No evidence of aquatic priming effects in hyporheic zone microcosms

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SUPPLEMENTARY INFORMATION

Detailed materials and methods

Production of a ^{13}C-labeled model allochthonous DOC substrate

To simulate allochthonous DOC in streams, a pre-degraded extract of plant material from crack willow (*Salix fragilis*) was produced. *S. fragilis*, and other *Salix* species, are common members of the riparian vegetation of European streams and rivers, including our studied stream, the Oberer Seebach (OSB) in Lunz am See, Austria (Lat: 47.852456°, Long: 15.065945°). Cuttings of *S. fragilis* (willow) were grown under a $^{13}CO_2$-enriched atmosphere (Isolife, The Netherlands) for 3 months. The highly ^{13}C-labeled leaf and stem material (>97 atom % 13C) was harvested, dried and ground to a fine powder using a ball mill (Retch MM2) and used to make a hot water extract. The willow powder was extracted in MilliQ water at 95°C for 60 minutes. Willow material grown under natural, outdoor conditions was extracted and pre-degraded in the same manner as the labeled material (in total 44 g). Three replicate degradation bioreactors were employed, consisting of plexiglass cylinders (Ø 14 cm, height 100 cm), equipped with aquarium pumps to aerate and recirculate water and filled with a porous ceramic substrate (Eheim MECH) for biofilm growth. A small amount of natural stream sediment, and 2 L of raw streamwater from the OSB were added as a microbial inoculum. 400 ml of unfiltered willow extract, corresponding to 16 g of dry plant material was processed in each bioreactor. One of the bioreactors included the ^{13}C-labeled willow extract. The gradual decrease in DOC concentration was monitored throughout the degradation process. The degradation was terminated when no further decrease in DOC concentration was observed during 3 consecutive days. The refractory DOC was considered relatively recalcitrant to further degradation by stream microbial communities. The resulting extracts were sterile filtered and kept at +4°C for a few days until use. The ^{13}C-labeled extract was

combined with part of the unlabeled degraded extract to a concentration of <10 atom % ^{13}C to accommodate the measurement constraints of the isotopic ratio mass spectrometer. A similar approach has previously been successfully applied to study uptake of DOC of different lability in streams [1,2].

Hyporheic microcosms

Sintered glass beads (SIRAN carriers, 2-3 mm Ø, Jaeger Biotech Engineering) were used as substrate for hyporheic biofilm communities in the microcosms. The beads were colonized inside mesh bags in the dark in the OSB for 31 days in October-November 2011. After this time, the beads were rinsed to remove detrital particles, mixed and gently filled into the microcosms which consisted of 200 ml borosilicate glass cylinders with tubing connectors at both ends (Fig. 1). The microcosms were then fed with unfiltered streamwater for an additional 16.5 days in order to acclimatize to laboratory conditions. The outflow of the microcosms during the streamwater phase was collected in a tank and used to make up the medium used in the following experimental phases (recirculated streamwater).

The hyporheic microcosms (Fig. 1) were subjected to a once-through flow of medium, consisting of recirculated streamwater mixed with willow DOM and labile DOM treatments during part of the experiment (see sections 2.3 and 2.4). The medium was pumped through the microcosms by peristaltic pumps (Ecoline VC MS/CA2, Ismatec) from 4 L polypropylene copolymer bottles (Nalgene). Each microcosm was connected to an individual bottle (Fig. 1), and the bottles with medium were replaced roughly every 48 hours. Bottles were autoclaved between changes, and all medium was sterile filtered to prevent bacterial growth in the bottles. The microcosms were covered to prevent algal growth during the entire experiment.

The experiment was carried out in a climatized room where the temperature was continuously monitored. The average temperature during the experiment was 18.8ºC (maximum 20.3, minimum 17.7). The empty bed contact time (residence time) of medium in the microcosms was 183 minutes (average, SD ± 11 min). This was calculated from the flow rate, which was 0.90 ml min^{-1} on average (SD ± 0.05, measured daily for each microcosm), and the volume of medium in each microcosm (the volume of the cylinder minus the volume displaced by the beads), which was 163.6 ml on average (SD ± 1.1).

Samples were taken at the inflow and outflow of each microcosm. Syringes (100 ml) were connected to sampling valves and allowed to passively collect sample liquid mediated by the pressure from the pump. Outflow samples were collected at the normal flow rate, while inflow samples were collected at a faster rate by diverting the flow for a short time (<5 minutes) via a three-way valve (see fig. 1).

Treatments

Three different labile DOM treatments simulating autochthonous inputs were employed in this experiment: Glucose, glucose combined with NO_3 and PO_4 (glucose+N+P), and an algal extract. The control treatment contained no labile carbon source. All treatments received the same background concentration of willow DOM. The target concentrations of carbon additions were 0.45 mg L^{-1} labile DOM and 0.9 mg L^{-1} willow DOM. The background stream DOC concentration averaged 1.09 mg L^{-1}. In the glucose+N+P treatment, $N\text{-}NO_3$ was added at a target concentration of 2200 µg L^{-1} above the background and $P\text{-}PO_4$ was added at 6.8 µg L^{-1}. These concentrations of inorganic nutrients in the glucose+N+P treatment were chosen to match the final concentration of mineral nutrients in the algal extract treatment, taking

into account also the concentration of organic N in the algal extract. The final concentrations of the treatments (Table 1) were within the range of naturally occurring concentrations of DOC and $N\text{-}NO_3$ recorded in the Oberer Seebach.

The algal extract was obtained from a 50 L culture of the green alga *Monoraphidium contortum* harvested at stationary phase. The algal cells were concentrated by centrifugation, washed twice in streamwater and sonicated to disrupt the cell walls. The resulting extract was sterile filtered and stored at 4ºC for a few days until use.

Experimental phases

The experimental preparations (described in detail in previous sections) included:

- Production of a ^{13}C-labeled willow DOM tracer.
- Production of labile DOM additions from an algal culture, glucose and mineral nutrients.
- Colonization of hyporheic biofilm communities in the streambed.
- Assembly of hyporheic microcosms.

The experiment included 4 phases during which sampling was carried out:

1. During the streamwater phase (16.5 days), the bioreactors were subjected to a flow of unfiltered streamwater from the OSB in order to acclimatize them to laboratory conditions.
2. During the pre-pulse phase (2 days), unlabeled willow DOM was added to all microcosms to acclimatize the bacterial communities to a higher total DOC concentration.

3. In the pulse phase (7 days), labile DOM substrate treatments were added along with the ^{13}C-labeled willow DOM to test for priming effects.

4. In order to detect delayed effects of the treatments, the pulse phase was followed by an extended ^{13}C labeled willow DOM-only phase (post-pulse phase, 14 days). See Fig. 2 for an illustration of experimental phases and timings of sampling points.

Liquid sample processing

Liquid samples were collected for analysis of the concentration and isotopic composition of DOC and DIC and the concentration of mineral nutrients (NO_3 and PO_4). The sample liquid was filtered using pre-combusted glass fiber filters (Whatman GFF, 25 mm) connected to the sampling syringe. DOC and DIC concentration samples were collected in acid washed and combusted glass vials (40 ml TOC, GE). Care was taken to avoid outgassing of CO_2 in the DIC samples by gently filling the sample liquid into the vials using a needle and eliminating any headspace in the vials. Samples were stored at + 4ºC and DOC and DIC concentrations were measured on a TOC Analyzer (Sievers 5310C, GE Analytical Instruments). DOC isotopic composition samples were collected in plastic tubes (15 ml, BD Falcon), acidified with a drop of concentrated HCl (37%), vortexed and frozen at -20ºC. Samples were subsequently lyophilized, resuspended in 1 ml MilliQ and DOC isotopic composition was measured on an LC-IRMS system (HPLC pump coupled via LC-Isolink in direct injection mode to Delta V Advantage IRMS, Thermo Scientific). DIC isotopic composition samples were collected in acid washed, pre-combusted glass vials (12 ml exetainer, Labco). The vials were closed with air-tight septa, pre-flushed with N_2 and pre-acidified with a drop of ortho-phosphoric acid. Approximately 1 ml (the exact volume was determined by weighing) of sample liquid

was injected into the vial, and the isotopic composition of the evaded CO_2 in the headspace was measured on a continuous-flow IRMS system (GasBench II gas preparation system coupled to Delta V Advantage IRMS, Thermo Scientific) within two weeks of collection (samples stored at +4ºC). Inorganic nutrient samples were collected in plastic tubes (15 ml, BD Falcon), stored at +4ºC and NO_3 and PO_4 concentrations were measured on a continuous flow analyzer (FlowSys 3rd generation, SYSTEA Analytical Technologies) within two weeks.

Dissolved oxygen was measured at the inflow and outflow using oxygen optode flow-through cells (PSt3 sensor, PreSens). Inflow and outflow sampling was performed within a short time frame (a few minutes) on each bioreactor, in order to minimize the effect of fluctuating temperature and air pressure on the concentration of dissolved gasses (O_2 and CO_2). Sampling was carried out at 14 time points during the experiment (Fig. 2).

Biomass sample processing

Biofilm biomass was sampled twice during the experiment. At the beginning, after the streamwater phase, five microcosms were destructively sampled and the beads were frozen at -80ºC until further processing. At the end of the post-pulse phase, all remaining microcosms were destructively sampled and the beads were frozen and stored at -80ºC.

Biomass samples were harvested from the glass beads by sonication and vortexing according to [3]. To detach biomass from the beads, approximately 6 g of beads per bioreactor were vortexed (10s, full power) in a sterile centrifuge tube (50 ml, BD Falcon) and sonicated (1 min, 1s pulse, 13% amplitude, Branson Digital Sonifier Probe) in MilliQ water (20 ml). The detachment procedure was repeated three times.

The supernatant was decanted, the beads rinse twice with MilliQ water and the supernatants decanted again. The resulting water-biofilm slurry was lyophilized and the dry powder was acidified with HCl (10% w/v), and dried again before determination of organic C content and isotopic composition by an elemental analyzer–IRMS (Euro EA 1110 C/N analyzer coupled to a DELTA^{plus} IRMS by ConFlo III interface, Thermo Scientific).

Respiration

Consumption of dissolved oxygen was used as a proxy for respiration in the microcosms. This method was preferred over using production of DIC because of the high background DIC concentrations (31-33 mg/l) resulting from carbonates in the streamwater that easily overshadow the relatively small increase in DIC due to respiration. However, to translate oxygen removal into respired CO_2, a respiratory coefficient (RQ) was derived from dividing the DIC produced by the O_2 consumed (in mol) for all microcosms and all time points (n=280). This yielded an average RQ of 0.81 (SD ±0.51) which corresponds well to RQs determined in other freshwater environments [4]. Thus, bulk respiration was calculated as:

$$C_R = (O_{in} - O_{out}) * 0.81 * \left(\frac{12.01}{31.998}\right)$$

Where C_R is the concentration of respired DIC (mg/l) and O_{in} and O_{out} are the measured oxygen concentrations at the input and output of the microcosms, respectively.

Removal and release of carbon and inorganic nutrients

The accumulated mass of CO_2 respired, and of the DOC and inorganic nutrients removed during the different phases of the experiment was calculated using the measured flow rates and integrated over the duration of each of the phases of the experiment.

The experiment was divided into 13 intervals, each encompassing one sampling point. The volume of medium that passed through each microcosm during each interval was calculated using the daily measured flow rates of each microcosm. The mass of C, N-NO_3 or P-PO_4 processed during each interval was calculated as:

$$M_X = \Delta C_X * \int_{t_0}^{t_{int}} Q \, dt \qquad (2)$$

Where M_X is the mass of C, N or P removed or released during an interval, ΔC_X is the difference in concentration of C, N or P between input and output of the microcosm (or the concentration of respired C, see section 2.8.1) measured at one sampling point, Q is the flowrate, and t_0 and t_{int} represent the duration of an interval. The masses calculated for each interval were then summed up over each experimental phase, and normalized to the mass of beads contained in each microcosm (a proxy for biomass), and finally expressed as an hourly rate.

Partitioning of carbon pools

Partitioning of respiration, DOC and biomass into willow-derived C and non-willow derived C was achieved using the isotopic signature of the carbon in the respective datasets in combination with the known isotopic label of the willow DOM. Other carbon pools include the labile DOM additions, background streamwater DOC and preexisting particulate OM and biomass in the microcosms.

For respiration it was necessary to correct the measured isotopic composition of the DIC to separate the isotopic label of the respired CO_2 from that of the background carbonates contained in the stream water. This could be achieved using a linear mixing model with two end members:

$$d_{out} = F_{BG} * d_{BG} + F_R * d_R \quad (3)$$

$$F_{BG} + F_R = 1 \quad (4)$$

Where d_{out} is the isotopic label of the output DIC in atom % ^{13}C, d_{BG} is the isotopic label of the background DIC (same as input DIC, containing no respired CO_2 from within the microcosms), F_{BG} is the fraction of background DIC and F_R is the fraction of respired C. F_R can also be expressed as:

$$F_R = \frac{C_R}{C_{BG} + C_R} \quad (5)$$

Where C_R is the concentration of respired CO_2 derived from oxygen concentration data (see section 2.8.1.), and C_{BG} is the concentration of background DIC, derived from bulk DIC concentration data of input samples. The isotopic label of the respired CO_2 could thus be calculated by combining equations 3, 4 and 5:

$$d_R = \frac{d_{out} - (1 - F_R) * d_{in}}{F_R} \quad (6)$$

To partition respiration, DOC and biomass into willow-derived and non-willow derived, a two end member linear mixing model was employed [5].

$$F_W = \frac{d_x - d_o}{d_W - d_o} \quad (7)$$

Where d_X is the measured isotopic signature of the C pool of interest (respired CO_2, DOC or biomass), d_W and d_o are the isotopic signatures of the willow DOM (9.35 atom % ^{13}C) and other C (natural abundance, 1.08 atom % ^{13}C), respectively. F_W and F_o are the fractions of willow DOM and other C in the mixture, respectively.

The priming effect was calculated as the surplus willow-derived respiration in the labile DOM treatments compared to the mean willow-derived respiration of the control treatment.

$$PE = (R_W)_{Treatment} - (\bar{R}_W)_{Control} \quad (8)$$

Statistical analyses

All calculations and tests were carried out in the statistical software environment R [6]. Significant differences between treatments were tested for using one-way anova (function aov() in R), followed by Tukey's honest significant differences (function TukeyHSD in R).

References

1. Kaplan, L. A., Wiegner, T. N., Newbold, J. D., Ostrom, P. H. & Gandhi, H. Untangling the complex issue of dissolved organic carbon uptake: a stable isotope approach. *Freshwater Biology* **53,** 855–864 (2008).
2. Wiegner, T., Kaplan, L., Newbold, J. & Ostrom, P. Synthesis of a C-13-labeled tracer for stream DOC: Labeling tulip poplar carbon with (CO2)-C-13. *Ecosystems* **8,** 501–511 (2005).
3. Hall, E. K. *et al.* Effects of resource chemistry on the composition and function of stream hyporheic biofilms. *Front. Microbio.* **3,** 35 (2012).
4. Berggren, M., Lapierre, J.-F. C. O. & del Giorgio, P. A. Magnitude and regulation of bacterioplankton respiratory quotient across freshwater environmental gradients. *ISME J* 1–10 (2011). doi:10.1038/ismej.2011.157
5. Fry, B. & Sherr, E. B. Delta-C-13 Measurements as Indicators of Carbon Flow in Marine and Fresh-Water Ecosystems. *Contributions in Marine Science* **27,** 13–47 (1984).
6. R Development Core Team. R: A language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org* (2014).

Table S1. Properties of the different DOM and inorganic nutrient additions in the experiment.

	Willow extract	Glucose	Algal extract	Recirculated stream water	NO_3 ($NaNO_3$)	PO_4 (KH_2PO_4)
Total organic C in stock solution (mg L^{-1})	148	4000.2	359.1	1.09	-	-
^{13}C atom %	9.35	1.09	1.09	1.08	-	-
Total N in stock solution (mg L^{-1})	2.80	-	78.2	NA	16479.5	-
Organic C: total N (molar ratio)	61.6	∞	5.4	NA	-	-
Avg. contribution to DOC conc. in medium (mg L^{-1})	0.88	0.44[a]	0.44[c]	1.09	-	-
Avg. contribution to N-NO_3 conc. in medium (µg L^{-1})	16.7	-	0.05[c]	1122.8	2187[b] /2091[c]	-
Avg. contribution to P-PO_4 conc. in medium (µg L^{-1})	13.1	-	6.82[c]	82.9	-	6.82[b]

[a] Only in Glucose treatment during priming phase
[b] Only in Glucose+N+P treatment during priming phase
[c] Only in Algal extract treatment during priming phase

Table S2. Metabolism of bulk dissolved organic carbon (without carbon pool partitioning using stable isotope signatures) and inorganic nutrients in the microcosms [a]

Treatment	Respiration (µg C h^{-1} g beads $^{-1}$)	DOC removal (µg C h^{-1} g beads $^{-1}$)	% input DOC respired (%)	% input DOC removed (%)	N-NO_3 removal (µg N h^{-1} g beads $^{-1}$)	P-PO_4 removal (ng P h^{-1} g beads $^{-1}$)
			Streamwater phase			
All (n=25)	0.0589 ±0.012	-0.000613 ±0.018	14.986 ±3.10	-0.0112 ±4.84	-0.00322 ±0.009	-1.21 ±0.59
			Pre-pulse phase			
All (n=20)	0.128 ± 0.037	0.14 ± 0.031	15.074 ±4.20	16.483 ±3.47	0.0223 ±0.0069	2.02 ±0.57
			Pulse phase			
Glucose	0.224 ±0.03 p=0.06	0.183 ±0.016 **p=0.05**	31.5 ±2.5 **p=0.0048**	25.7 ±1.1 p=0.14	0.0116 ±0.0042 p=0.97	-0.57 ±0.53 **p=0.0029**
Glucose+N+P	0.219 ±0.010 p=0.13	0.179 ±0.014 p=0.11	29.5 ±1.3 p=0.2	24.1 ±1.2 p=0.86	-0.0198 ±0.037 p=0.081	-0.736 ±0.56 **p=0.0012**
Algal extract	0.240 ±0.020 **p=0.0056**	0.184 ±0.0085 **p=0.034**	31.9 ±0.68 **p=0.0021**	24.6 ±1.9 p=0.6	0.0339 ±0.027 p=0.72	-1.0 ±0.7 **p=3e-04**
Control	0.189 ±0.015	0.161 ±0.0077	27.4 ±1.4	23.3 ±2.1	0.0184 ±0.0032	1.04 ±0.59
			Post-pulse phase			
Glucose	0.208 ±0.021 p=0.94	0.139 ±0.014 p=0.21	28.7 ±1.3 p=0.98	19.3 ±2.3 p=0.18	-0.0139 ±0.0040 p=0.94	-0.761 ±0.23 p=0.89
Glucose+N+P	0.220 ±0.015 p=0.37	0.149 ±0.024 p=0.069	29.6 ±1.7 p=0.5	19.9 ±1.8 p=0.1	-0.0125 ±0.0059 p=1	-0.819 ±0.27 p=0.99
Algal extract	0.222 ±0.023 p=0.29	0.12 ±0.027 p=0.84	30.2 ±1.3 p=0.16	16.3 ±3.2 p=0.92	-0.0116 ±0.0075 p=1	-0.812 ±0.124 p=0.98
Control	0.201 ±0.0075	0.107 ±0.032	28.4 ±0.63	15 ±4.5	-0.0121 ±0.0024	-0.869 ±0.29

[a] P-values refer to differences of the labile DOM treatments to the control treatment (ANOVA). Significance ($p<0.05$) is indicated by bold font.
[b] Values refer to the last 72 hours of the stream water phase

Table S3. Metabolism of willow DOM[a]

Treatment	Willow-derived respiration (µg C h^{-1} g beads $^{-1}$)	Willow DOC removal (µg C h^{-1} g beads $^{-1}$)	% input willow DOC respired (%)	% input willow DOC removed (%)	Priming effect (µg C h^{-1} g beads $^{-1}$)
		Pulse phase			
Glucose	0.130 ±0.012 p=1	0.164 ±0.015 p=0.82	62.4 ±1.9 p=0.19	78.5 ±2.4 p=1	0.000931 ±0.012 p=1
Glucose+ N+P	0.137 ±0.0091 p=0.6	0.156 ±0.021 p=0.98	68.9 ±2.8 p=1	77.7 ±5.3 p=1	0.00815 ±0.0091 p=0.6
Algal extract	0.139 ±0.013 p=0.43	0.143 ±0.036 p=0.97	70.8 ±6.6 p=0.8	72.1 ±13 p=0.47	0.0101 ±0.013 p=0.43
Control	0.129 ±0.0060	0.15 ±0.020	68.3 ±4.8	78.7 ±3.6	-
		Post-pulse phase			
Glucose	0.175 ±0.015 p=1	0.142 ±0.024 p=0.7	103 ±15 p=0.56	82.4 ±1.8 p=1	0.00198 ±0.015 p=1
Glucose+ N+P	0.185 ±0.017 p=0.62	0.134 ±0.024 p=0.38	106 ±17 p=0.38	75.7 ±7.2 **p=0.05**	0.0114 ±0.017 p=0.62
Algal extract	0.182 ±0.017 p=0.78	0.153 ±0.028 p=0.98	99.1 ±12 p=0.8	82.2 ±2.2 p=0.99	0.00884 ±0.017 p=0.78
Control	0.173 ±0.0093	0.159 ±0.019	91.1 ±11	82.9 ±1.8	-

[a] P-values refer to differences of the labile DOM treatments to the control treatment (ANOVA). Significance ($p<0.05$) is indicated by bold font.

Table S4. Metabolism of non-willow carbon pools (added labile DOM, background streamwater DOM and biofilm OM)[a]

Treatment	Respiration (μg C h^{-1} g beads $^{-1}$)	DOC removal (μg C h^{-1} g beads $^{-1}$)	% input DOC respired (%)	% input DOC removed (%)
		Pulse phase		
Glucose	0.09 ±0.02 **p=0.004**	0.019 ±0.004 p=0.91	18.7 ±3.6 **p=0.0001**	3.81 ±0.92 p=0.92
Glucose+ N+P	0.0817 ±0.0068 p=0.08	0.023 ±0.009 p=0.74	15.1 ±1.59 p=0.14	4.43 ±1.9 p=0.81
Algal extract	0.1 ±0.009 **p=0.001**	0.041 ±0.032 p=0.13	18.2 ±1.07 **p=0.002**	7.63 ±6.22 p=0.18
Control	0.060 ±0.01	0.011 ±0.022	11.9 ±1.63	2.19 ±4.54
		Post-pulse phase		
Glucose	0.032 ±0.011 p=0.85	-0.0035 ±0.03 p=0.18	5.91 ±1.9 p=0.96	-0.67 ±5.36 p=0.18
Glucose+ N+P	0.035 ±0.010 p=0.56	0.015 ±0.025 **p=0.043**	6.33 ±2.0 p=0.81	2.42 ±3.9 p=0.055
Algal extract	0.034 ±0.009 p=0.19	-0.034 ±0.034 p=0.84	7.26 ±1.32 p=0.31	-6.48 ±8.1 p=0.81
Control	0.028 ±0.005	-0.053 ±0.047	5.42 ±1.01	-10.6 ±10.2

[a] P-values refer to differences of the labile DOM treatments to the control treatment (ANOVA). Significance ($p<0.05$) is indicated by bold font.

Table S5. Biomass total carbon and willow DOM-derived carbon content[a]

Treatment	Total biomass (µg C g beads $^{-1}$)	Willow-derived biomass (µg C g beads $^{-1}$)	Biomass increase during experiment (µg C g beads $^{-1}$)
Glucose	114 ± 34.7 p= 0.23	14.2 ± 6.3 p= 0.49	42.5 ± 34.7 p= 0.052
Glucose+N+P	103 ± 23.3 p= 0.65	13.6 ± 3.53 p= 0.69	31 ± 23.3 p= 0.23
Algal extract	91.7 ± 11 p= 0.97	13.1 ± 1.96 p= 0.79	19.9 ± 11 p= 0.64
Control	83 ± 17.7	10.6 ± 2.19	11.3 ± 17.7 p= 0.93
Before	71.7 ± 19.3 p= 0.93	-	-

[a] P-values refer to differences of the labile DOM treatments to the control treatment (ANOVA), except for biomass increase, where all treatments are compared to the before biomass.

Supplementary figures

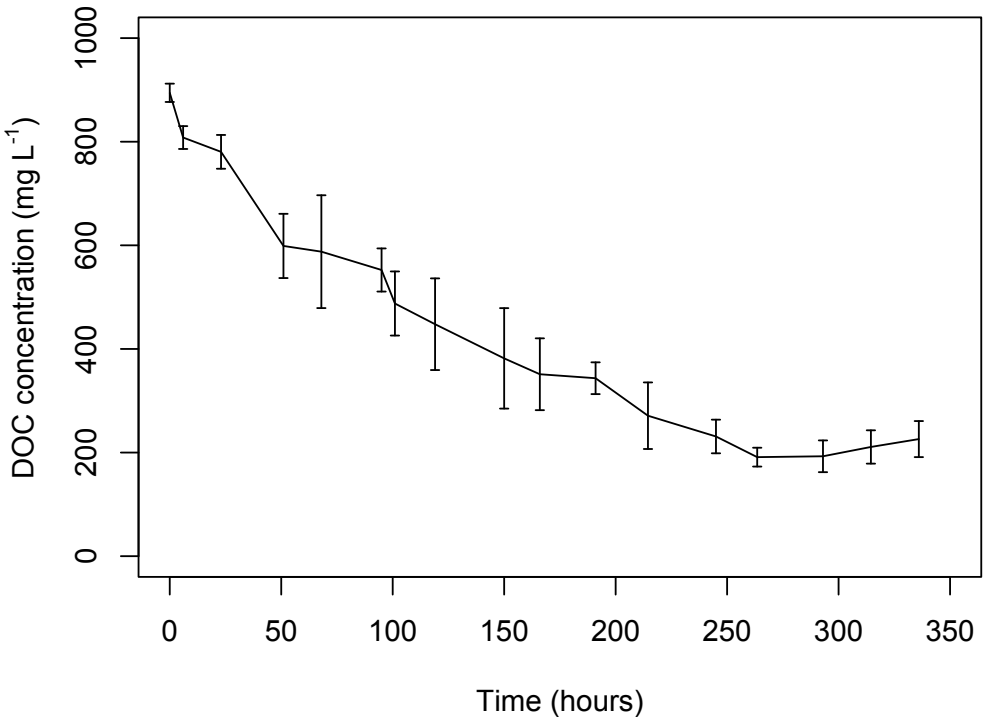

Figure S1: Degradation of *Salix fragilis* (willow) extract to produce a model stream al-DOM substrate. The error bars represent ± 1 standard deviation of three replicate degradation bioreactors.

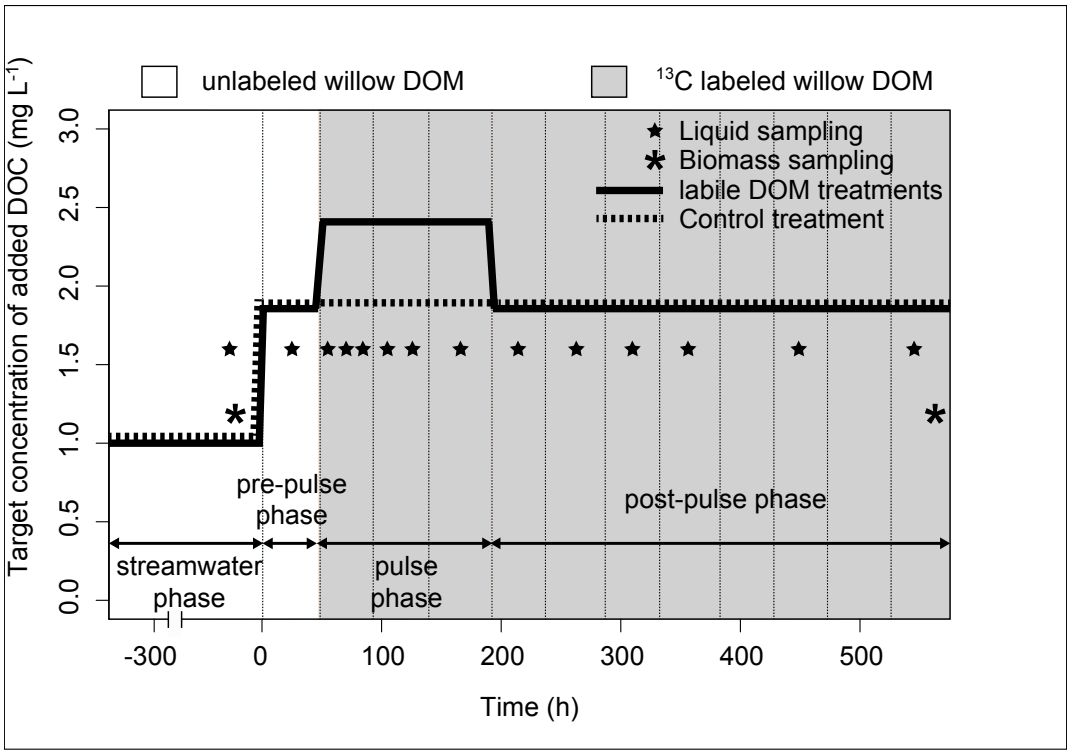

Figure S2: The timeline and different phases of the experiment. The stars indicate sampling points of liquid samples while the asterisks indicate sampling of biomass. The dotted vertical lines indicate the timings of bottle changes.

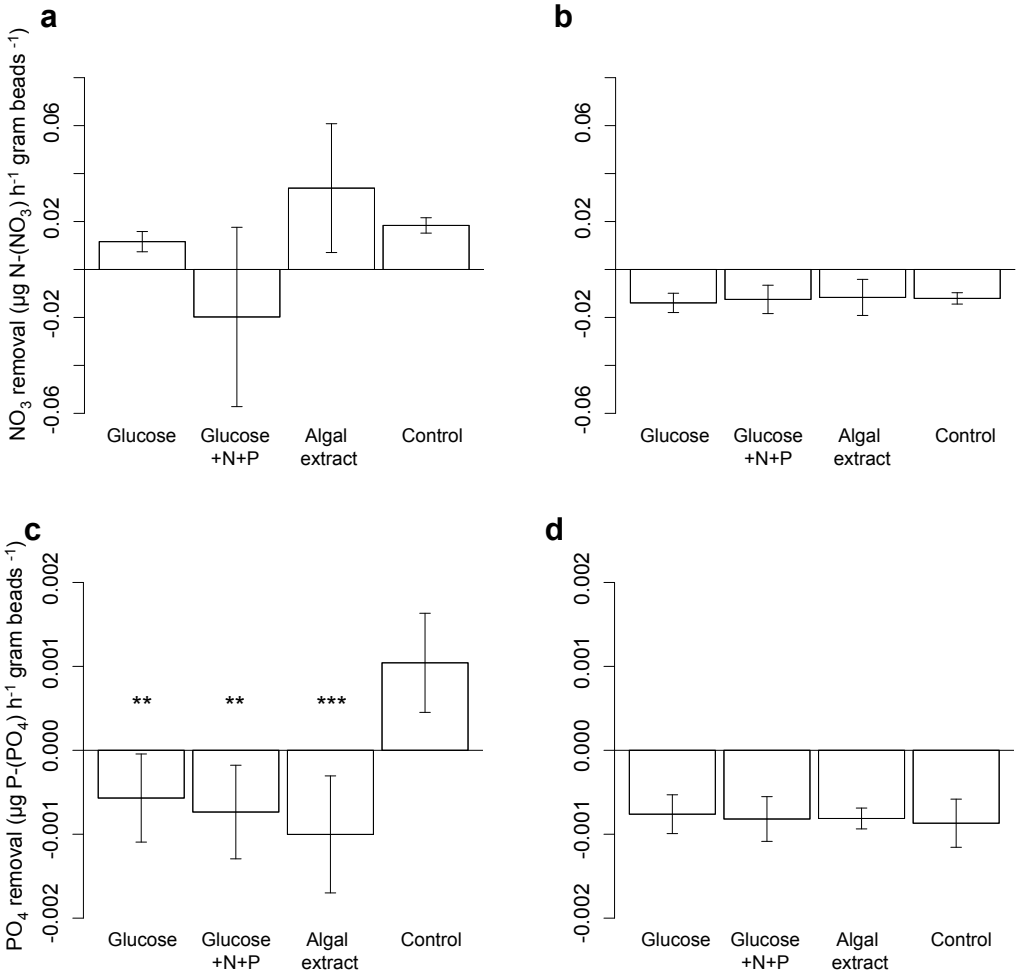

Figure S3: The removal and release of inorganic nutrients in the microcosms. The bars represent removal of NO_3 (**a** & **b**) and PO_4 (**c** & **d**) during the pulse phase (**a** & **c**) and the post-pulse phase (**b** & **d**). Negative removal corresponds to a net release of inorganic nutrients. The error bars represent ± 1 standard deviation. The stars over the bars indicate the significance of the difference of the labile DOM treatments compared to the control using one-way ANOVA (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$).