others 2019).

Ecosystem Carbon Fluxes and Efficiencies

Flow food webs based on carbon fluxes were assembled for the reference and treatment streams at the end of the experiment, the period for which we were able to distinguish biofilm autotrophs and bacteria δ^{13}C using PLFAs (see above). Differences in resource use efficiencies between the reference and treatment due to sucrose addition relied therefore on a simple comparison. We calculated the resource use efficiency of macroinvertebrate communities (sum of individual taxon contributions) as the ratios between macroinvertebrate secondary production and resource production (net primary production, bacterial production, DOM and sucrose fluxes), with all fluxes expressed in g C m^{-2} day^{-1}. Net primary production was estimated

as 0.5 ± 0.3 × GPP from whole stream metabolism. Heterotrophic respiration (HR) was estimated from whole ecosystem respiration minus autotrophic respiration. Heterotrophic (bacterial) production (HP) was derived from HR and heterotrophic growth efficiency (HGE range 5 to 20%) as follows: $\mathrm{HP} = \frac{-\mathrm{HR} \times \mathrm{HGE}}{1-\mathrm{HGE}}$.

For more details, see Demars (2019) and Demars and others (2020).

Data Analyses of the Experiment (Stream Reach Scale)

We calculated the relative changes in macroinvertebrate densities, carbon fractions of autotrophic and natural terrestrial organic matter (OM) in macroinvertebrates using the values of the reference (R) and treatment (T) reaches, before (B) and after (A) sucrose addition as follows:

$$\text{Effect size} = \frac{(R_\mathrm{B} - R_\mathrm{A}) - (T_\mathrm{B} - T_\mathrm{A})}{T_\mathrm{B}}$$

Differences between the reference and treatment (when data prior to sucrose addition were not available) were quantified similarly as $(T_\mathrm{A} - R_\mathrm{A})/T_\mathrm{A}$. We calculated the effect size 10,000 times by varying randomly the values of the parameters according to their mean and standard deviation using a normal distribution and Monte Carlo simulations in R version 3.5.0 (R Core Team 2018; Method S2). We reported the 25th, 50th and 75th centiles (median and interquartile range). Because the experiment was unreplicated we did not report P values (see Hurlbert 1984). In the period prior to sucrose addition we used filamentous green algae and bryophyte δ^{13}C to characterise the autotrophs in the reference and treatment reaches.

Results

Macroinvertebrate Density, Biomass and Production

The macroinvertebrate total densities of the BACI were 1300, 4000, 2000 and 6000 individuals m^{-2}, respectively (see Table 1). Sucrose addition may have decreased the average individual macroinvertebrate density by 81%, relative to the changes in the reference stream, but this decrease was very uncertain (about 500% interquartile range)—Figure S2. Chironomidae increased by 83% but with very large uncertainties (interquartile range 760%). At the end of the experiment, macroinvertebrate community biomass (dry mass) were 1.6 ± 0.8 g m^{-2} and 2.7 ± 1.1 g m^{-2} in the reference and treatment streams, respectively, and macroinvertebrate production was lower in the reference stream (20 ± 4 mg C m^{-2} day^{-1}) than in the treatment stream (36 ± 8 mg C m^{-2} day^{-1}).

Estimation of Isotopic Tissue Turnover in Macroinvertebrates

The tissue isotopic turnover rate λ ranged from 0.06 to 0.18 day^{-1} based on macroinvertebrate biomass of 0.05 to 10.2 mg dry mass ind^{-1} (Table 1, Table S1). The estimates of isotopic tissue turnover of consumers (τ) for the isotopic ratio δ^{13}C were on average 96% (range 84–103%) over 14 days and 98% (range 93–101%) over 21 days, both very close to equilibrium. We used the isotopic tissue turnovers of consumers at $t = 21$ days to correct the observed values of F_S and δ^{13}C for the diet partitioning analyses for the treatment (Cairn impact, Table S1).

Tracing Sucrose into Macroinvertebrates

The BACI design allowed the quantification of the fraction of carbon derived from sucrose in the treatment reach for individual taxa with high accuracy (Fig. 2). The proportion of added sucrose (F_S) in the macroinvertebrates varied widely between taxa, with F_S exceeding 30% in half of the taxa (Fig. 2). Among the ten most frequent and abundant taxa, Simuliidae had the highest proportion (0.81 ± 0.03), followed by *Protonemura*, *Leuctra*, Baetidae and *Rhyacophila* (0.40–0.53). *Elmis*, *Dicranota*, and Oligochaeta, consumed little or no sucrose derived food (see Table 1). Chironomidae assimilated C derived from sucrose only moderately (0.33 ± 0.11), but because they were more abundant than other taxa, Chironomidae held 60% of the sucrose retained in the macroinvertebrate assemblage (191 ± 62 mg C m^{-2}, Fig. 2). The mass of sugar retained by all macroinvertebrates was only 292 ± 107 mg C m^{-2}, or about 25 g C for the treated stream reach, representing 0.2% of the sucrose flux over the three-week addition. Carbon flux from filter feeders were extremely small because the densities of blackflies were low (average 88–231 individuals m^{-2}).

Diet of Individual Macroinvertebrate Taxa

All invertebrates were within the bounds of the end members (sources), allowing to partition the diet of macroinvertebrate taxa (Fig. 3, Table S2 and Table S3). In the reference stream (at the end of the

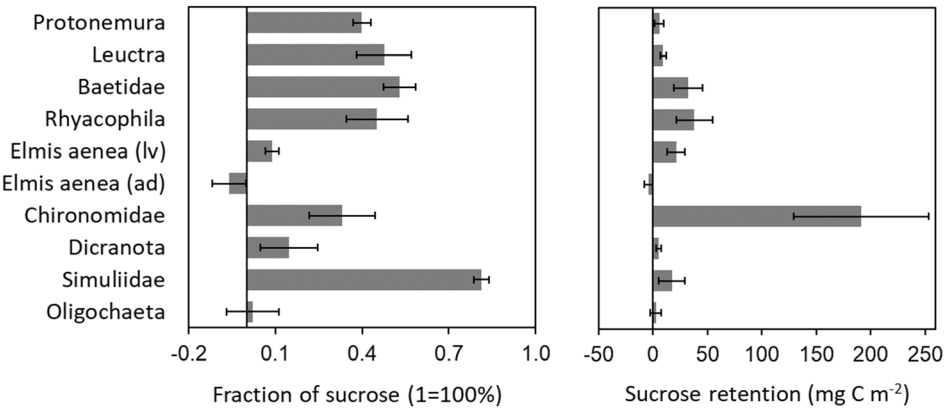

Figure 2. Fraction of sucrose and sucrose retention in the treatment reach for macroinvertebrate taxa (± sem) computed from the BACI experimental design. lv = larvae, ad = adult.

experiment) most taxa assimilated a mixed diet including a large fraction of bacteria. *Protonemura* were mostly feeding on autotrophic C while Oligochaeta fed mostly on natural allochthonous organic matter—see Fig. 4. In the treatment, several taxa including *Protonemura*, *Elmis aenea* and Chironomidae derived their carbon from a large fraction of autotrophic C (Fig. 4), possibly larger than in the reference stream for *Elmis aenea* (Figure S3). Four taxa were more abundant and determined the retention of primary sources: Baetidae and Oligochaeta in the reference and *Elmis aenea* and Chironomidae in the treatment (Fig. 4). The fraction of bacterial carbon in macroinvertebrates may be larger in the treatment relative to the reference in only three taxa: *Leuctra*, Baetidae and *Rhyacophila* (Figure S3). Simuliidae switched to a diet dominated by sucrose (Fig. 4). The relative changes in autotrophs and natural terrestrial organic matter in invertebrate diet based on the more robust BACI design reflected similar patterns, except for Leuctra and Chironomidae (decrease in autotrophic C assimilation), adult *Elmis aenea* (decrease in terrestrial organic matter assimilation) and Chironomidae (increase in terrestrial organic matter)—Fig. 5.

Food Web Source Links to Macroinvertebrates

In the reference stream, macroinvertebrates fed predominantly on carbon directly derived from the detrital pathways (65 ± 25%): natural terrestrial organic matter (30 ± 15%) and bacteria (35 ± 20%), while autotrophs only contributed 35 ± 15%, see Fig. 6. The carbon *originally* derived from natural terrestrial organic matter contributed 54 ± 19% to macroinvertebrates: 30% directly, 0.51 × 0.35 = 18% through bacteria and 0.51 × 0.37 × 0.35 = 7% through autotrophs (Fig. 6 and Table S4 for individual taxa).

In the treatment stream, it was possible to separate all sources with the following contribution to macroinvertebrate diet: autotrophs (48 ± 17%; including 22 ± 8% for bryophytes and 25 ± 11% for algae and biofilm autotrophs), sucrose (1 ± 1%), bacteria (28 ± 11%) and allochthonous natural organic matter (23 ± 11%), see Fig. 6. Hence, the detrital pathways (52 ± 16%) directly assimilated by macroinvertebrates were equivalent to the green pathway (autotrophs) in the treatment. The carbon *originally* derived from natural terrestrial organic matter contributed 30 ± 12% to macroinvertebrates: 23% directly, 0.18 × 0.28 = 5% through bacteria and 0.18 × 0.26 × 0.25 = 1% through algae and biofilm autotrophs, as bryophytes did not uptake bacterial CO_2 in the treatment streams (Fig. 6). Sucrose was the original source of 25 ± 8% of the carbon assimilated by macroinvertebrates: 1% directly, 0.62 × 0.28 = 17% through bacteria and 0.13 × 0.25 = 3% through algae and biofilm autotrophs (Fig. 6). So natural organic matter and sucrose contributed 51 ± 14% of macroinvertebrate diet in the treatment stream.

Hence, the addition of sucrose did not increase (as expected) the contribution of the detrital pathways to macroinvertebrate diet in the treatment stream (52 ± 16%) relative to the reference stream (65 ± 25%). The carbon *originally* derived from DOM (including sucrose) was virtually the same in the reference (54 ± 19%) and treatment stream (51 ± 14%).

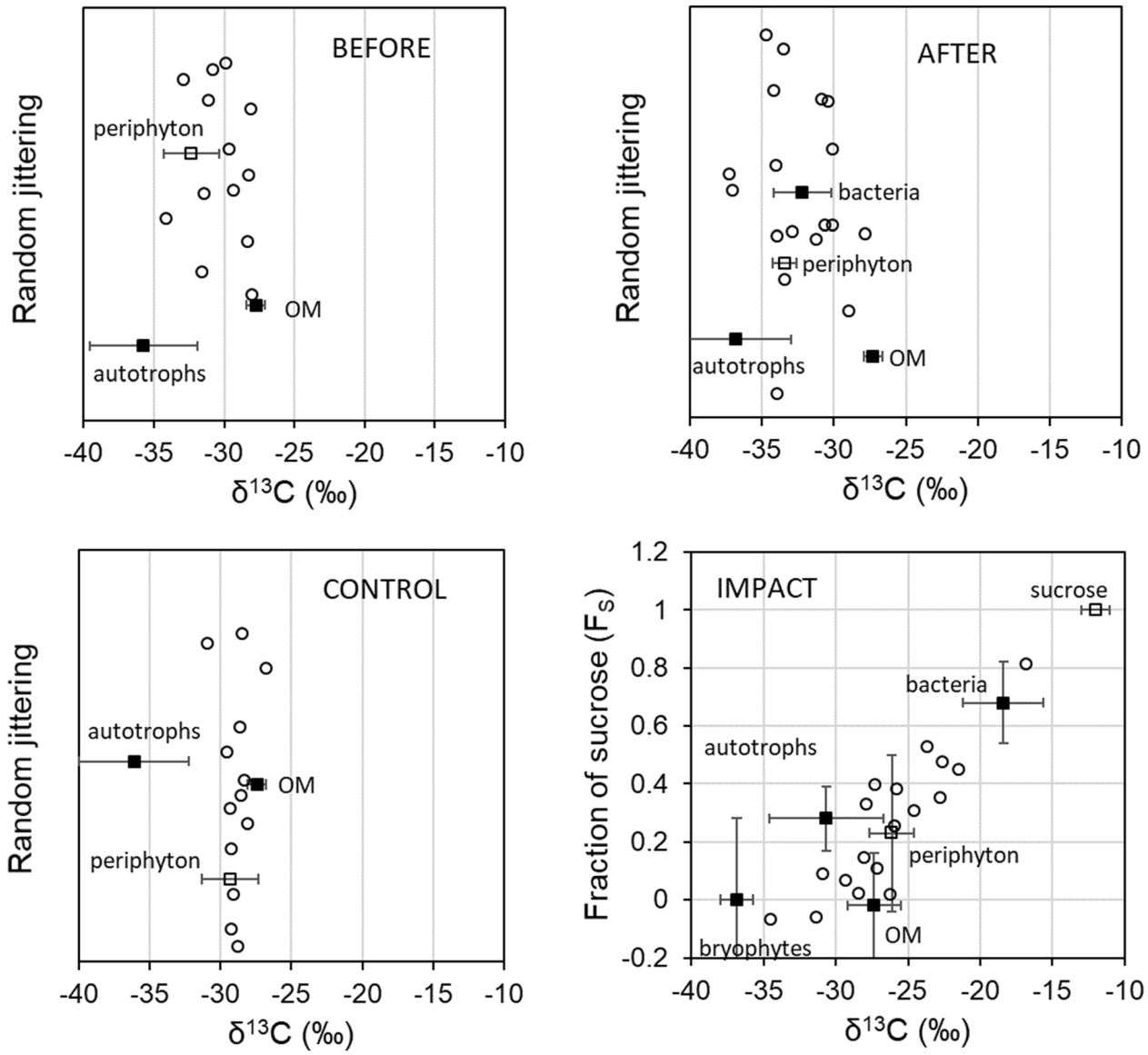

Figure 3. Macroinvertebrate (circles) and sources (squares with error bars ± SD) in the reference stream Birnie Burn (Before, After) and treatment stream Cairn Burn (Control, Impact) of the before and after control impact (BACI) design. A random jittering was applied on the y axes of the Before, After, Control graphs to better visualise the data points. The source end members (filled squares) were autotrophs (including filamentous green algae, periphyton autotrophs and bryophytes—except in Impact where bryophytes were separated), organic matter (OM) and bacteria for all macroinvertebrates except for Simuliidae ($\delta^{13}C = -16.85$, $F_S = 0.81$) which also had sucrose as an end member in the treatment (Cairn impact). Periphyton was not used as a source in this study because it was a mixture of autotrophs, bacteria and natural terrestrial organic matter. The macroinvertebrate isotopic ratios of the impacted stream were corrected to be at equilibrium following the diet shift. No trophic enrichment factors (TEF) or isotopic fractionation were applied. The source bacteria could not be identified in the period prior to sucrose addition (Before, Control)—see ''Method'' section.

Resource Use Efficiencies in Flow Food Webs

Macroinvertebrate production and links to sources were added to the base of the food webs of the reference and treatment streams (Fig. 7). In the reference stream, the direct assimilation of autotrophic C in macroinvertebrates was 7 ± 3 mg C m^{-2} day^{-1} and represented 3 ± 2% of net primary production (Fig. 7). Regarding bacterial C, 1.0 ± 0.7% to 4.7 ± 3.3% of heterotrophic production was assimilated by macroinvertebrates. In

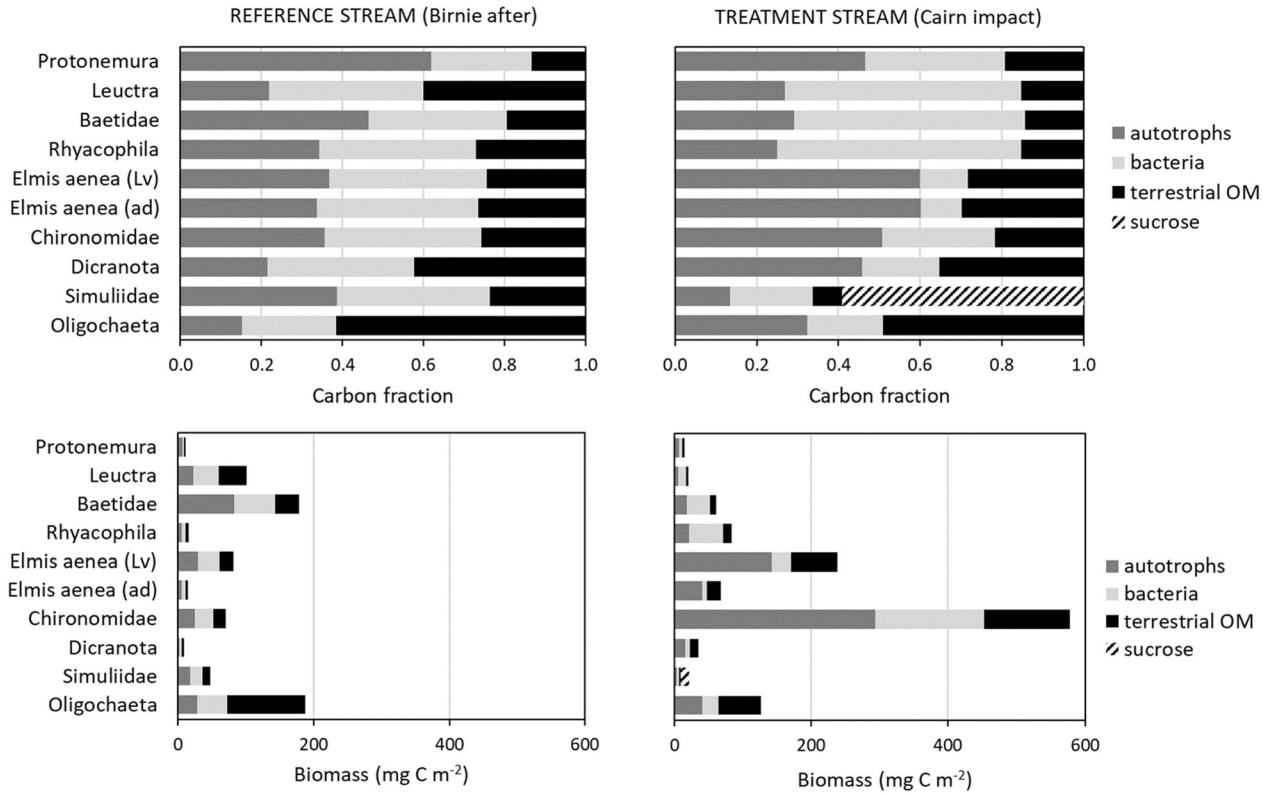

Figure 4. Fractions of C sources (1 = 100%) in macroinvertebrates and their specific biomass (mg C m^{-2}) in the reference and treatment reaches after three weeks of sucrose addition. Note only Simuliidae had sucrose as an additional direct source in the treatment (Cairn impact). lv = larvae, ad = adult, OM = organic matter.

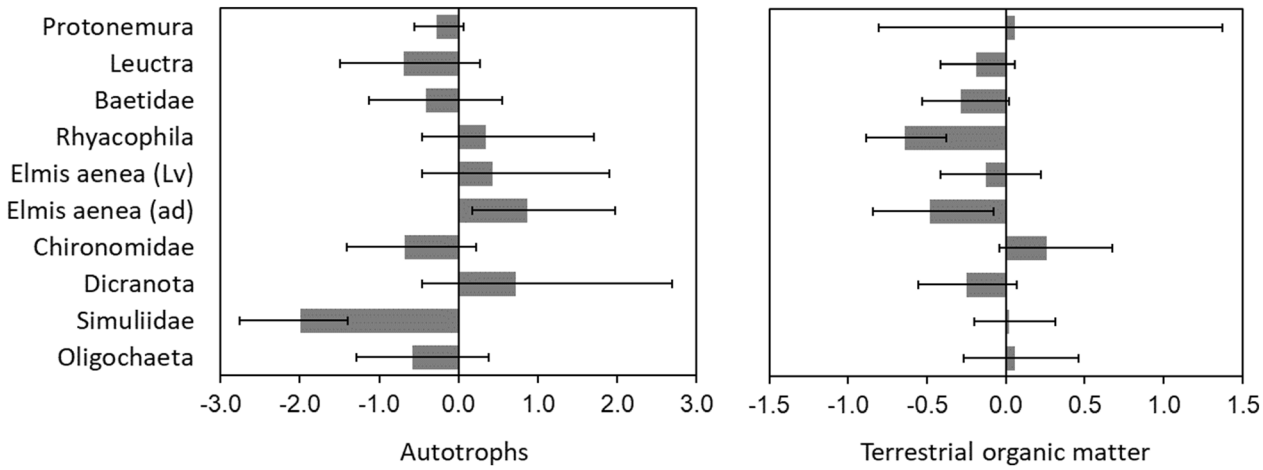

Figure 5. Relative changes (size effects, 1 = 100%) in the fractions of autotrophs and natural terrestrial organic matter in the diet of macroinvertebrates due to sucrose addition. Size effects were calculated as the median from 10,000 Monte Carlo simulations using the BACI experimental design. Error bars represent the interquartile range. lv = larvae, ad = adult.

the treatment, the trophic transfer efficiencies were 1.8 ± 1.4% for autotrophic C and 1.5 ± 0.9% to 6.7 ± 3.8% for bacterial C—Fig. 7. The trophic transfer efficiencies appeared similar between the reference and the treatment streams. In the reference stream, assuming invertebrate production of the studied reach is similar throughout the full stream length (about 500 m^2 stream area), the daily carbon retention by macroinvertebrates represented only about 0.14% of the soil organic carbon

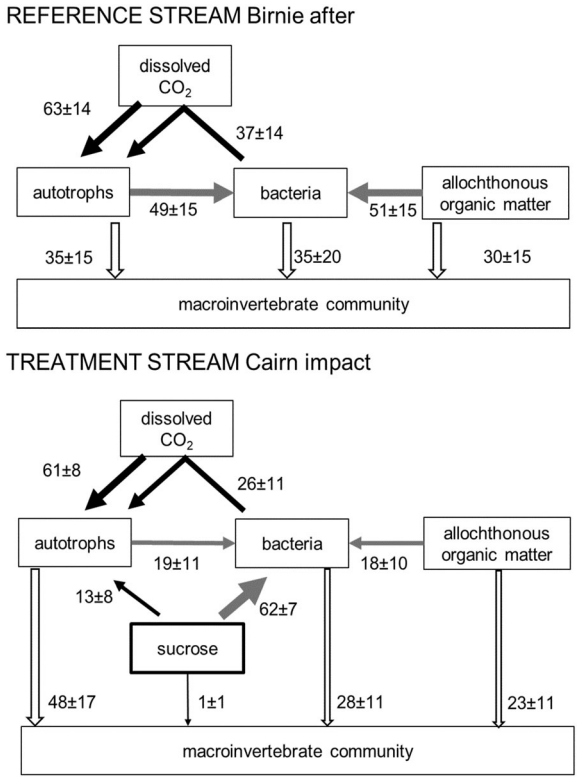

Figure 6. Carbon source partitioning of autotrophs (black arrows), bacteria (grey arrows) and macroinvertebrate community (white arrows; weighted sum of individual contributions) in the reference (top) and treatment (bottom) after three weeks of sucrose addition. Numbers represent the proportion (%) of the sources and their uncertainties (SD). The calculations were based on carbon stable isotope ratio analyses ($\delta^{13}C$) and, in the treatment, also with the fraction of sucrose in the different compartments using the Bayesian mixing model MixSIAR.

delivered to the stream at the catchment scale (DOC flux at the outlet + heterotrophic respiration). The daily soil-derived carbon retention by macroinvertebrates at the catchment scale was only 3–12% of bacterial retention, and less than 1% of bacterial respiration and CO_2 biotic emissions in the reference stream (Fig. 7).

Autotrophic use of CO_2 respired by bacteria was 13 ± 11 times larger in the reference and 24 ± 20 times larger in the treatment than bacterial C uptake by invertebrates (that is, the classic microbial loop). This autotrophic use of CO_2 respired by bacteria allowed a significant fraction of natural organic carbon to make its way to macroinvertebrates via autotrophs fixing CO_2 respired by bacteria: 6.6 ± 4.2% in the reference and 2.2 ± 1.8% in the treatment (10 ± 6% including sucrose).

Discussion

The flow food web including three primary sources (autotrophs, bacteria and natural organic matter) was dominated by the detrital pathway for macroinvertebrates. The small change in carbon supply (+ 12% ambient DOC), mimicking a natural pulse of dissolved organic matter, induced changes in the diet of individual taxa but did not have any disproportionate effects on the overall relative contribution of the detrital pathways versus green pathway. Daily carbon retention by macroinvertebrates represented only a tiny fraction of the flux of soil-derived DOC available at the base of the stream food web.

Twist in the Allochthony Debate

Bacteria play a central role in the food cycle (Lindeman 1942) and the use of fatty acid compound-specific stable isotope ratios allowed the quantification of the detrital pathway of carbon flow to invertebrates (65 ± 25% and 52 ± 16% in the reference and treatment, respectively). On an annual basis, the detrital pathway is likely to be much more prominent because gross primary production was extremely low during the winter (Demars 2019). The periphyton was clearly a mixture of primary sources and its use in flow food web cannot disentangle the origin of carbon, unless primary sources can be identified from compound-specific stable isotopes (for example, fatty acids or amino acids, Liew and others 2019; Demars and others 2020). When there are significant carbon exchanges between autotrophs and bacteria, bulk analyses of autotrophs and allochthonous organic matter can only tell us something about the quality of the food directly assimilated by macroinvertebrates, and not the origin of the carbon (allochthony *versus* autochthony). For now, the role of autochthonous carbon in consumers may have been overestimated in studies relying mostly from reviews of stable isotope bulk analyses (for example, Thorp and Delong 2002; Brett and others 2017). A range of approaches is now available to trace biomolecules through the food webs to improve further our understanding (for example, Nielsen and others 2018; Liew and others 2019; Whiteman and others 2019). Food web studies derived from carbon stable isotope ratios are based on assimilated carbon, which may differ from consumed (or ingested) carbon due to preferential uptakes as shown for bacteria (for example, Guillemette and others 2016). Further studies should

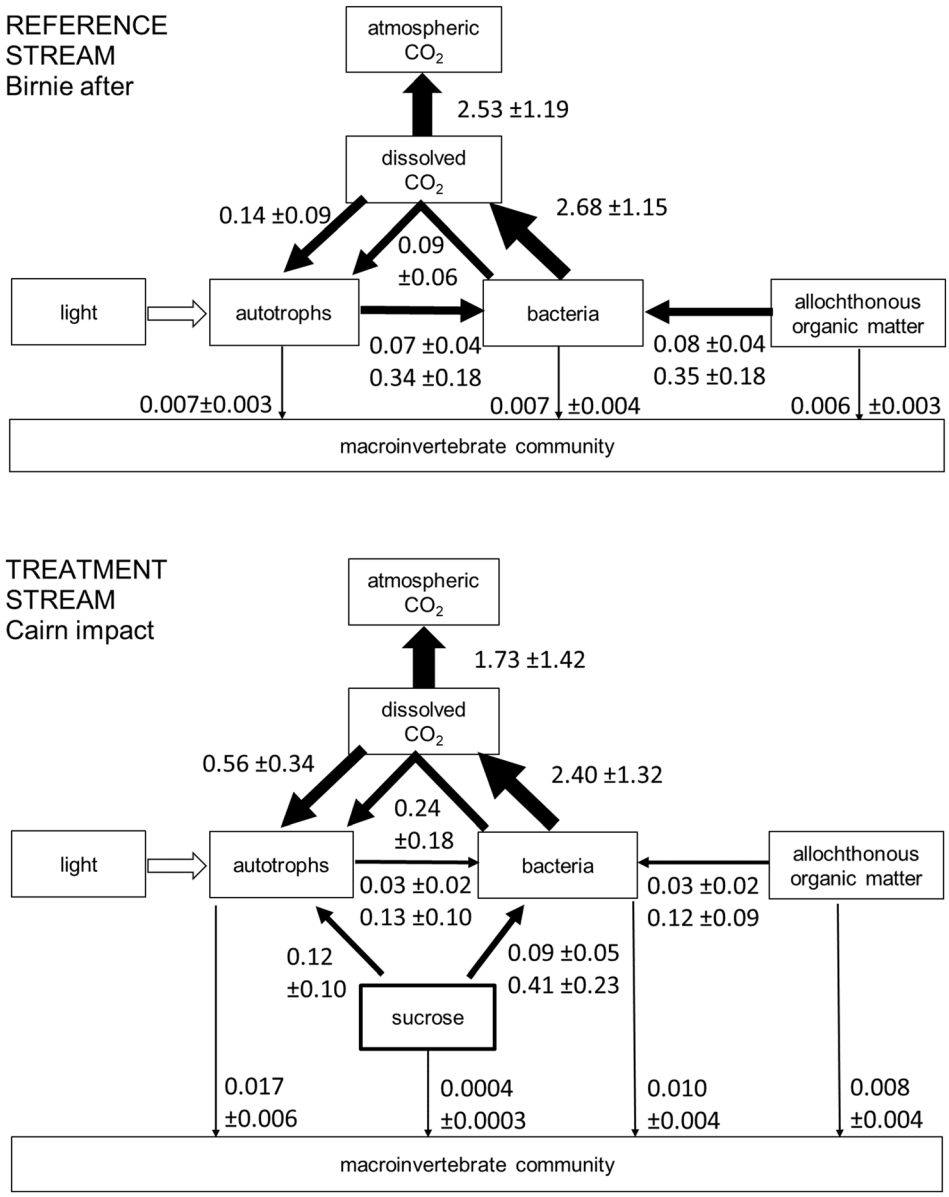

Figure 7. Flow food webs, bacterial respiration and biotic CO_2 emissions under stable flows: in-stream biotic carbon fluxes (g C m^{-2} day^{-1}, black arrows) in the reference stream (Birnie after) and treatment stream (Cairn impact) after three weeks of sucrose addition, based on source partitioning using stable isotopes (Fig. 6) and production estimates. The C fluxes going to autotrophs, bacteria and macroinvertebrates represent rates of biomass accrual, that is, net primary production, heterotrophic production and macroinvertebrate secondary production. Two estimates were given for bacterial production based on heterotrophic growth efficiencies of 0.05 (low) and 0.2 (moderate). Note the supply of natural terrestrial organic matter in the reference stream (85 ± 15 g C m^{-2} day^{-1}) was higher than in the treatment (50 ± 15 g C m^{-2} day^{-1}), also from lateral inflows, explaining the similar rates of bacterial production and respiration.

strive to directly quantify the fluxes of respired CO_2 and its $\delta^{13}C$.

Dissolved Organic Matter Retention by Macroinvertebrates

Although this study shows that assimilation of DOC by macroinvertebrates was low relative to total DOC fluxes, there are indirect macroinvertebrate-mediated pathways that may still be significant (for example, Malmqvist and others 2001; Parr and others 2019, 2020). In our study macroinvertebrate recycling of limiting nutrients is unlikely to play a large role as the small addition of sucrose led to large shift in autotroph C:N:P stoichiometric ratios indicating nutrient limitation (Demars and others

2020). We also found no evidence, in a grazer exclusion experiment, of top-down grazing by macroinvertebrates on biofilm biomass accrual, the composition of PLFAs and stable isotope ratios of PLFAs from the tiles deployed for six weeks (see Demars and others 2020).

Macroinvertebrate Diet

The assimilation of labile carbon (here sucrose) varied greatly between species, as observed in previous studies (for example, Hall 1995; Hall and Meyer 1998; Collins and others 2016). The functional plasticity of macroinvertebrate diet is also well known (for example, Friberg and Jacobsen 1994; Dangles 2002; Merritt and Cummins 2007). Blackflies (Simuliidae) can directly assimilate dissolved organic matter, extracellular polysaccharides and colloidal particles (Couch and others 1996; Hershey and others 1996; Ciborowski and others 1997; Wotton 2009). The added sucrose solution was made every two days with stream water and it is likely that sucrose was adsorbed onto colloidal particles prior to filtration by Simuliidae.

Chironomids relied to a large extent on autochthonous carbon in the treatment, against our expectations. In the treatment, *Microspora* was noticeably gelatinous to the touch and hosted chironomids in high densities, possibly feeding directly on degenerate filaments or exopolymeric substances secreted by the algae under strong nutrient limitations (Demars and others 2020). Stable isotope analyses suggested bryophytes contributed substantially to the diet of Limnephilidae ($62 \pm 17\%$) and *Elmis aenea*, both larvae ($34 \pm 16\%$) and adults ($42 \pm 16\%$), justifying bryophytes as a separate source from the other autotrophs in the treatment reach, after sucrose addition. Contribution from methane-oxidising bacteria (known for their very low $\delta^{13}C$) was dismissed in these small streams draining catchments with soils rich in organic matter, because measured $\delta^{13}C$ of total inorganic carbon (75–95% as CO_2) reflected natural soil (or soil derived) organic matter in a stream running on the other side of the hill (Palmer and others 2001).

Macroinvertebrate Density, Biomass and Production

In response to sucrose addition, chironomids may have shown a small density increase relative to the other taxa. Their growth rate may have been higher, as indicated by their larger average size in the treatment (0.37 mg C ind^{-1}) relative to the reference (0.17 mg C ind^{-1}). A three-year addition of sucrose (0.25–1.00 mg C L^{-1}) in a forested and flow regulated stream resulted in higher bacterial (*Sphaerotilus*), chironomids and trout production (Warren and others 1964). Similar results were reported from shaded experimental side channel experiments where increased DOC (2–3 mg C L^{-1} of sucrose) led to higher bacterial abundances and higher densities of Chironomidae (Fuller and others 2004). A massive addition of dextrose (20 mg C L^{-1}) in a forested stream for eight-week periods also led to the production of thick microbial mats of sheathed bacteria and aquatic fungus on which macroinvertebrates thrived (including chironomids, Wilcox and others 2005). Our experiment simulating a natural pulse of dissolved organic matter in an open stream with bryophytes and filamentous green algae (*Microspora*) produced more subtle effects on invertebrates than in previous studies, as expected (Collins and others 2016).

Our method to estimate individual macroinvertebrate biomass was very rapid but lacked estimation of uncertainties. The calculations of macroinvertebrate growth rate for secondary production were also rapidly executed from individual taxa using empirical equations derived from a global synthesis (Golubkov 2000; Gladyshev and others 2016). Our secondary production estimates did not deviate, however, from expectations based on more accurately determined secondary productions using more time consuming approaches (Figure S1, Patrick and others 2019).

Conclusions

Our flow food web approach, with bacteria playing a pivotal role, showed the dominant contribution of the detrital pathways for macroinvertebrates in the reference, a first-order open blackwater stream. The small change in carbon supply (+ 12% ambient DOC), simulating a natural pulse of dissolved organic matter, induced changes in the diet of individual taxa, but did not have any strong effects on the overall relative contribution of the detrital pathways *versus* the green pathway. Autotrophic use of CO_2 respired by bacteria played a much larger role than bacterial C assimilation by invertebrates (that is, the classic microbial loop, Meyer 1994), showing a much more strongly inter-connected base of the food web than generally depicted (for example, Thorp and Delong 2002). Autotrophic use of CO_2 respired by bacteria allowed a significant fraction of natural organic carbon (7% in the reference) to make its way to

macroinvertebrates via autotrophs fixing CO_2 respired by bacteria.

Our findings suggest that daily carbon retention by macroinvertebrates represented only a tiny fraction of the flux of soil derived organic carbon available at the base of the stream food web. Hence, while anthropogenic changes in allochthonous organic matter may impact the diet of individual species of stream macroinvertebrates, with possible knock on effects for upper trophic levels and riparian ecosystems, macroinvertebrates may not regulate directly to any great extent the flux of stream DOM towards downstream ecosystems. The reciprocal exchange of carbon between autotrophs and heterotrophs should take centre stage in flow food web studies if we are to understand the impact of changing DOM supply driven by climate and land use change on stream macroinvertebrates.

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DATA AVAILABILITY

The raw data necessary to reproduce all figures and calculations were given in supplementary information and Demars and others (2020).

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