Supplementary material: Biofilm-specific uptake does not explain differences in whole-stream DOC tracer uptake between a forest and an agricultural stream

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Supplementary material

Solute travel time distributions

To characterize the transport characteristics in the streams, we analyzed the solute travel time distribution (TTD) which describes hydrologic conditions and the morphological setup. Different from slug injections, the TTD of continuous tracer injections cannot be directly inferred from the breakthrough curve of the tracer. As transport of solutes in streams can be conceptualized as a linear time-invariant system, it is possible to obtain the TTD from the transfer function $g(\tau)$ from the measured upstream C_{up} and downstream C_{down} concentration time series:

$$C_{down}(t) = \int_0^t g(\tau) c_{up}(t-\tau) d\tau$$

Estimating $g(\tau)$ directly from observed data requires regularization as otherwise high frequency noise would be amplified yielding results that are physically not meaningful. We applied a projected gradient method with smoothness constraints, which ensures smoothness and non-negativity of the estimated transfer functions.

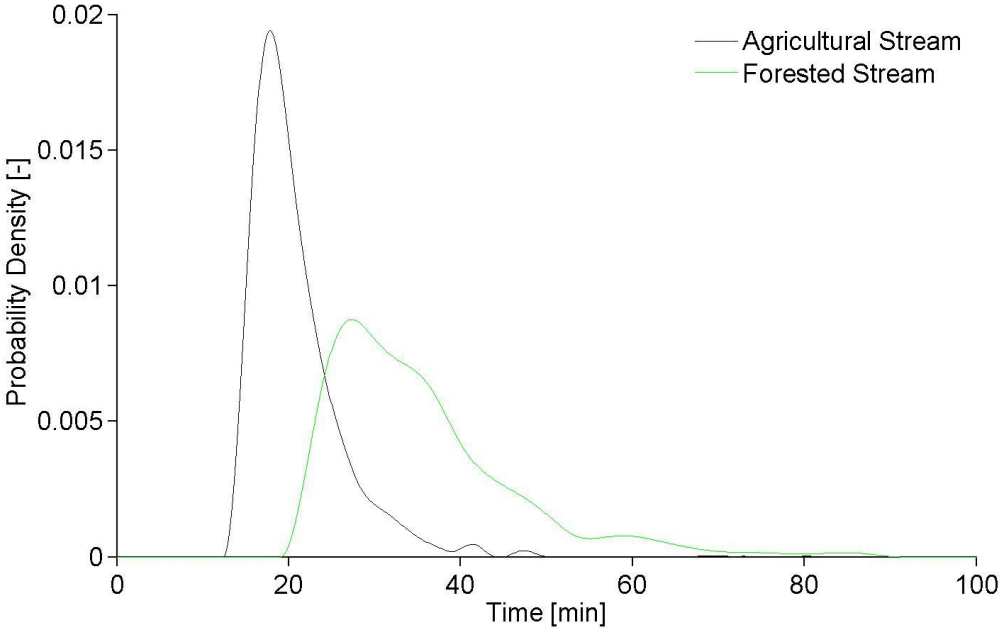

Fig. S1. Travel time distributions of the two investigated streams.

Biofilm metabolism

Biofilm metabolism was measured as dissolved oxygen (DO) production and consumption with a flow-through chamber benchtop system at the field site. Before measurement, the sampling water was filtered (Whatman GF/F), aerated for 10 min and subsequently equilibrated to avoid oversaturation. The water was pumped at 1 mL min^{-1} through the flow-through chambers and DO concentration was measured at the outflow via optical sensors (PreSens FTC, PreSens, Regensburg, Germany). The chambers were placed in a styrofoam box filled with cooled stream water to maintain constant stream water temperatures and to allow to keep the system submerged to avoid air-bubbles inside the measurement system. The styrofoam box was darkened with black tape and an LED panel was embedded inside the lid (at an height of 28 cm above the metabolism chambers). For the measurements, five tiles with biofilms and one blank tile were placed inside the flow through chambers. Measurements were performed at dark conditions to measure the O_2 consumption (respiration) of the biofilm community and at light conditions (377 µE m^{-2} s^{-1} PAR) to measure the O_2 production by autotrophs. The measurements were terminated when oxygen concentration reached equilibrium for at least 10 min (with measurement intervals of 30 sec). Prior to the measurements the offset of every channel to the reference channel was determined in measurements at dark and light conditions with blank ceramic tiles inside each chamber. The areal biofilm metabolism was calculated as follows for dark and light condition measurements, respectively:

$$\Delta DO_{areal} = \frac{(DO_{BF} - DO_{Blank} - offset) * Q}{a}$$

with Δ*DO* [mg O_2 L^{-1}] as the change in oxygen concentration compared to blank measurements and corrected by the offset, with *Q* as a flow-through rate (mL min^{-1}) and with a as the area of the biofilm tiles. Before the calculations, the measured DO was corrected by temperature fluctuations and the air pressure. After the measurements, biofilm biomass on the tiles was determined as described in the methods section and the next sections.

Biofilm bacterial production

After storage of samples at 4 °C until the next day, tiles of 1 cm^2 surface area were transferred to scintillation vials and covered with 4 ml sterile-filtered stream water. Triplicate aliquots and one formalin-treated control (3.7%, final concentration) were spiked with ^{14}C-leucine (12.2 MBq μmol^{-1}, Sigma, 5 µM final concentration). Samples were incubated in the laboratory at in situ temperature for one hour in the dark under continuous shaking. Incorporation was stopped with formalin, and 0.6 ml 50% trichloracetic acid (TCA) was added. Proteins were extracted for 15 min on ice. Biofilms were removed from stones by ultrasonication for 1 min (20 kHz, 20%; HTU Soni130, Heinemann, Germany). Tiles were removed and rinsed, and the supernatant was filtered onto 0.2 µm Nuclepore membranes. Filters were rinsed twice with 1 ml 5% TCA and once with 80% ethanol. After dissolving the filters in 0.5 ml Soluene (Packard) and adding 2.5 ml Hionic Fluor (Packard) to each scintillation vial, radioactivity was measured using a Liquid Scintillation Analyzer (2300 TR, Packard). The external standard ratio method was used for quenching. Carbon production was calculated using the equations of Simon and Azam (1989).ß

OTU richness of biofilm bacteria and algae

Biofilms were scraped off the tiles for DNA extraction using the NucleoSpin Plant II kit (Macherey Nagel GmbH, Düren, Germany) following the manufacturer's recommendations. Selected marker genes were amplified by polymerase chain reaction using a Verity 96-well thermal cycler (Applied Biosystems Inc., Foster City, CA, USA): Bacterial 16s ribosomal DNA (rDNA) was partially amplified with the primer pair 27f (AGA GTT TGA TCM TGG CTC AG) and 907r (CCG TCA ATT CMT TTG AGT TT) (Lane 1991). For algae, 18s rDNA was partially amplified using the primer pair P73 (forward; AAT CAG TTA TAG TTT ATT TGR TGG TAC C) and P47 (reverse; TCT CAG GCT CCC TCT CCG GA) (Dorigo et al. 2002). Forward primers were tagged with fluorescent dye HEX for later sizing fragments. Each 20 µL PCR mix consisted of 10 µL PhireHotStart II master mix (Thermo Scientific™), 1 µL of each primer (5 nM stock solution), 0.6 µL DMSO, 6.9 µL HPLC-grade water and 1 µL of DNA template (diluted 1:20 with HPLC-grade water). The thermal protocol comprised of an initial denaturation step (95°C, 60

sec), 35 cycles of denaturation (95°C, 15 sec), annealing (52°C for bacteria and 54°C microalgae, 10 sec) and elongation (72°C, 15 sec) followed by a final extension step (72°C, 60 sec). Ten µL of crude PCR product were digested with MspI (FastDigest™, Thermo Scientific™) restriction enzyme in 30 µL reactions for ten minutes and precipitated by adding 90 µL ethanol (96%) and 7.5 µL 150 mM EDTA followed by 15 min incubation at room temperature and centrifugation for 30 min (2,500 g). Supernatants were discarded; DNA pellets were washed with 70 µL ethanol (70%) and centrifuged for 10 min (2,500 g). Supernatants were discarded, and DNA pellets were air-dried in the dark. DNA was denatured with 20 µL HiDiformamide (Life Technologies Corp.) supplemented with 0.2 µL GeneScan 500 ROX size standard (Life Technologies Corp.) at 90°C for 2 min immediately followed by chilling (0°C, 1 min). Samples were analyzed on an ABI prism 3130 automated capillary sequencer (Applied Biosystems Inc., Foster City, CA, USA). To validate sequencing results, each sample was analyzed in technical duplicate. Restriction fragments were sized using the GeneMapper 4.0 software (Applied Biosystems Inc., Foster City, CA, USA) and exported in data formats appropriate for fingerprinting analyses. Terminal restriction fragment (T-RF) datasets were imported into the T-REX online software (Culman et al. 2009). The noise was filtered from raw data using a threshold of 1.2 applied to peak height. T-RFs were aligned with a threshold of 0.25, and relative T-RF abundances were inferred from peak heights previously normalized over all samples. T-RFs occurring in 10% of samples were omitted to reduce data complexity. Number of operational taxonomic units (OTUs) was used to describe bacterial or algal OTU richness in a sample

Permutational ANOVA on the differences between biofilm characteristics within different experiment parts

Table S1. Significant results or trends (results with $p > 0.1$ are not shown) of the permutational multivariate analysis of variance (PERMANOVA) of biofilm structure in streams and flumes, assessing effects stream type (forest or agricultural stream), placement of the tiles (stream or flume), and treatment effect on biofilm structure (before or after DOC tracer addition). Non-significant comb. = Non-significant main effects or factor interactions, **$p < 0.01$, *** $p < 0.001$.

Factor	Df	F	R^2	p
Stream type (ID)	1	32.3	0.37	0.001***
Placement of tiles (P)	1	6.0	0.07	0.001***
ID x Treatment	1	1.3	0.05	0.004**
ID x P	1	2.3	0.03	0.051
ID x P x Treatment	1	2.0	0.02	0.059
Non-significant combinations		< 1.9	0.12	> 0.1
Residuals	30		0.34	
Total	45		1	

tDOC stable isotope and uptake calculations

Stable isotope data were measured as the relative difference between ratios of the sample and the PeeDee Belemnite standard as

$$\delta^{13}C_{sample}\ [‰] = [(^{13}C/^{12}C_{Sample} / {}^{13}C/^{12}C_{Standard})\text{-}1]x10^{3} \quad \text{eq. 1}$$

Isotope data of enriched samples was expressed as the ^{13}C fraction of the sample, where $R(\frac{13C}{12C})_{Standard}$ is the isotope-number ratio of the standard (Coplen 2011):

$$x(^{13}C)_{Sample} = \frac{1}{1+\frac{1}{(\delta^{13}C_{Sample}+1)\times R(\frac{13C}{12C})_{Standard}}} \quad \text{eq. 2}$$

To correct for background values based on upstream or control flume measurements, we calculated the excess ^{13}C fraction as:

$$x(^{13}C) = x(^{13}C)_{Sample} - x(^{13}C)_{Background} \quad \text{eq. 3}$$

Background-corrected average tracer flux of ^{13}C in was calculated following Mulholland et al. (2004) based on the excess ^{13}C fraction, DOC concentration and discharge (Q) with values from the control flumes or upstream stations, respectively:

$$^{13}C_{Flux} = x(^{13}C) \times DOC\ concentration \times Q \quad \text{eq. 4}$$

We calculated whole-stream DOC spiraling metrics as described in Mulholland (2004). Briefly, uptake length of DOC (S_W, m) was calculated as the inverse of the slope of ln($^{13}C_{Flux}$) versus distance from the ^{13}C-DOC injection. Whole-stream tDOC tracer uptake rate (U_{tot}, mg m^{-2} d^{-1}) was calculated as:

$$U_{tot} = \frac{^{13}C_{Flux}\times k_{tot}}{w} \quad \text{eq. 5}$$

where k_{tot} is the fractional uptake rate between each downstream station and w is the average stream wetted width. The mass transfer velocity (V_f) was calculated by dividing U_{DOC} by the average DOC concentration.

Uptake of the tDOC tracer into flume and stream biofilms was calculated following Mulholland (2004). We first calculated the tracer standing stock in the biofilm biomass ($^{13}C_{Biofilm}$, mg ^{13}C m^{-2}):

$$^{13}C_{Biofilm} = Biomass \times (\%C/100) \times Biofilm_{Excess} \quad \text{eq. 6}$$

where *Biomass* (mg dry weight m^{-2}) is the epilithon standing stock, *%C* C elemental concentrations (%) and $Biofilm_{Excess}$ the excess ^{13}C fraction of biofilms. Subsequently, we calculated C_{flux} (mg DOC s^{-1}) as

$$C_{Flux} = DOC \times Q \quad \text{eq. 7}$$

where DOC is DOC concentration (mg L^{-1}) and Q is discharge (L s^{-1}) at plateau samplings.

DOC uptake into biofilms (U_b, mg DOC m^{-2} d^{-1}) was then calculated as

$$U_b = \frac{^{13}C_{Biofilm}}{(^{13}C_{Flux}/C_{flux})} \quad \text{eq. 8}$$

Mineralization of tDOC was calculated analogous to S_w of ^{13}C-DOC but taking the slope of ln ^{13}C-DIC flux versus distance from the injection point. Based on this, we calculated a mineralization rate (mg ^{13}C-DIC m^{-2} d^{-1}) analogous to eq. 5 , expressing the release of ^{13}C-DIC from our tDOC tracer per unit stream bed and time.

Benthic substrates

Table S2. Mean percentage coverage of benthic substrates estimated from : 13 cross sections in the forest stream reach and 17 cross sections in the agricultural stream reach. Fine sediment was a mix of mineral and organic material.

Substrate	Forest	Agriculture
Sand	7	6
Gravel	41	53
Stones	42	17
Coarse woody debris / roots	7	0
Coarse particulate organic matter	3	1
Fine sediment	0	7
Macrophytes	0	16

tDOC addition and SI enrichment success

Fig. S2. A self-made diffusor ensured that isotopically enriched tDOC tracer solution was completely mixed with stream water after passing the mesocosm flumes.

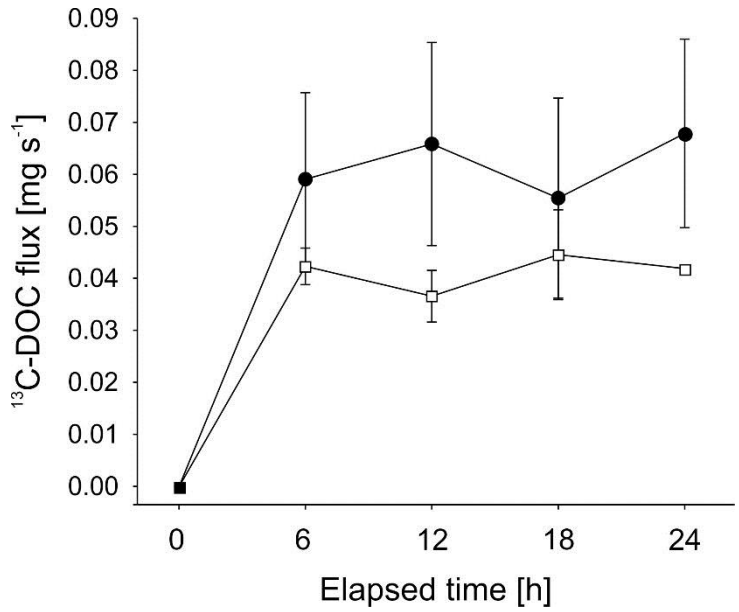

Fig. S3. Background-corrected ^{13}C-DOC tracer flux in the flumes of the forest stream (dots) and the agricultural stream (rectangles). Shown are means ±1SD from triplicated measurement conducted during the 24 hours of additions. The plot indicates constant tDOC label fluxes over time.

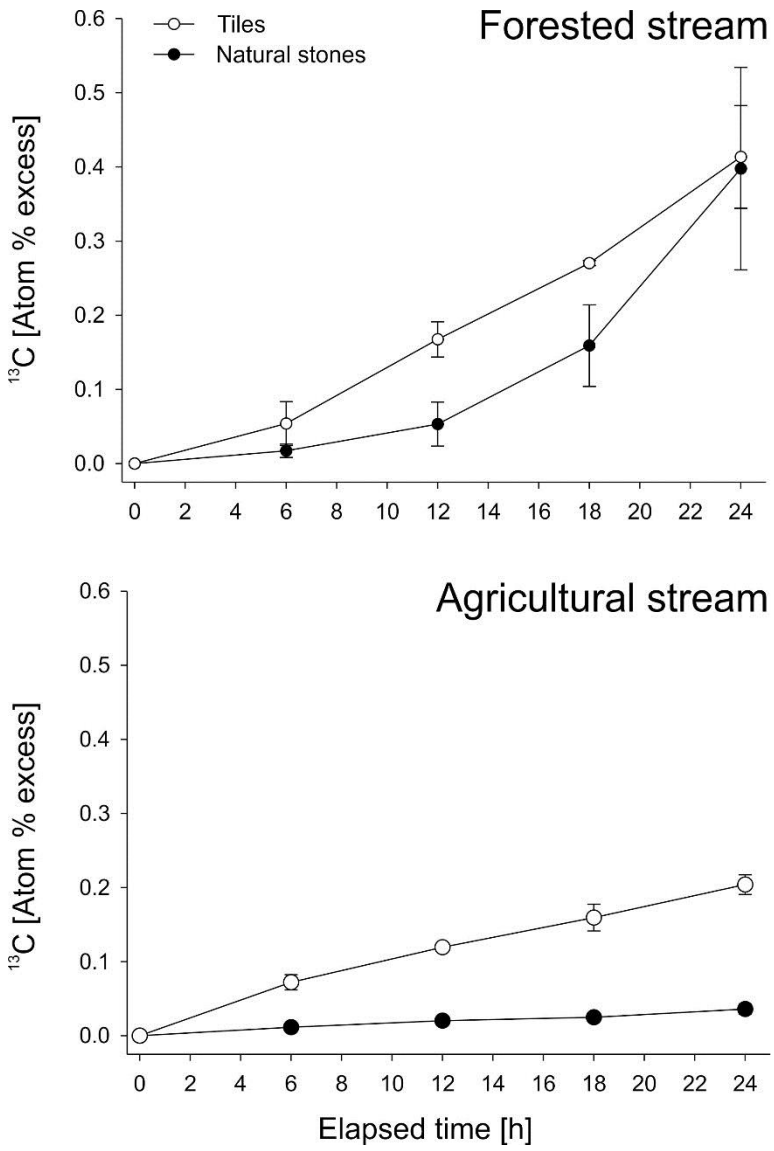

Fig. S4. Temporal development of ^{13}C concentrations in biofilms from experimental tiles and natural pebbles in the studied forested and agricultural stream

Table S3. Summary of the linear models of natural ln-transformed, background-corrected ^{13}C-DOX fluxes against distance downstream from the release for each stream and replicate. * p = 0.01 – 0.05; *** p < 0.001

Stream	Replicate	k_{tot} [m^{-1}]	Std. err. of k_{tot} [m^{-1}]	Adjusted R^2
Agriculture	1	$1.0*10^{-3}$	$3.5*10^{-4}$	0.59*
	2	$1.8*10^{-3}$	$4.2*10^{-4}$	0.73*
	3	$1.9*10^{-3}$	$4.9*10^{-4}$	0.71*
Forest	1	$4.0*10^{-3}$	$3.9*10^{-4}$	0.94***
	2	$3.6*10^{-3}$	$3.8*10^{-4}$	0.93***
	3	$3.8*10^{-3}$	$4.7*10^{-4}$	0.92***

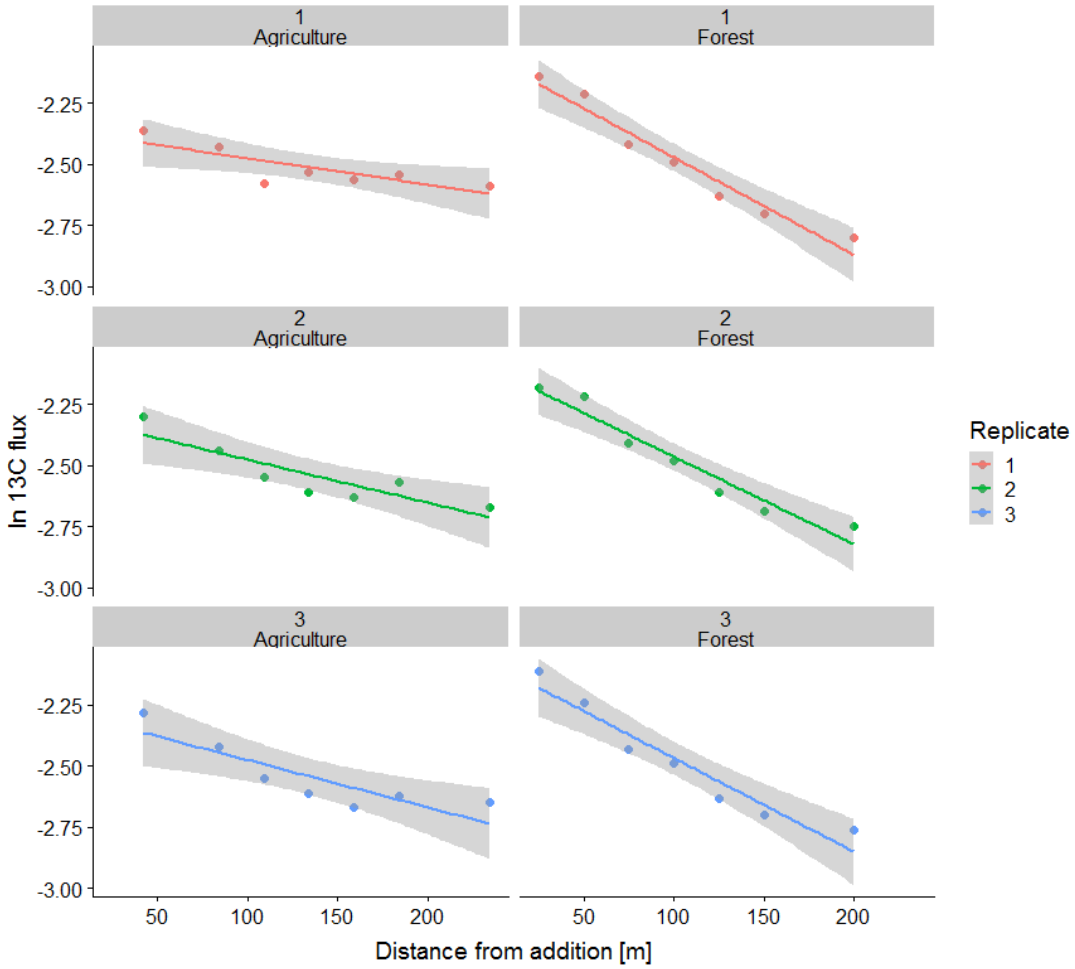

Fig. S5. Regressions of natural ln-transformed, background-corrected ^{13}C-DOX fluxes against distance downstream from the release for each stream (Agriculture or Forest) and replicate (1, 2, or 3), slopes of which are k_{tot}.

References

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