Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supplementary Information

Using recirculating flumes and a response surface model to investigate the role of hyporheic exchange and bacterial diversity on micropollutant half-lives

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Pictures of the flume setup

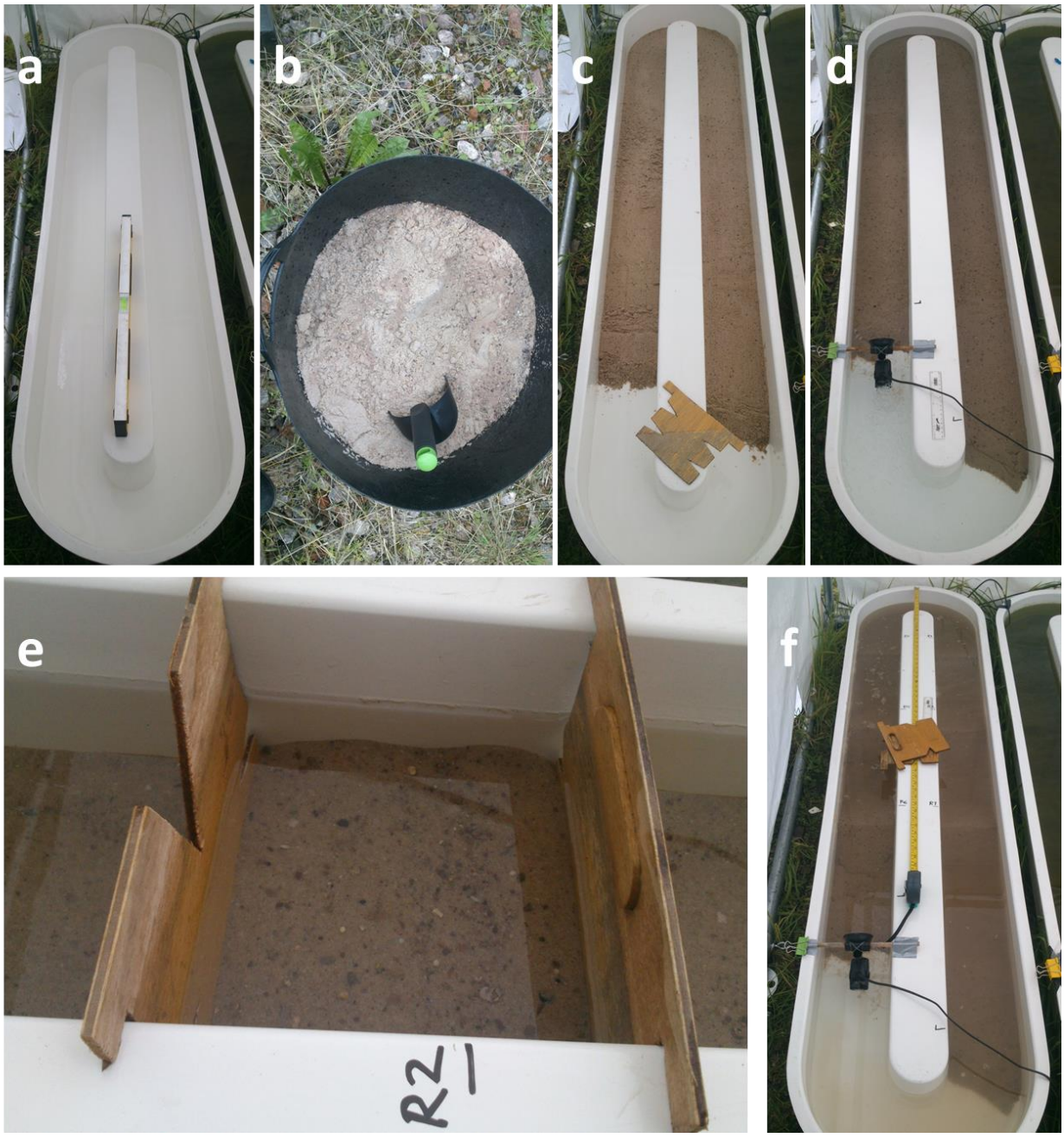

Figure S 1. Photos of the flume setup procedure and formation of bedforms: (a) placing the acid-rinsed flume inside the tent and leveling it; (b) mixing the Inocula with the sand in acid-rinsed containers; (c) distributing the sediment-mix inside the flume and leveling the sediment to 3.5 cm height, as in treatments B0; (d) filling the flume with di-ionised water, installing the pump and switching on the flow; (e) at day -3 formation of bedforms using custom-made wooden plates; (f) flume ready for injection.

Chemicals and Reagents

Nutrient solutions

Table S 1. Composition of the three nutrient solutions added to all flumes throughout the experiment.

substance	mass added per flume	target concentration	vendor
	N1 (addition day -12)		
$CaCl_2$	15285 mg	254.7 mg L^{-1}	Fisher scientific UK
$NaHCO_3$	1498 mg	25.0 mg L^{-1}	Sigma-Aldrich
KCl	2722 mg	45.4 mg L^{-1}	BDH VWR Chemicals
$MgSO_4*7H_2O$	6583 mg	109.7 mg L^{-1}	BDH VWR Chemicals
Na_2SO_4	6852 mg	114.2 mg L^{-1}	Fisher scientific UK
KH_2PO_4	125 mg	2.1 mg L^{-1}	Fisher scientific UK
NH_4Cl	1719 mg	28.6 mg L^{-1}	BDH VWR Chemicals
$C_6H_{12}O_6$	9000 mg	150.0 mg L^{-1}	Fisher scientific UK
$MnCl_2*4H_20$	472 mg	7.9 mg L^{-1}	Sigma-Aldrich
$NaNO_3$	9289 mg	154.8 mg L^{-1}	Fisher scientific UK
$CuSO_4$	58 µg	0.96 µg L^{-1}	VWR International
Na_2SeO_3	23 µg	0.38 µg L^{-1}	VWR International
$Na_2Mo_4*H_2O$	62 µg	1.03 µg L^{-1}	VWR International
$Na_2WO_4*2H_2O$	178 µg	2.97 µg L^{-1}	VWR International
$NiCl_2$	163 µg	2.72 µg L^{-1}	VWR International
H_3BO_3	360 µg	6 µg L^{-1}	VWR International
$ZnCl_2$	430 µg	7.16 µg L^{-1}	VWR International
$CoCl_2$	324 µg	5.4 µg L^{-1}	VWR International
ribovlavin	30 µg	0.5 µg L^{-1}	VWR International
biotin	300 µg	5 µg L^{-1}	VWR International
folic acid	300 µg	5 µg L^{-1}	VWR International

nicotinic acid	300 µg	5	µg L^{-1}	VWR International
pantothenic acid	300 µg	5	µg L^{-1}	VWR International
pyridoxal-HCl	300 µg	5	µg L^{-1}	VWR International
thiamine	300 µg	5	µg L^{-1}	VWR International
choline chloride	300 µg	5	µg L^{-1}	VWR International
myoinositol	600 µg	10	µg L^{-1}	VWR International
vitmain B12	1 µg	0.01	µg L^{-1}	VWR International
lipoic acid	376 µg	6.26	µg L^{-1}	VWR International
p-aminobenzoic acid	376 µg	6.26	µg L^{-1}	VWR International
	N2 (addition day 10)			
NH_4Cl	2315.6 mg	38.6	mg L^{-1}	BDH VWR Chemicals
	N3 (addition day 46)			
NH_4NO_3	690.88 mg	11.5	mg L^{-1}	n.a.
KH_2PO_4	66.72 mg	1.1	mg L^{-1}	Fisher scientific UK

Target micropollutants

Table S 2. The 31 trace organic micropollutants spiked to the flumes at day 0. Highlighted in grey are the artificial sweetener acesulfame (ACS) and the anti-epileptic carbamazepine (CBZ), which were chosen as model compounds for the present study. Pharmaceuticals were purchased from Sigma-Aldrich (Darmstadt, Germany) and Toronto Research Chemicals Inc. (North York, Canada). Compound purities were >98% with the exception of Sitagliptin (96.4%).

Vendor	**Compound name**	**Product code**	**purity**		**Concentration in spiking solution mg/L**
Sigma	Acesulfame	PHR1266-500MG	0.9996		200.98
Sigma	Acetaminophen	A3035-1VL	1.013		199.56
TRC	Amisulprid	A633250	0.98		200.90
Sigma	Atenolol	74827-100MG	0.988	HPLC	199.58
TRC	Benproperine	B161500	0.98		200.96
Sigma	Benzotriazol	B11400-100G	1		200.00
Sigma	Bezafibrate	72516-500MG	0.995	HPLC	200.00
Sigma	Carbamazepine	94496-100MG	0.998		198.60
TRC	Celiprolol	C254500	0.98		200.28
Sigma	Citalopram	PHR1640-1G	0.998		198.89
Sigma	Clofibric acid	90323-100MG	1		202.00

Sigma	Diclofenac	D6899-10G	1	TLC	200.14
Sigma	Flecainid	F6777-25MG	0.985	TLC	199.60
Sigma	Fluoxetine	PHR1394-1G	0.9995		200.28
Sigma	Furosemide	F4381-1G	1		201.00
Sigma	Gemfibrozil	91823-100MG	1		200.00
Sigma	Hydrochlorothiazide	H4759-5G	1		200.00
Sigma	Ibuprofen	I4883-1G	1	GC	201.00
TRC	Irbesartan	I751000	0.98		198.94
Sigma	Ketoprofen	34016-100MG-R	0.999		199.80
TRC	Metaxalone	M225850	0.98		200.90
Sigma	Metformin	PHR1084-500MG	0.998		200.03
Sigma	Metoprolol	M5391-1G	1.004	titration	199.91
TRC	Naproxen	N377526	0.98		199.92
Sigma	Propranolol	P0884-1G	1		199.90
Sigma	Sitagliptin	PHR1857-1G	0.964		200.33
Sigma	Sotalol	S0278-25MG	0.995		199.20
Sigma	Sulfamethoxazole	31737-250MG	0.998		199.60
Sigma	Sulpiride	S2190000	1		201.00
Sigma	Valsartan	PHR1315-1G	0.989		199.78
Sigma	Venlafaxin	Y0000587	1		198.87

Background conditions

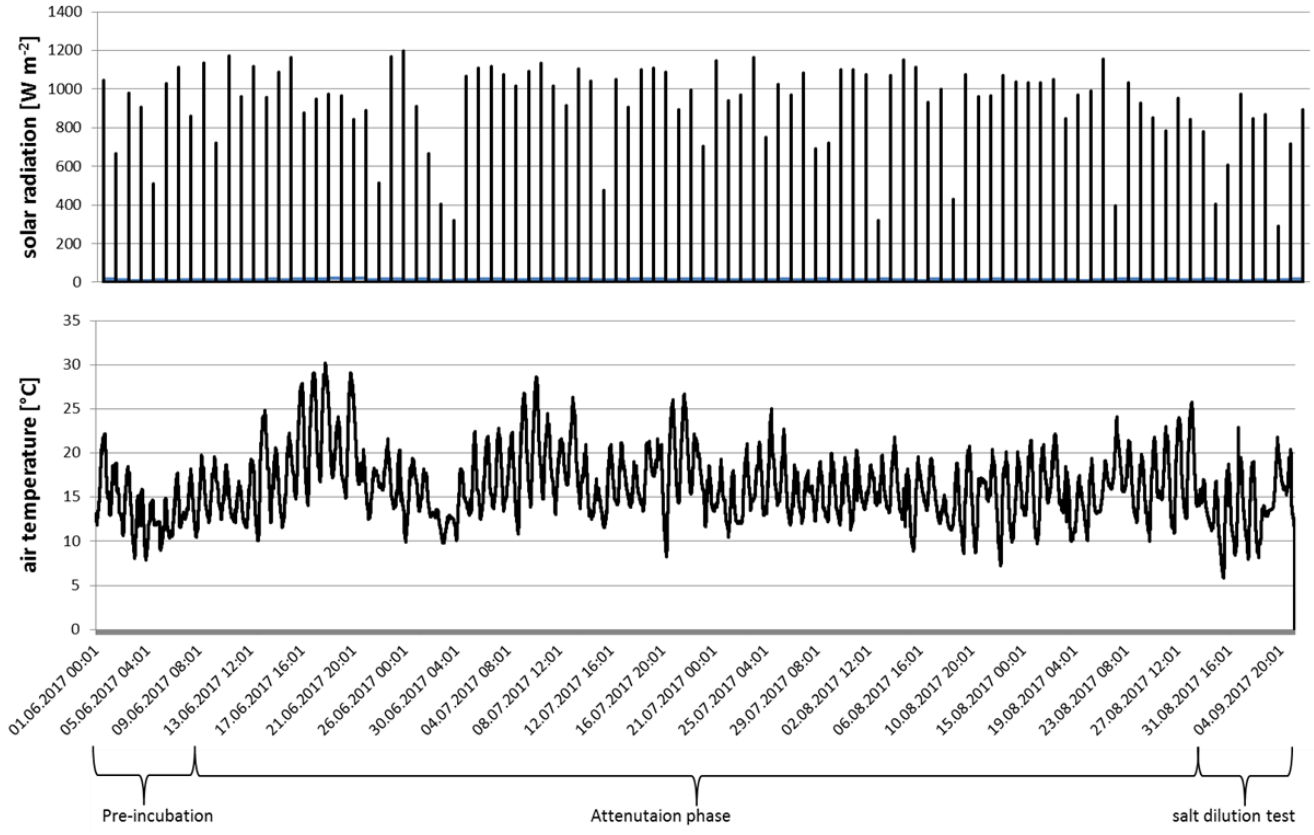

Figure S 2. Solar radiation [W m^{-2}] and air temperature [° C] measured at a weather station approximately 400 m from the experimental site throughout the experimental period (01.06.2017 - 04.09.2017).

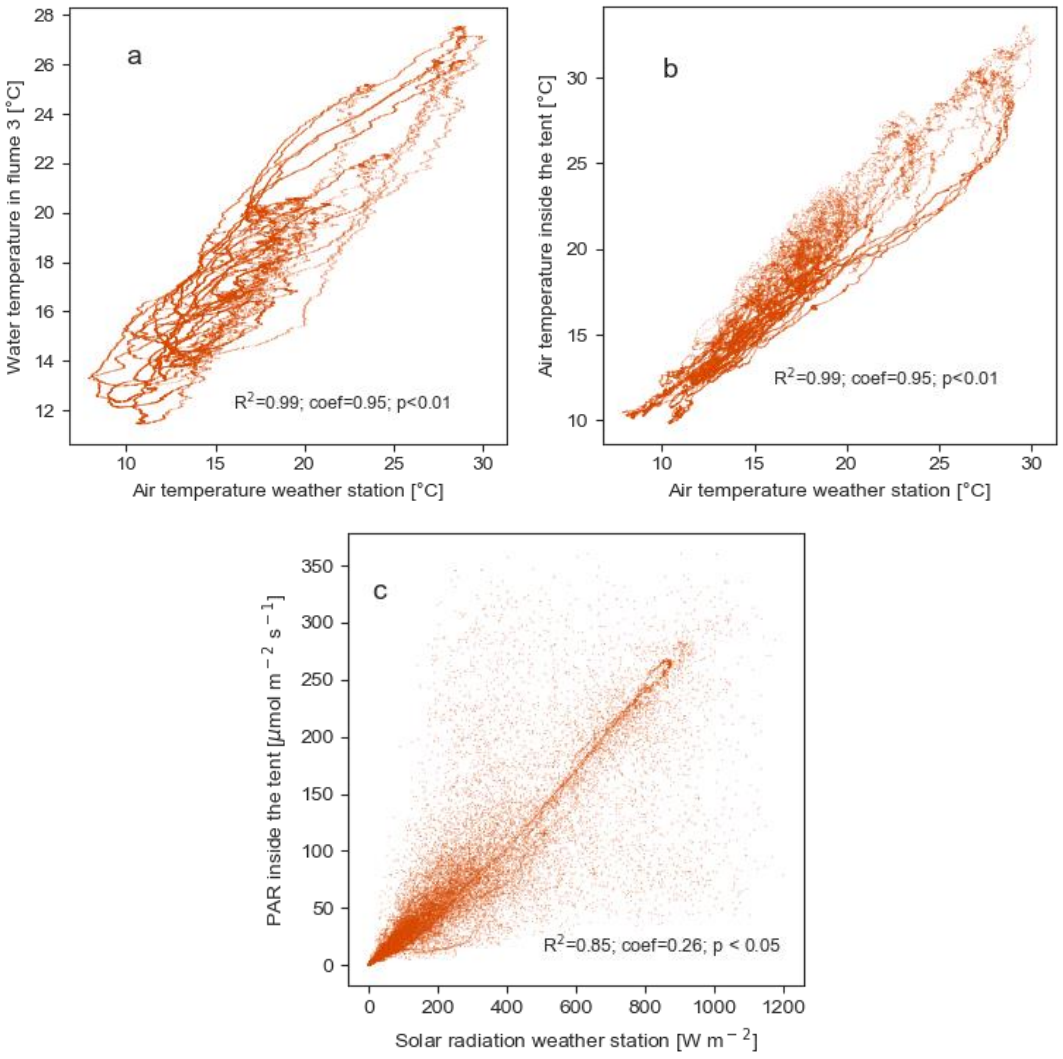

Figure S 3. Linear regression of air temperature measured at the weather station (400 m from experimental site) and water temperature in the flumes (a; R^2=0.99; coef=0.95, p<0.01) and air temperature inside the tent (b; R^2=0.99; coef=0.95, p<0.01), respectively. Linear regression of solar radiation measured at a weather station and PAR measured inside the tent (c; R^2=0.85; coef=0.26; p<0.01).

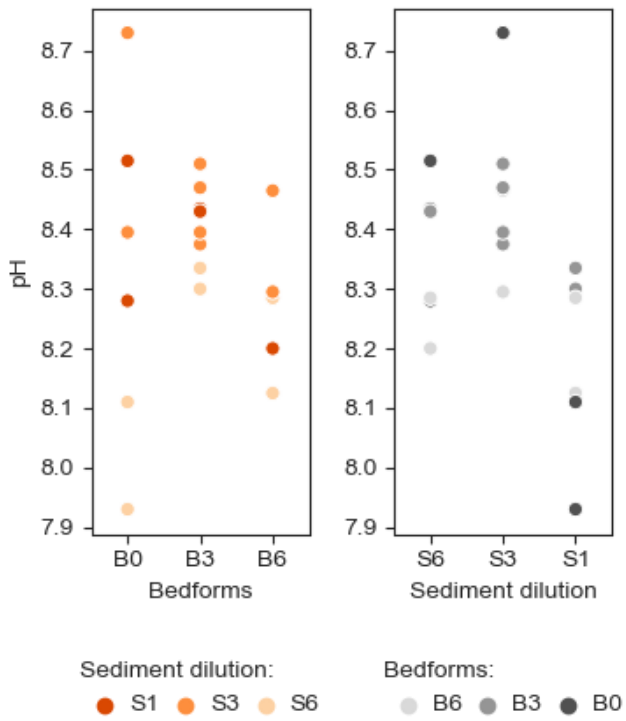

Figure S 4. Average pH of measurements at day -4 and day 45 in each flume. The bedform treatment had no significant effect on the pH. Within the sediment dilution treatment S3 flumes had a significantly higher mean pH than S1 flumes (ANOVA $p < 0.05$) in a Tukey post-hoc analysis. The interaction term was not significant.

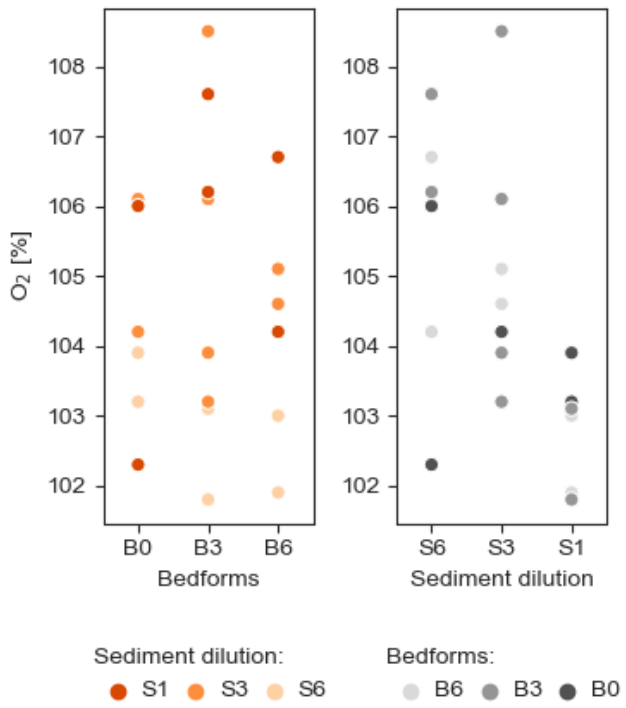

Figure S 5. Average O_2 of measurements at day 28, 36, 44 and day 86 in each flume. The bedform treatment had no significant effect on the pH. Within the sediment dilution treatment S3 flumes had a significantly higher mean O_2 than S1 flumes (ANOVA $p < 0.05$) in a Tukey post-hoc analysis. The interaction term was not significant.

Nutrient analysis

Surface water and porewater samples for analysis of NO_3^- (NO3), NO_2^- (NO2), NH_4^+ (NH4), total dissolved nitrogen (TN), PO_4^{3-} (PO4) and dissolved organic carbon (DOC) were stored at -20 °C and surface water samples were filtered through 0.45 µm nylon filters (Thames Restek, UK) prior to analysis. Concentrations of NO3, NO2, NH4 and PO4 were determined using a Skalar (Breda, Netherlands) SAN++ continuous flow analyzer and concentrations of DOC and TN were determined using a Shimadzu (Kyoto, Japan) TOC-L analyzer.

Nutrients

Initially the same amount of nutrients were added to the surface water of all flumes (Table S 1). Nutrient dynamics in the surface water differed little between the bedform treatments, but were highly impacted by the sediment dilution treatment (Figure S 6). This is why at the day of injection of micropollutants (day 0), nutrient concentrations differed between sediment dilution levels. Generally the rate of reduction during pre-incubation (day -12 to day 0) was higher, the lower the dilution. This is,

concentrations of nutrients at day 0 were lowest in low dilution flumes (S1) and highest in high dilution flumes (S6). In S3 and S6 flumes, the decrease in NO3 and NH4 coincides with a formation of NO2. These nitrogen dynamics resemble the N-cycles described for aquatic ponds or aquaria during establishment of the bacterial colonies responsible for nitrogen turnover, also called the "new tank syndrome".[1-3] The ammonium oxidizing communities, mostly *Nitrosomas*, develop first due to high availability of NH4 and oxidize it to NO2. As NO2 levels rise nitrite oxidizing communities, mostly *Nitrobacter,* develop and turn NO2 into NO3.[3] This is confirmed, as high turbidity was observed frequently in the S3 and S6 flumes during pre-incubation, which is often described as a sign for proliferation of bacteria in the surface water. However, formation of NO2-N exceeds the decrease of NH4-N. As NO2 is an intermediate in both, the nitrification and denitrification process,[4] NO3 reduction likely contributes to the temporary high NO2 concentrations. Other causes for NO2 accumulation such as O_2 limitations, low pH or high light intensities can be excluded.[5] Formation of NO3 was not observed, indicating that the sum of denitrification rates and NO3 assimilation rates generally exceeded nitrification rates. Following the argumentation, that NO2 is formed as a result of the establishment of bacterial communities, we can assume that the community in the low-dilution treatments S1 had developed much quicker, as no NO2 was observed in the surface water. Slightly elevated concentrations in the porewater at day 0 indicate a potential NO2 peak before day 0 in the S1 treatments. The fact, that no formation of NO2 was observed after the second addition of NH4 (d 10) in any flume, points out that the nitrifying communities had been fully developed and in balance by then. Interestingly, despite similar level of NO2 peak-concentrations in S3 and S6, subsequent NO2 depletion occurred faster in S3 than S6. This phenomenon is likely caused by the difference in bacterial diversity between treatments. The rapid decrease in PO4 concentration during pre-incubation in all treatments is most likely caused by a combination of sorption to binding sites in the sediment and microbial assimilation.[6,7] Over the 78 days of attenuation phase, some of the PO4 is released back to the surface water. This is either caused by reductive dissolution from Fe and Mn-oxides or re-mineralisation of formerly assimilated PO4 under anaerobic conditions. Particularly high concentrations were observed in the porewater of S1 flumes. This indicates that PO4 release processes were strongest in the low-dilution treatment. Similar to the nutrients, DOC was depleted during pre-incubation to a large extent. The depletion rate again was increasing from S6 over S3 to S1 treatments.

Nutrient concentrations in the porewater partly differed from surface water concentrations and porewater sampled from flat sediment showed different dynamics than porewater sampled from

bedforms. At day 0, NO3 concentrations in porewater were mostly below surface water concentrations due to consumption, while NO2 concentrations were mostly higher in the porewater due to formation in the sediment.[2] NH4 concentrations in contrast seemed in balance between surface water and porewater at day 0. However, it is worth mentioning that while in S1 and S6 nutrient concentrations were on average mostly higher in the bedform compared to the flat porewater, the opposite was observed for the S3 treatment. The differences in NH4 between surface water and porewater observed at day 14 are hard to interpret as they are likely caused by a combination of biological processes and varying advective transport porewater velocities of the additional NH4 introduced at day 10. Generally, the sediment was a hotspot for nutrient turnover and turnover conditions varied within the sediments.

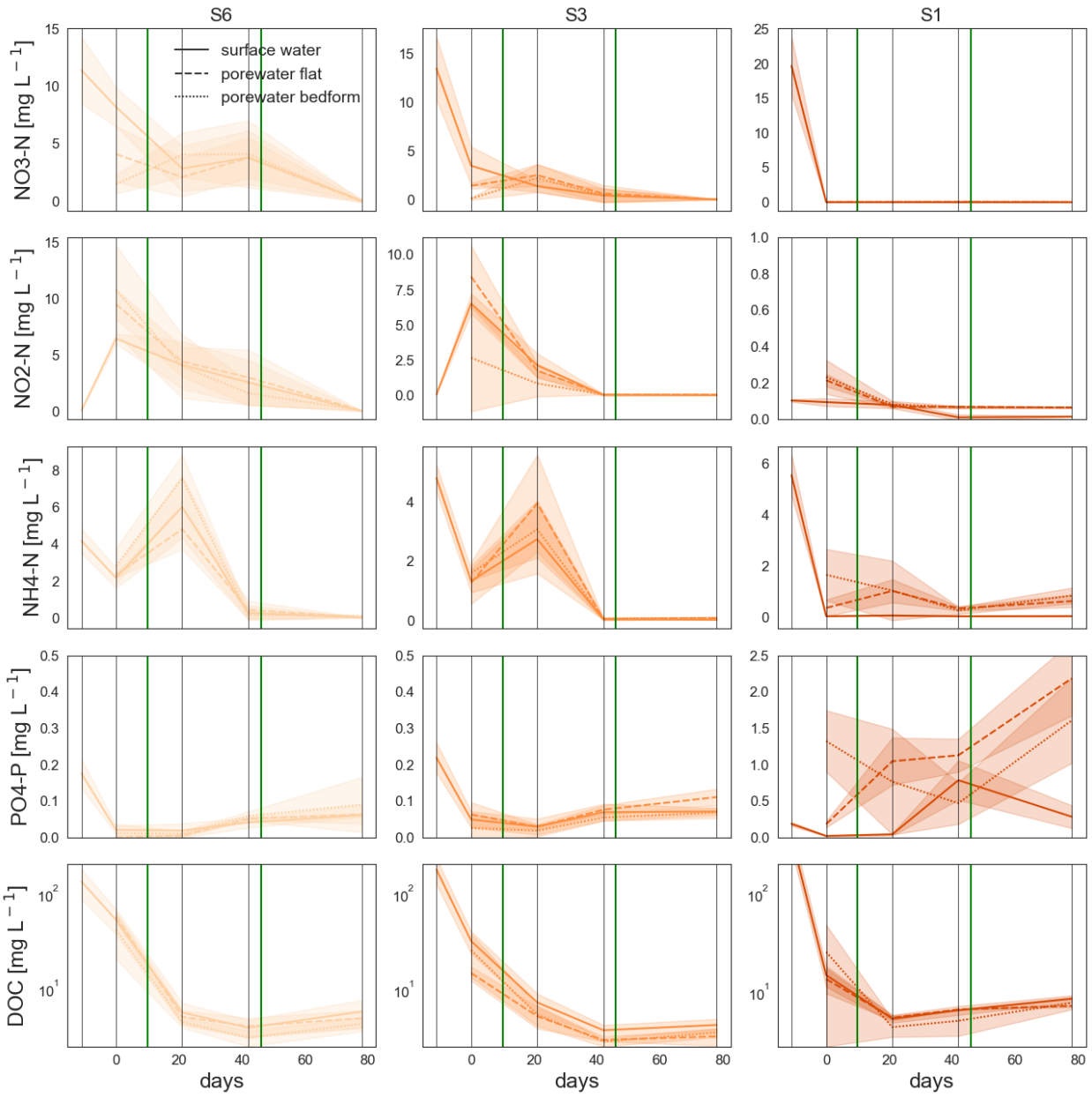

Figure S 6. Nutrient concentrations in surface water, porewater from flumes without bedforms and porewater from bedforms plotted by sediment dilution treatments (S1, S3, S6). Translucent areas indicate standard deviations. Green lines indicate addition of nutrient solutions N2 (day 10) and N3 (day 46), respectively (Table S 1). Grey lines indicate sampling points (day -11, 0, 21, 42 and 78). Note the different scales and logarithmic scales for DOC concentrations.

Salt tracer dilution test

From the salt dilution curves, surface water-pore water exchange flux Q_{in} [L d^{-1}] and exchange volume V_s [L] were calculated according to Equ1 and Equ2. V_s is the volume of porewater that is affected by

hyporheic exchanges, V_w is the surface water volume, C_0 the electrical conductivity at the beginning of the test and C_{eq} the electrical conductivity at equilibrium. K represents the rate constant of the concentration change over time. The average residence time [d] was calculated as the ratio between exchange volume and exchange flux.

Equ 1 $$V_s = \frac{V_w(C_0 - C_{eq})}{C_{eq}}$$

Equ 2 $$Q_{in} = kV_w \frac{C_0 - C_{eq}}{C_0}$$

At the time of the salt tracer dilution test, only the sediment dilution variable determined the surface water- porewater exchange flux significantly (ANOVA; $p<0.05$) (Figure S 7). No significant effect of any variable was found on the exchange volume and the residence time.

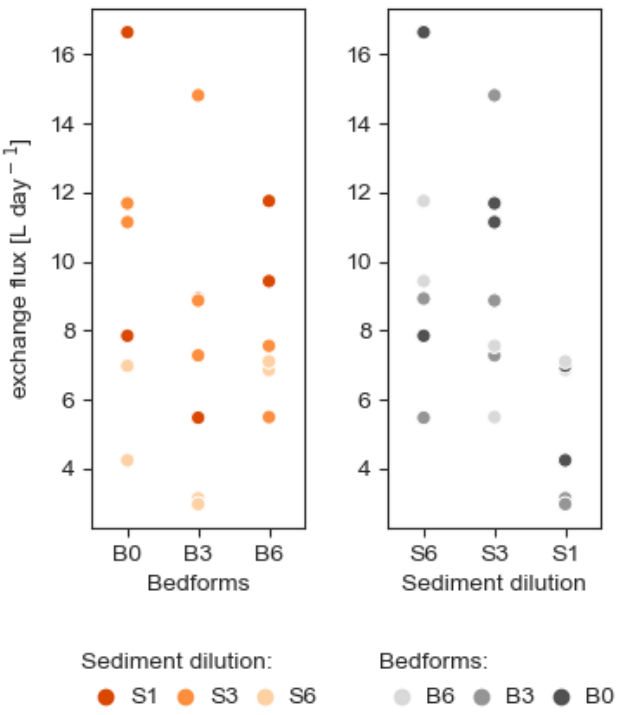

Figure S 7. Flux [L d^{-1}] after day 78 estimated from the EC dilution curves of the salt tracer dilution test plotted by bedform and sediment dilution levels. No variable has a significant effect on the flux (p-value ≤ 0.05).

16S rRNA copies

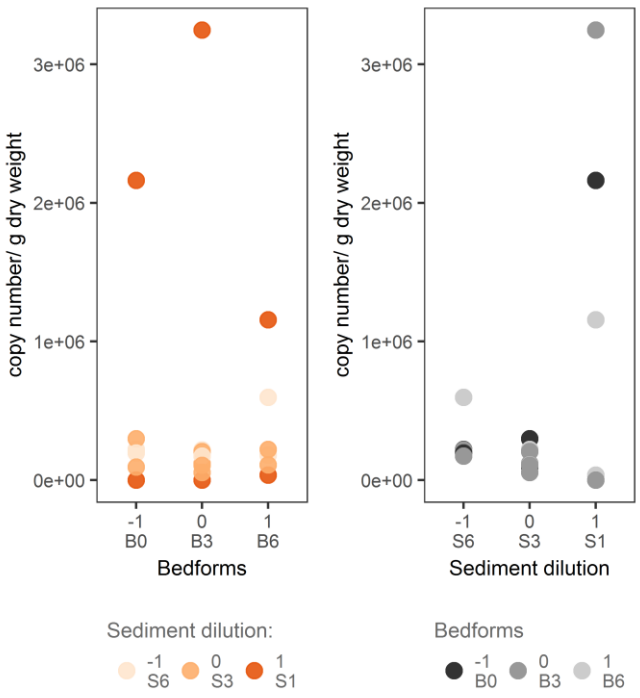

Figure S 8. Number of copies per g of dry sediment of 16S rRNA gene obtained from real-time PCR at day 21 of the attenuation phase

Changes in bedform morphology

Table S 3. Average change in morphology of all bedforms and surface water velocities in all flumes. Numbers in italics are estimates.

Day of measurement	**-3**	**27**	**46**	**82**	**Reduction by day 27**	**Reduction by day 46**	**Reduction by day 82**
Height of the bedforms (H) [cm]	8	7.8 ±0.8	7.3 ±0.8	6.5 ±0.9	3%	9%	19%
Length of bedforms (W) [cm]	12	*12*	*12.5*	*13*			
Sediment depth valleys (V) [cm]	2	*2*	1.94	1.84		3%	8%
Length of slope (S) [cm] (calculated)	13.4	13.3	13.6	13.8			
Water level from bottom (L) [cm]	12	11.5 ±0.4	11.7 ±0.4	11.2 ±0.5	4%	3%	7%
surface water velocity bedforms [cm s^{-1}]		8.2 ±1.2	8.3 ±1.1	6.6 ±1.8	0%	-1%	19%
Sediment depth flat (F) [cm]	3.5	3.8 ±0.3	3.7 ±0.2	3.4 ±0.2	-9%	-6%	3%
surface water velocity flat [cm s^{-1}]		9.2 ±1.7	10.3 ±1.4	7.4 ±2.2		-11%	20%

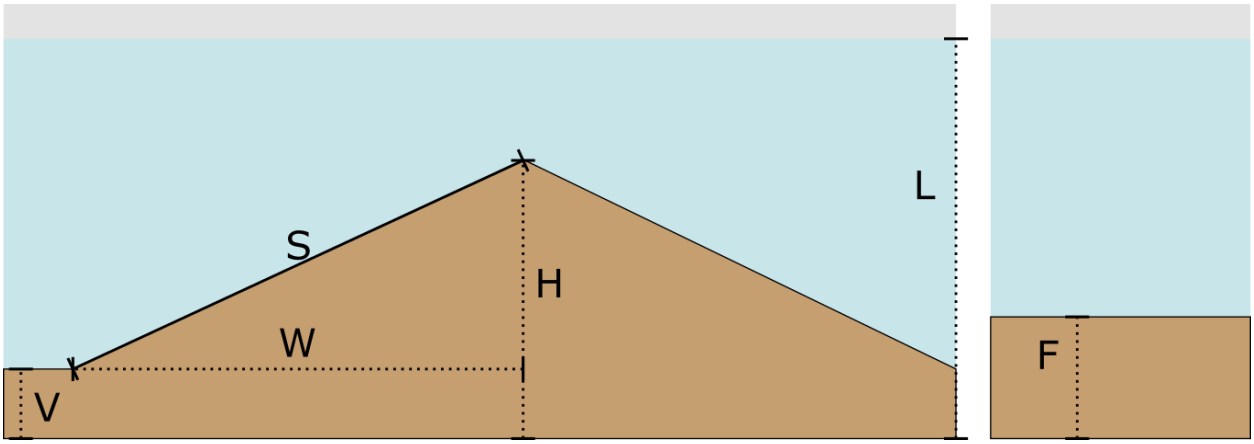

Figure S 9. Scheme of bedform measures.

Floating algae formation in S1 flumes

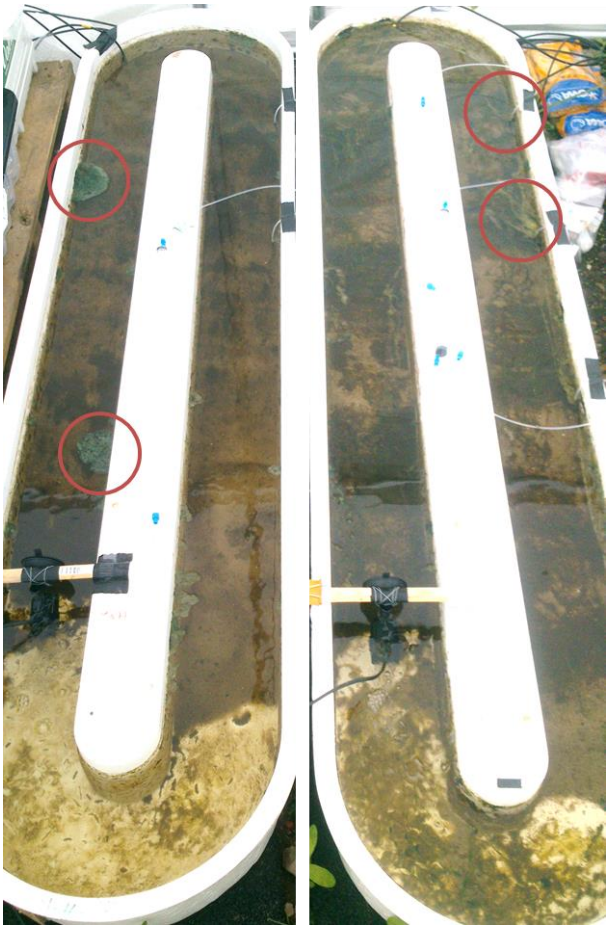

Figure S 10. Pictures of flumes 3 (S1/B0) and 4 (S1/B6) at day 39 showing the formation of floating algae which occurred in the second half of the attenuation phase in all S1 flumes.

Sorption of CBZ

In order to determine sorption capacity of the sediment, sorption experiments of CBZ were conducted following OECD 106 guideline.[8] Twenty grams of sediment samples taken from the sediment collected in River Erpe and three sediment mixtures (S1, S3, S6) were added into a glass bottle with 100 mL of 0.01 M of calcium chloride and 0.02 % sodium azide in deionized water. A mixture of carbamazepine (CBZ), ibuprofen and sulfamethoxazole was spiked into the sediment solution to produce a series of bottle incubations with final concentrations between 60 to 6000 µg/L in triplicates. A micropollutant-spiked control solution without added sediment acted as reference. The sediment and solution bottle incubations were continuously mixed using an orbital shaker at 100 rpm for 24 hours at room temperature. Water samples (5 mL) were taken after 0, 0.5, 2, 4 and 24h. Subsequently, water samples were passed through 0.20 µm regenerated cellulose membrane filter (Perfect-Flow, WICOM Germany) and transferred directly to vials for analysis. Sorbed micropollutant concentrations onto the sediment particles were calculated by using mass balance of initial and final micropollutants concentration differences. Sorption percentage was calculated at each time point to assess sorption kinetics and equilibrium concentrations were used to fit Freundlich isotherms.

The results showed that sorption of CBZ was negligible in the sediment taken from flumes with sediment dilutions S1, S3 and S6 within 24 hours equilibrium period (Figure S 11). As CBZ is a neutral compound it is reasonable to assume that the limited sorption in the flume sediments is a result of the low OC. The three flume sediments (S1, S3 and S6) used in this study recorded very low cation exchange capacity (CEC) values (0 - 3.6 cmol/kg) due to the very low organic matter content. Freundlich isotherms were not fitted to data from sediment flumes (S1, S3 and S6) due to very low sorption and consequently large data scatter in equilibrium concentrations (Cs and Cw) (Figure S 12). The equilibrium concentration in the water phase (Cw) was generally higher than the concentration in the sediment (Cs) for flume samples, thus very low sorption occurred in flume sediment.

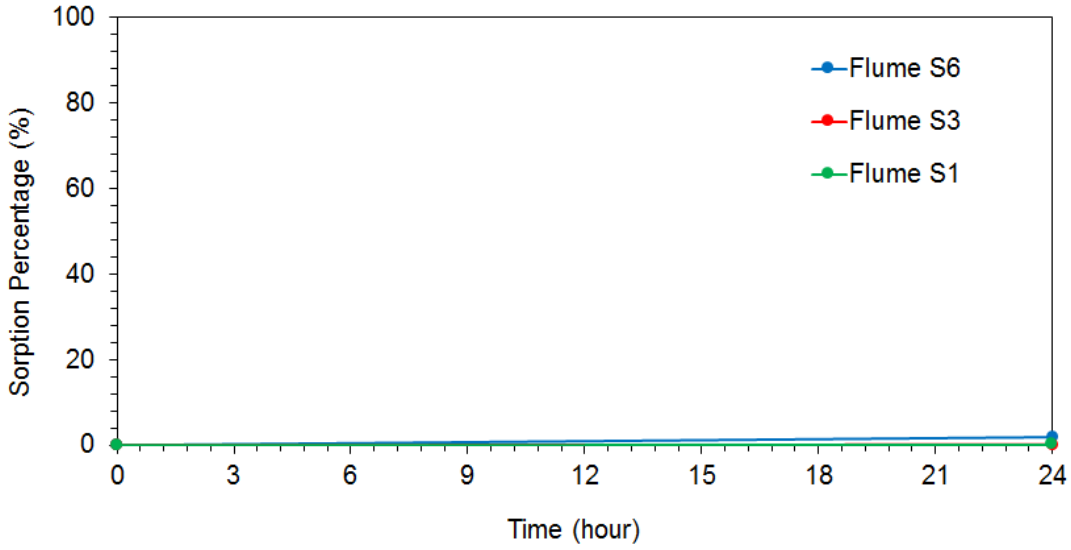

Figure S 11. Kinetics of carbamazepine (CBZ) for batch sorption experiments. Negligible sorption recorded for sediment taken from flumes with sediment dilutions S1, S3 and S6

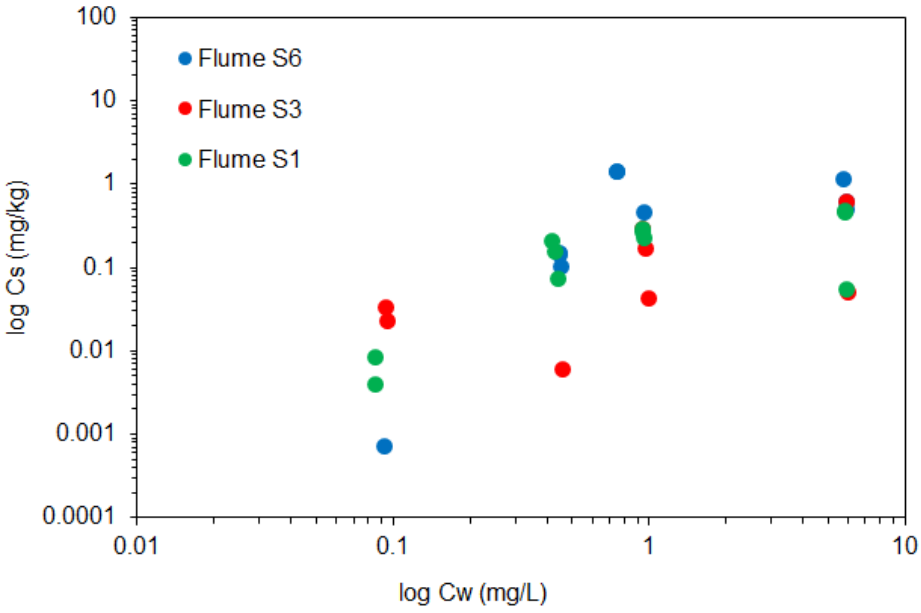

Figure S 12. Equilibrium concentrations of CBZ in the water phase (Cw) and in the sediment phase (Cs) in the sorption test

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