Supporting Information. Benoît O.L. Demars, Nikolai Friberg, and Barry Thornton. 2020. Pulse of dissolved organic matter alters reciprocal carbon subsidies between autotrophs and bacteria in stream food webs. *Ecological Monographs.*

Appendix S1

Supplementary figures

Supplementary tables

Additional references

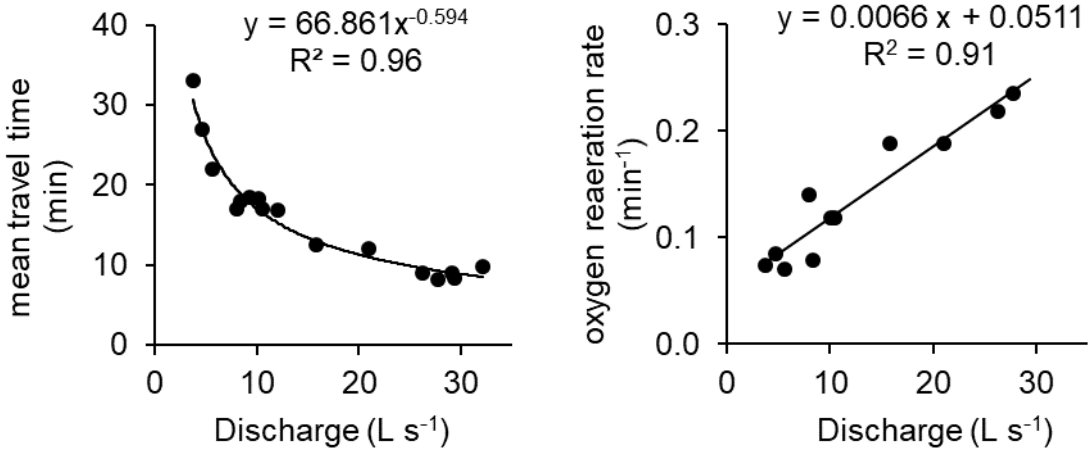

Fig. S1. Discharge as predictor of mean travel time and oxygen reaeration measured from NaCl and propane tracer studies in the Cairn Burn treatment reach. Data from the other two reaches were presented in Demars (2019).

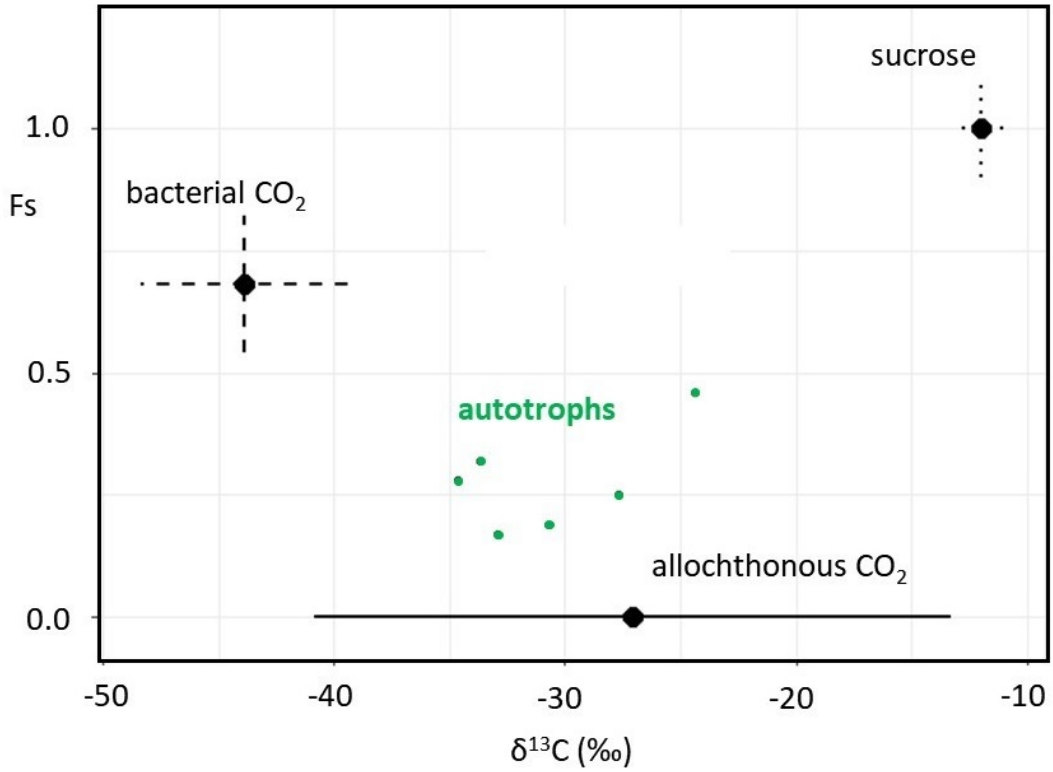

Fig. S2. Cairn burn treatment reach: carbon isotopic signature and fraction of sucrose (Fs) in autotrophs (filamentous green algae and periphyton autotrophs, individual samples or PLFAs represented by small dots) and their sources corrected for carbon fractionation (large dots with error bars, ±1SD). Note all the individual samples lay within the triangle defined by the sources. Edited graph from MixSIAR (Stock and Semmens 2016).

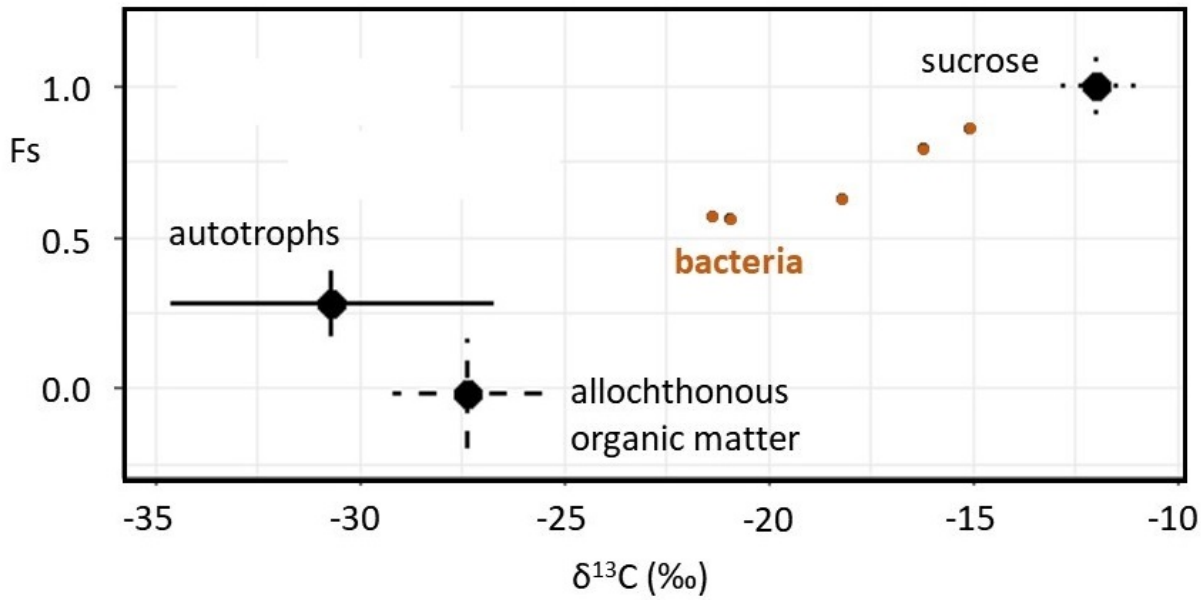

Fig. S3. Cairn burn treatment reach: carbon isotopic signature and fraction of sucrose (Fs) in bacteria (individual PLFAs represented by small dots) and their sources corrected for carbon fractionation (large dots with error bars, ±1SD). Note all the individual samples (PLFAs) lay within the triangle defined by the sources. Edited graph from MixSIAR (Stock and Semmens 2016).

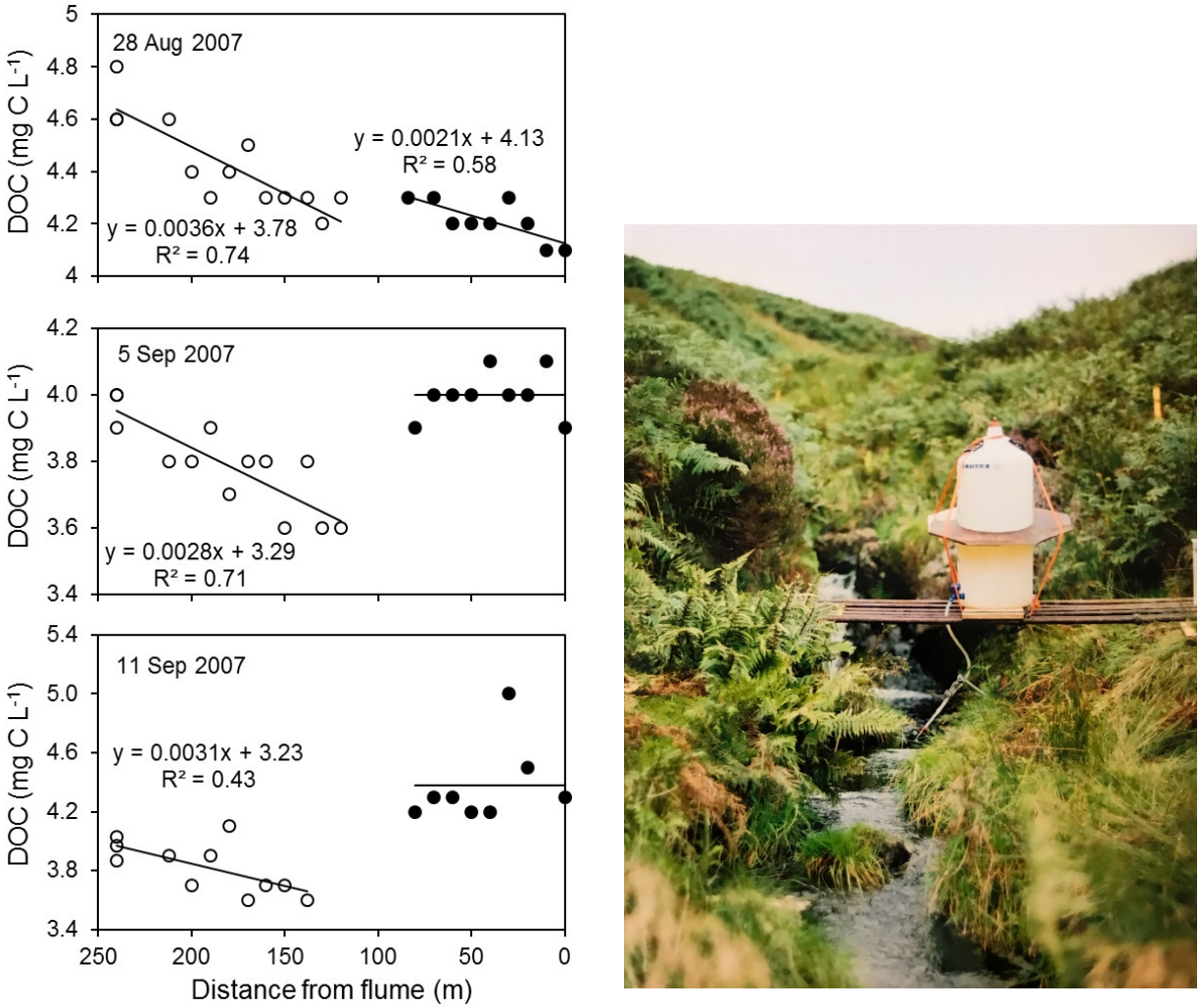

Fig. S4. Cairn Burn control (open symbols) and treatment reach (filled symbols). Effect of continuous sucrose addition at the top of the treatment reach (84 m upstream from flume) on dissolved organic carbon (DOC) after one (+0.22 mg C L^{-1}, top panel), two (+0.47 mg C L^{-1}, middle panel) and three weeks (+0.88 mg C L^{-1}, bottom panel) of treatment under decreasing stream flow rate (average effect +0.52 mg C L^{-1} above background concentration). The effect of sucrose addition was calculated by extrapolating the regression curves to the intersection point at 84 m upstream from the flume. The observed decrease in DOC concentration was due to in-stream DOC processes and possible hydrological dilution (see Dunn et al. 2006, Stutter et al. 2012). The treated reach shows signs of saturation on 5 and 11 September 2007 where the line simply represents the average. The picture on the right illustrates the 60 L carboy set as a Mariotte bottle delivering a continuous flow of sucrose solution to the stream (Cairn Burn).

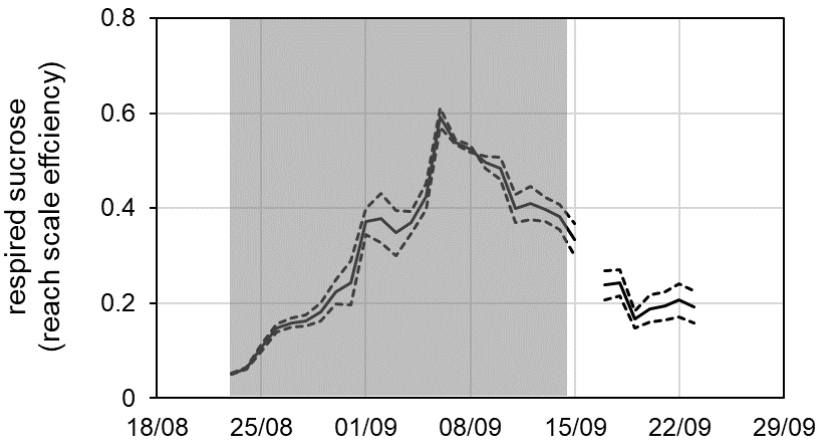

Fig. S5. Efficiency of respired sucrose in the 84 m long treatment reach (with mean travel time of 15 minutes) during (shaded area, 23/08-14/09) and shortly after sucrose addition, calculations based on heterotrophic respiration in the treatment reach relative to the Birnie control (same results with Cairn control, not shown). The black line was calculated with an autotrophic respiration (AR) of 0.5×GPP and dashed lines with AR=0.2×GPP and 0.8×GPP (see method). On average 35±20% of the daily flux of sucrose was respired within that reach during the sucrose addition.

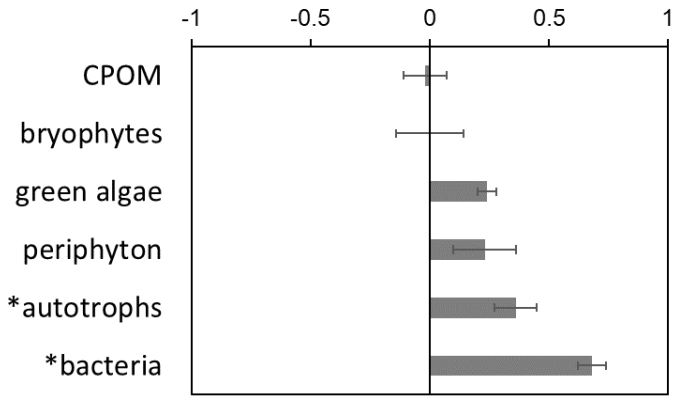

Fig. S6. Proportion of carbon derived from added sucrose in food web basal resources based on $\delta^{13}C$ (see TABLE S1) and PLFA $\delta^{13}C$ for autotrophs and bacteria in periphyton (indicated by *, See TABLE S2). Error bars represent sem. The proportions were calculated from the BACI design, except for periphyton autotrophs and bacteria (periphyton collected at the end of the experiment in the control and treatment reach, see method).

Table S1. Consumer resources' stable carbon isotope ± SD and fraction of carbon derived from added sucrose F_S ± SD assimilated (data used for Fig S6). CPOM = coarse particulate organic matter. The $\delta^{13}C$ of dissolved organic matter was determined for the Cairn burn prior to sucrose addition as -28.5 ±0.3 ‰ (Stutter et al., 2013). The $\delta^{13}C$ of sucrose was determined as -12.0 ±1 ‰ (Jahren et al 2006, Augspurger et al 2008, Kankaala et al 2010, De Castro et al 2016).

		Birnie $\delta^{13}C$, ‰		**Cairn $\delta^{13}C$, ‰**		**F_S**
		before	after	control	impact	impact
CPOM	average	-27.73	-27.28	-27.42	-27.34	-0.02
	uncertainty	0.19	0.65	1.81	1.84	0.18
	n	4	3	4	4	
Bryophytes	average	-35.98	-36.70	-36.15	-36.85	0.00
	uncertainty	2.04	5.97	2.73	1.14	0.28
	n	6	3	4	3	
Filamentous green algae	average	-35.24	-38.90	-36.06	-32.97	0.24
	uncertainty	0.46	0.99	0.22	1.68	0.07
	n	3	3	4	4	
Periphyton	average	-32.32	-33.42	-29.29	-26.13	0.23
	uncertainty	3.49	0.84	2.87	1.51	0.27
	n	3	3	6	4	
***Autotrophs**	average	ND	-34.04	ND	-26.05	0.36
	uncertainty		1.48		2.35	0.13
	n		2		2	
***Bacteria**	average	ND	-32.20	ND	-18.38	0.68
	uncertainty		1.99		2.79	0.14
	n		5		5	

*, derived from the PLFA $\delta^{13}C$ compound specific stable isotope analysis of periphyton (see Table S2); the values were corrected by +3‰ to be comparable with bulk tissue (see methods); ND, not determined (because not enough biofilm had developed on the tiles after three weeks, see method)

Table S2. Periphyton phospholipid fatty acids (PLFAs, 14 to 19 carbon chain length) and fraction of carbon derived from added sucrose F_S in the treatment (1=100%). *, PLFAs identified as bacterial based on the literature and a very high fraction of carbon derived from sucrose. **, PLFA identified as specific to cyanobacteria and algae.

		Birnie, control		Cairn, treatment		Cairn	Taxonomic specificity	References
PLFA	**common name**	%	$\delta^{13}C$, ‰	%	$\delta^{13}C$, ‰	F_S		
14:0	myristic acid	19.3	-38.97	16.2	-37.33	0.07	bacteria, cyanobacteria, algae	2
iso 15:0	*methyltetradecanoic acid	0.3	-35.62	1.7	-19.23	0.79	bacteria, cyanobacteria	1,2,3,4
anteiso 15:0	*methyltetradecanoic acid	0.1	-31.79	1.3	-21.22	0.63	bacteria	1,3
15:0	*pentadecanoic acid	0.4	-36.68	0.5	-24.40	0.57	bacteria, cyanobacteria, algae	2
16:1ω7c	*palmitoleic acid	7.9	-35.34	10.0	-23.97	0.56	bacteria, cyanobacteria, algae	2, 4
16:1ω5c	*hexadecenoic acid	0.5	-36.57	1.9	-18.10	0.86	bacteria, cyanobacteria	1,5
16:0	palmitic acid	55.7	-38.85	47.3	-37.05	0.08	not specific	2
18:3ω6,8,13	**α-linolenic acid	1.3	-37.04	2.0	-29.05	0.36	algae, cyanobacteria	2
18:2ω(14,8 or 13,8)	octadecadienoic acid	0.7	-45.59	0.3	-46.66	-0.04	no information	
18:2ω6,9	linoleic acid	1.1	-43.75	1.1	-41.05	0.09	not specific	1,6,7,8
18:1ω7, 18:1ω9	vaccenic acid, oleic acid	8.9	-41.04	10.8	-34.66	0.25	not specific	6,7,8,9
18:1ω13	octadecenoic acid	0.8	-33.07	1.0	-38.90	-0.32	marine red algae	10
18:0	stearic acid	2.9	-42.63	5.4	-38.64	0.14	not specific	2
19:1ω8	nonadecenoic acid	0.7	-35.32	1.9	-28.75	0.32	no information	

(1) Augspurger et al. 2008, (2) Risse-Buhl et al. 2012, (3) Boschker et al. 1999, (4) Taipale et al. 2015, (5) Collins et al. 2016, (6) Strandberg et al. 2015,(7) Finlay 2004, (8) Sahu et al. 2013, (9) Zhang et al. 2015, (10) Mohy El-Din and El-Ahwany 2016

Table S3. Raw data used to determine the proportion of sources in autotrophs with MixSIAR (Stock and Semmens 2016) in the two streams at the end of the experiment: Birnie control and Cairn treatment (illustrated in Fig S3). The data are individual samples, the sources and carbon fractionation (TEF=trophic enrichment factor in MixSIAR terminology) are averages, their standard deviations and number of samples (or PLFAs). Since PLFA $\delta^{13}C$ values are depleted by 3‰ relative to bulk tissue (see main text) a correction was applied in the table to homogenise the data: the PLFA values were adjusted by +3‰. The mean PLFA value of the source bacterial_CO2 were adjusted through the TEF by +3‰. The control has two sources and one tracer ($\delta^{13}C$) and the treatment has three sources and two tracers ($\delta^{13}C$ and fraction of sucrose, Fs).

		Birnie control			Cairn treatment				
		d13C			**d13C**		**Fs**		
data	filamentous green algae	-40.00			-33.66		0.32		
		-38.62			-30.69		0.19		
		-38.07			-34.63		0.28		
					-32.9		0.17		
	PLFA (α-linolenic acid)	-35.08			-24.39		0.46		
		-32.99			-27.71		0.25		
		Meand13C	**SDd13C**	**n**	**Meand13C**	**SDd13C**	**MeanFs**	**SDFs**	**n**
sources	bacterial_CO2	-35.20	1.99	5	-21.38	2.79	0.68	0.14	5
	allochthonous_CO2	-1.55	13.34	5	-1.55	13.34	0	0.001	5
	sucrose				-12	1	1	0.10	5
		Meand13C	**SDd13C**		**Meand13C**	**SDd13C**	**MeanFs**	**SDFs**	
TEF	bacterial_CO2	-22.5	3.5		-22.5	3.5	0	0	
	allochthonous_CO2	-25.5	3.5		-25.5	3.5	0	0	
	sucrose				0	0	0	0	

Table S4. Raw data used to determine the proportion of sources in bacteria with MixSIAR (Stock and Semmens 2016) in the two streams at the end of the experiment: Birnie control and Cairn treatment (illustrated in Fig S4). The data are individual samples (PLFAs), the sources and carbon fractionation (TEF=trophic enrichment factor in MixSIAR terminology) are averages, their standard deviations and number of samples (or PLFAs). Since PLFA $\delta^{13}C$ were assumed to be depleted by 3‰ relative to bulk tissue (see main text) a correction was applied in the table to homogenise the data: the PLFA values were adjusted by +3‰. The mean PLFA value of the source autotrophs (filamentous green algae and PLFA α-linolenic acid) were calculated from the raw data presented in Table S3. The sources were assumed to be processed by bacteria without carbon fractionation (see main text). The control has two sources and one tracer ($\delta^{13}C$) and the treatment has three sources and two tracers ($\delta^{13}C$ and fraction of sucrose, Fs).

		Birnie control			**Cairn Treatment**				
		d13C			**d13C**		**Fs**		
data	bacterial PLFAs	-32.62			-16.23		0.79		
		-28.79			-18.22		0.63		
		-33.68			-21.40		0.57		
		-32.34			-20.97		0.56		
		-33.57			-15.10		0.86		
		Meand13C	**SDd13C**	**n**	**Meand13C**	**SDd13C**	**MeanFs**	**SDFs**	**n**
sources	autotrophs	-36.95	2.85	5	-30.66	3.94	0.28	0.11	6
	allochthonous_OM	-27.28	0.65	3	-27.34	1.84	-0.02	0.18	4
	sucrose				-12.00	1.00	1.00	0.10	5
		Meand13C	**SDd13C**		**Meand13C**	**SDd13C**	**MeanFs**	**SDFs**	
TEF	autotrophs	0	0		0	0	0	0	
	allochthonous_OM	0	0		0	0	0	0	
	sucrose				0	0	0	0	

Table S5. Nutrient cycling study parameters (data used for Fig 4). SE=standard error, ND=not determined (because the water samples were not analysed for the conservative tracer Cl)

Dates dd/mm/yyyy	background concentration mg L^{-1}	added geometric mean mg L^{-1} or g m^{-3}	exponential regression slope m^{-1}	SE of slope m^{-1}	Sw m	Sw low m	Sw high m	Q L s^{-1}	max width cm	u cm s^{-1}	z cm	v_f mm s^{-1}	U mg m^{-2} h^{-1}
NO_3-N													
Cairn													
10/07/2007	0.089	0.607	0.00100	0.00027	1005	791	1377	29.5	102	16	19	0.029	9.2
31/07/2007	0.093	0.254	0.00075	0.00013	1330	1139	1598	29.7	102	17	17	0.022	7.3
14/08/2007	0.091	0.350	0.00078	0.00008	1287	1161	1445	21.2	102	12	18	0.016	5.3
28/08/2007	0.094	0.386	0.00124	0.00016	808	717	924	16.0	102	11	14	0.019	6.6
04/09/2007	0.088	0.410	0.00133	0.00012	754	691	828	10.6	102	8	13	0.014	4.4
11/09/2007	0.088	0.386	0.00183	0.00021	547	492	617	8.1	102	8	10	0.014	4.6
Birnie													
10/07/2007	0.192	0.841	ND	ND	ND	ND	ND	25.5	77	14	24	ND	ND
31/07/2007	0.160	0.489	0.00058	0.00026	1733	1201	3115	25.7	77	13	26	0.019	11.1
14/08/2007	0.203	0.576	0.00017	0.00031	5798	2051	-7017	18.4	77	9	26	0.004	3.0
28/08/2007	0.202	0.525	0.00038	0.00017	2640	1811	4867	13.9	77	7	25	0.007	5.0
04/09/2007	0.159	0.384	0.00032	0.00010	3121	2379	4536	9.2	77	6	21	0.004	2.2
11/09/2007	0.167	0.438	0.00049	0.00015	2032	1548	2955	7.0	77	4	20	0.004	2.7
PO_4-P													
Cairn													
10/07/2007	0.008	0.037	0.00397	0.00103	252	200	340	29.5	102	16	19	0.115	3.3
31/07/2007	0.004	0.012	0.00409	0.00036	245	225	268	29.7	102	17	17	0.119	1.6
14/08/2007	0.003	0.008	0.00843	0.00320	119	86	191	21.2	102	12	18	0.175	1.7
28/08/2007	0.003	0.007	0.01160	0.00370	86	65	127	16.0	102	11	14	0.182	1.7
04/09/2007	0.002	0.006	0.01771	0.00148	56	52	62	10.6	102	8	13	0.184	1.5
11/09/2007	0.004	0.004	0.03175	0.00437	31	28	37	8.1	102	8	10	0.251	3.2
Birnie													
10/07/2007	0.001	0.076	ND	ND	ND	ND	ND	25.5	77	14	24	ND	ND
31/07/2007	0.002	0.038	0.00330	0.00039	303	271	344	25.7	77	13	26	0.110	0.9
14/08/2007	0.002	0.039	0.00416	0.00047	241	216	271	18.4	77	9	26	0.099	0.7
28/08/2007	0.001	0.030	0.00694	0.00078	144	129	162	13.9	77	7	25	0.125	0.7
04/09/2007	0.001	0.016	0.01236	0.00064	81	77	85	9.2	77	6	21	0.147	0.7
11/09/2007	0.003	0.010	0.01955	0.00229	51	46	58	7.0	77	4	20	0.177	2.2

Table S6. Nutrient (C, N, P) tissue content (% mass/mass) and molar stoichiometric ratios (C:N:P) of resources (data used for Fig. 5). CPOM=coarse particulate organic matter

		Birnie Burn		Cairn Burn	
		before	after	control	impact
CPOM	C	43.66	43.03	43.88	44.90
	N	1.09	0.90	1.13	1.63
	P	0.07	0.06	0.08	0.11
	C:N:P	1565:33:1	1914:34:1	1381:31:1	1096:34:1
Bryophytes	C	37.47	37.62	39.02	39.60
	N	1.80	1.91	2.25	2.70
	P	0.10	0.09	0.18	0.18
	C:N:P	1001:41:1	1118:49:1	574:28:1	576:34:1
Filamentous green algae	C	43.12	39.36	40.99	38.66
	N	3.50	3.62	4.18	3.48
	P	0.19	0.22	0.32	0.16
	C:N:P	587:41:1	460:36:1	330:29:1	632:49:1
Periphyton	C	29.15	23.57	25.25	32.04
	N	3.23	2.20	3.28	3.33
	P	0.20	0.19	0.25	0.19
	C:N:P	372:35:1	327:26:1	262:29:1	428:38:1

Table S7. Relative effect size (proportional changes, 1=100%) in selected ecosystem properties (see Fig. 8, Table S8). Stream reach scale data based on the before and after control impact experimental design. The control reach was in Birnie Burn and the treatment reach in Cairn Burn. δx/x represent relative uncertainties based on 1 standard deviation (equivalent to 68% confidence interval).

	Relative effect size	δx/x
gross primary production	0.12	0.35
ecosystem respiration	0.70	1.39
net ecosystem production	1.25	1.65
net primary production	0.12	8.10
heterotrophic respiration	0.89	1.50
heterotrophic production	0.89	1.50
organic carbon uptake length SwOC	-0.40	2.76
organic carbon mineralisation velocity vf-OC	0.92	1.52
CO_2 emissions	-0.20	3.30
proportion of biotic CO_2 emissions	0.88	5.48
proportion of DOC flux respired	1.12	1.61
light use efficiency by autotrophs	0.37	3.10
organic carbon use efficiency by bacteria	1.12	1.61

Table S8. Values and uncertainties of ecosystem properties (stream reach scale), Before After Control Impact (BACI) design, presented in Fig 8 and Table S7, with additional information regarding PAR and DOC. HGE = heterotrophic growth efficiency, PAR=photosynthetic active radiation, DOC=dissolved organic carbon, δx/x = standard deviation / value,. Multiply by 100 to express the relative uncertainties in percentages.

		Values, x				Relative uncertainties, δx/x			
		Birnie control		Cairn treatment		Birnie control		Cairn treatment	
	Units, x	B	A	C	I	B	A	C	I
gross primary production	g C m^{-2} day^{-1}	0.41	0.47	1.58	1.81	0.07	0.07	0.02	0.02
ecosystem respiration	g C m^{-2} day^{-1}	-4.93	-3.00	-3.22	-3.54	0.40	0.40	0.40	0.40
net ecosystem production	g C m^{-2} day^{-1}	-4.52	-2.53	-1.65	-1.73	0.44	0.47	0.78	0.82
net primary production	g C m^{-2} day^{-1}	0.21	0.23	0.79	0.91	0.60	0.60	0.60	0.60
heterotrophic respiration	g C m^{-2} day^{-1}	-4.72	-2.77	-2.44	-2.64	0.42	0.43	0.54	0.55
heterotrophic production (HGE=0.05)	g C m^{-2} day^{-1}	0.25	0.15	0.13	0.14	0.42	0.43	0.54	0.55
heterotrophic production (HGE=0.20)	g C m^{-2} day^{-1}	1.18	0.69	0.61	0.66	0.42	0.43	0.54	0.55
organic carbon uptake length Sw_{OC}	m	3214	2531	4257	1886	0.67	0.68	0.75	0.76
organic carbon mineralisation velocity $v_{f\text{-}OC}$	m day^{-1}	0.82	0.55	0.53	0.76	0.45	0.47	0.56	0.57
CO_2 emissions	g C m^{-2} day^{-1}	-18.6	-9.9	-29.0	-14.3	0.50	0.50	0.50	0.50
proportion of biotic CO_2 emissions	%	24.2	25.6	5.7	12.1	0.66	0.69	0.93	0.96
proportion of DOC flux respired	%	2.7	3.3	2.2	5.3	0.44	0.46	0.56	0.57
light use efficiency by autotrophs	%	0.2	0.3	0.7	1.0	0.60	0.60	0.60	0.60
organic carbon use efficiency by bacteria (HGE=0.05)	%	0.1	0.2	0.1	0.3	0.44	0.46	0.56	0.57
organic carbon use efficiency by bacteria (HGE=0.20)	%	0.7	0.8	0.6	1.3	0.44	0.46	0.56	0.57
PAR	g C m^{-2} day^{-1}	106	80	118	90	0.05	0.05	0.05	0.05
DOC outlet	g C m^{-2} day^{-1}	173	82	108	47	0.15	0.15	0.15	0.15
DOC supply	g C m^{-2} day^{-1}	177	85	110	50	0.15	0.15	0.15	0.14

Additional references

Augspurger, C., G. Gleixner, C. Kramer, and K. Kusel. 2008. Tracking carbon flow in a 2-week-old and 6-week-old stream biofilm food web. Limnology and Oceanography **53**:642-650.

Boschker, H. T. S., J. F. C. de Brouwer, and T. E. Cappenberg. 1999. The contribution of macrophyte-derived organic matter to microbial biomass in salt-marsh sediments: Stable carbon isotope analysis of microbial biomarkers. Limnology and Oceanography **44**:309-319.

Collins, S. M., J. P. Sparks, S. A. Thomas, S. A. Wheatley, and A. S. Flecker. 2016. Increased light availability reduces the importance of bacterial carbon in headwater stream food webs. Ecosystems **19**:396-410.

Demars, B. O. L. 2019. Hydrological pulses and burning of dissolved organic carbon by stream respiration. Limnology and Oceanography **64**:406-421.

Dunn, S. M., S. I. Vinogradoff, G. J. P. Thornton, J. R. Bacon, M. C. Graham, and J. G. Farmer. 2006. Quantifying hydrological budgets and pathways in a small upland catchment using a combined modelling and tracer approach. Hydrological Processes **20**:3049-3068.

Finlay, J. C. 2004. Patterns and controls of lotic algal stable carbon isotope ratios. Limnology and Oceanography **49**:850-861.

Mohy El-Din, S. M., and A. M. D. El-Ahwany. 2016. Bioactivity and photochemical constituents of marine red seaweeds (Jania rubens, Corallina mediterranea and Pterocladia capillacea). Journal of Taibah University for Science **10**:471-484.

Risse-Buhl, U., N. Trefzger, A. G. Seifert, W. Schonborn, G. Gleixner, and K. Kusel. 2012. Tracking the autochthonous carbon transfer in stream biofilm food webs. Fems Microbiology Ecology **79**:118-131.

Sahu, A., I. Pancha, D. Jain, C. Paliwal, T. Ghosh, S. Patidar, S. Bhattacharya, and S. Mishra. 2013. Fatty acids as biomarkers of microalgae. Phytochemistry **89**:53-58.

Stock, B. C., and B. X. Semmens. 2016. MixSIAR GUI User Manual. Version 3.1., https://github.com/brianstock/MixSIAR/, doi:10.5281/zenodo.47719.

Strandberg, U., S. J. Taipale, M. Hiltunen, A. W. E. Galloway, M. T. Brett, and P. Kankaala. 2015. Inferring phytoplankton community composition with a fatty acid mixing model. Ecosphere **6**:16.

Stutter, M. I., S. M. Dunn, and D. G. Lumsdon. 2012. Dissolved organic carbon dynamics in a UK podzolic moorland catchment: linking storm hydrochemistry, flow path analysis and sorption experiments. Biogeosciences **9**:2159-2175.

Taipale, S. J., E. Peltomaa, M. Hiltunen, R. I. Jones, M. W. Hahn, C. Biasi, and M. T. Brett. 2015. Inferring phytoplankton, terrestrial plant and bacteria bulk delta C-13 values from compound specific analyses of lipids and fatty acids. PLoS ONE **10**:e0133974.

Zhang, X. W., T. S. Bianchi, and M. A. Allison. 2015. Sources of organic matter in sediments of the Colville River delta, Alaska: A multi-proxy approach. Organic Geochemistry **87**:96-106.