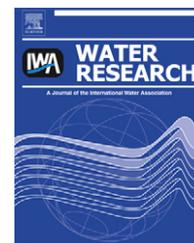


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River phosphorus cycling: Separating biotic and abiotic uptake during short-term changes in sewage effluent loading

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ABSTRACT

Medium to small scale point sources continue to threaten river ecosystems through P loadings. The capacity and timescales of within-river processing and P retention are a major factor in how rivers respond to, and protect downstream ecosystems from, elevated concentrations of soluble reactive P (SRP). In this study, the bio-geochemical response of a small river (~40 km² catchment area) was determined before, during and after exposure to a fourteen day pulse of treated sewage effluent using an upstream reach as a control. A wide array of approaches (batch and column simulations to in-situ whole stream metabolism) allowed independent comparison and quantification, of the relative contribution of abiotic and biotic processes in-river P cycling. This enabled, for the first time, separating the relative contributions of algae, bacteria and abiotic sorption without the use of labelled P (radioisotope). An SRP mass balance showed that the ecosystem switched from a P sink (during effluent inputs) to a P source (when effluent flow ceased). However, 65–70% of SRP was retained during the exposure time and remained sequestered two-weeks after-effluent flow ceased. Batch studies treated with biocide gave unrealistic results, but P uptake rates derived by other methods were highly comparable. Downstream of the effluent input, net P uptake by algae, bacteria and sediment (including the biofilm polysaccharide matrix) were 0.2 (±0.1), 0.4 (±0.3), and 1.0 (±0.9) mmol m⁻² day⁻¹ during effluent exposure. While autotrophic production did not respond to the effluent exposure, heterotrophic production increased by 67% relative to the control and this translated into a 50% increase in biological P uptake rate. Therefore, both biological and abiotic components of stream ecosystems uptake P during exposure to treated sewage effluent P inputs, and maintain a long ‘memory’ of this input in terms of P storage for considerable time-scales after loading.

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1. Introduction

Phosphorus is the key limiting nutrient controlling biological processes in many river systems (Slavik et al., 2004) and is the focus of research and legislation aiming to reduce loads to these systems (e.g. European Waste Water Treatment

Directive, CEC 1991). Despite past efforts in addressing point sources and a current shift towards tackling agricultural diffuse pollution, there remains a complex interplay of P sources to rivers (Withers and Jarvie, 2008). Point sources should not be considered a past problem since much of what is considered ‘diffuse’ sources concerns an abundance of

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smaller point sources in rural landscapes (farm yard drains, septic tank outflows, etc.). Hence, understanding the behaviour of point sources and their impact on river ecosystems remains an important goal, particularly under summer low flow conditions when biota are most sensitive (Rodda, 2007; Van Vliet and Zwolsman, 2008). For example, while P concentration in the water column decreases rapidly following P removal at point sources, river bed sediment P dynamic may not respond as fast (Demars et al., 2005; Demars and Harper, 2005; Jarvie et al., 2006). An improved understanding of biotic and geochemical processes of river P retention would enable better prediction of current and future buffering capacity as pollutant, land management and climatic pressures evolve.

A river's capacity for internal nutrient processing provides an important function of buffering downstream ecosystems by removing (in the case of N; Mulholland et al., 2008), or delaying (in the case of P; Dorioz et al., 1989) increases in nutrient concentrations due to upstream pollution. Short-term classic radioisotope studies with ^{32}P have revealed crucial insights in our understanding of P cycling in streams, particularly the biological uptake and translocation of P into the food web (Ball and Hooper, 1963; Newbold et al., 1983). Traditional P cycling methods (not relying on ^{32}P), using: a) laboratory closed systems such as batch bottles, chambers or re-circulating flumes (House et al., 1995; Jarvie et al., 2005; Gainswin et al., 2006); b) outdoor open channels and in-situ whole river studies (House et al., 1995; Gücker and Pusch, 2006; Demars, 2008), have not yet resolved the relative proportions of biotic and abiotic uptake. Only a few studies have tried to partition the relative role of the biota, but these relied on the use of biocide in batch bottles or flow-through channels and did not distinguish the role of autotrophs from heterotrophs (e.g. D'Angelo et al., 1991; Haggard et al., 1999; McDowell, 2003; Lottig and Stanley, 2007). Therefore, more amenable methods still need to be developed (Webster et al., 2009; Small et al., 2009) to quantify the relative role of the biota (autotrophs and heterotrophs) in P cycling rates. There are also large discrepancies between laboratory and in-situ whole stream P cycling rates (Demars, 2008) that need to be further investigated.

In this work we therefore sought to (i) compare several in-situ whole stream and laboratory P exchange capacity methods between river bed sediment and water column; (ii) separate the relative role of algae, bacteria and abiotic P uptake-release dynamics using independent approaches. Our field manipulation experiment exposed a rural river reach (37 km² mixed-agriculture catchment) to a 14 day pulse of treated sewage effluent. This novel approach enabled study of the river bed retention of P during exposure to elevated P, and fate of the transient storage of P post treatment.

2. Material and methods

To structure the work and experimental work done we first describe, the water and sediment of the field site and its characteristics to provide essential and detailed experimental context. Then, we present two methods to estimate total whole river P cycling (Section 2.2.). This is followed by two

batch laboratory methods (i.e. within a closed system in a bottle) to study abiotic P exchange capacity of river bed sediment with water column, with a method allowing comparison with in-situ whole stream measurements (Section 2.3.). Finally, we undertook three independent methods to quantify P algal and bacterial uptake (Section 2.4.). The combination of the best results was used to partition the algal, bacterial and abiotic (total-biotic) P uptake rate (Section 3.5.). The design of the study is further illustrated in Fig. 1 and summarised in Table 1.

2.1. Experimental site, water and sediment sampling

In 2004 the Tarland village (~600 people) Waste Water Treatment Works (WWTP) was upgraded as part of an ongoing catchment-wide programme to improve water quality. Treated effluent was then discharged to a self-contained wetland, but the operator included a switch valve to discharge treated effluent directly to the stream (under licensed consent). The WWTP is a combined sewer overflow and so intermittent discharges to the stream occur during high rainfall events when the storage tank facility is exceeded. Short (few minutes) intermittent overflows were also observed under dry weather when WWTP effluent discharge was around 4 L s⁻¹ due to an overflow at the switch valve.

Our experiment diverted the treated effluent directly to the stream for 14 days in Aug 2006, after which it went back to the wetland (Fig. 1). The experiment had three periods: (A) prior to-, (B) during- and (C) after-effluent discharge. Stream water samples were collected at three to six hourly intervals upstream and two downstream reaches from the WWTP effluent pipe using auto-samplers (Fig. 1). River stage was continually monitored at the downstream end of reach 2 in a stream section rated by depth velocity profiling. Discharge of

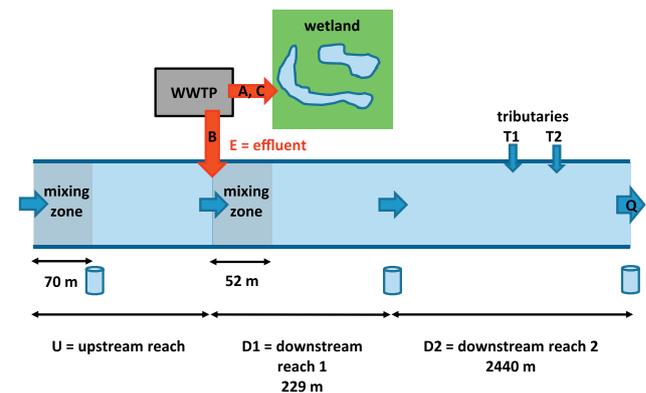


Fig. 1 – Experimental design (see Table 1). Three sections (U, D1, D2) of Tarland stream were studied before (A), during (B) and after (C) WWTP effluent diversion from a wetland to the stream. Whole stream mass balance measurements are reported from these sections. Note however that the measurements based on whole stream metabolism and nutrient spiralling (U and D1) excluded the mixing zone necessary for tracer studies (NaCl and propane). Discharge (Q) was measured continuously at the downstream end. The position of auto-samplers is indicated by a cylinder symbol.

Table 1 – Experimental design. The present study sought to compare whole river in-situ P dynamics with laboratory essays (Sections 2.2. and 2.3.), and to disentangle algal, bacterial and abiotic P uptake (Section 2.4.). The measurements were taken upstream and downstream a WWTP (see Fig. 1), in three consecutive periods, before (A), during (B) and after (C) effluent diversion.

Measurements	Upstream	Downstream 1	Downstream 2	Period
2.2. Whole river P cycling				
2.2.1. Mass balance ^a (MB = ΣCQ)	n/a	✓	✓	A, B, C
2.2.2. Whole stream SRP addition studies (nutrient spiralling)	✓	✓	n/a	A, B
2.3. Laboratory sediment P sorption capacity				
2.3.1. Batch ^b bottle P exchange capacity with biocide (EPC ₀)	✓	✓	n/a	A, B, C
2.3.2. Kinetic bottle P sorption capacity with biocide	n/a	✓	n/a	Simulated B
2.4. Biological P uptake rate				
2.4.1. Laboratory column sorption experiment (no biocide)	n/a	✓	n/a	Simulated A, B, C
2.4.2. Biofilm accrual on bricks	✓	✓	n/a	A, B, C
2.4.3. Whole stream metabolism ^c (WSM)	✓	✓	n/a	A, C
Stoichiometric approach based on WSM and nutrient spiralling	n/a	✓	n/a	B (C)

n/a, not applicable.
a C = concentration, Q = discharge.
b Batch of eight different water SRP concentrations.
c Respiration and photosynthesis.

reach 1 was catchment-scaled from reach 2 measurements, which agreed within 5% with depth velocity profile and salt dilution gauging. The 37 km² catchment is subject to additional agricultural pressures with land areas of arable and intensive grassland of 23% and 40%, respectively (see Stutter et al., 2008).

The upper 2 cm of sediment from the river bed was sampled in the upstream reach and downstream reach 1 using a flat plastic scoop. At the upstream and downstream locations ten spot samples across 10 m² area of river bed were combined at each site. Sediments were passed through a 2-mm aperture sieve and air-dried (30 °C). The decision to dry sediments was taken on the basis that different storage times for sediments sampled over time prior to determining isotherms in a single batch would introduce as much errors as the drying process itself (cf McDowell, 2003). The sediments are characterised as having low organic C contents <2 g kg⁻¹ and oxalate extractable Fe and Al contents of 9 and 3 g kg⁻¹, respectively (Stutter et al., 2008). Sediments typically had size class contributions clay, silt, fine sand, medium to coarse sand of 1, 10, 28, 61%, respectively. Further analytical details are given in Supporting Information.

2.2. Whole river P cycling

Two approaches were used to determine in-situ SRP cycling rates: (i) a mass balance approach using auto-samplers at the top and bottom of downstream reaches and (ii) detailed SRP addition studies (Fig. 1, Table 1).

2.2.1. SRP mass balance

The mass balance (MB; $\mu\text{mol s}^{-1}$) of the downstream river reaches (subscripts 1 and 2) was undertaken according to:

$$MB_1 = C_{D1} Q_{D1} - (C_U Q_U + C_E Q_E), \quad (1)$$

where C and Q equal the SRP concentration ($\mu\text{mol L}^{-1}$) and discharge (L s^{-1}), respectively, and subscripts D1, U and E

denote downstream reach 1, upstream and effluent, respectively. For the downstream reach 2 (D2) this was expanded to include the inputs from two tributaries (T1 and T2):

$$MB_2 = C_{D2} Q_{D2} - (C_U Q_U + C_E Q_E + C_{T1} Q_{T1} + C_{T2} Q_{T2}). \quad (2)$$

The combined tributary inputs averaged 17% of the P flux at D2. Mass balances were scaled to $\text{mmol P m}^{-2} \text{day}^{-1}$ on the basis of the average measured river width of 2.9 m.

2.2.2. Nutrient addition approach

Whole river phosphate cycling studies following Webster and Valett (2006) were performed before and after the start of the effluent diversion upstream and downstream of the WWTP (2, 4, 6, 24 and 72 h). This was undertaken on the same river reaches used for whole stream metabolism measurements. These studies allowed the measurement of background and subsequent changes in: nutrient uptake length (average distance travelled by a molecule of phosphate before uptake by the river bed); phosphate uptake rate; and phosphate uptake velocity (a measure of uptake rate, relative to availability in the water, which normalises for stream velocity and depth). The nutrient spiralling studies were undertaken during 'period A' and described the background uptake-release dynamic equilibrium. The studies done at 24 and 72 h downstream of the WWTP described the net uptake rate (Haggard et al., 2001), directly comparable to the mass balance approach. The uptake velocity is comparable across streams and sensitive to anthropogenic pressures (e.g. Newbold et al., 2006).

2.3. Laboratory sediment abiotic P sorption capacity

Two batch laboratory approaches were undertaken in order to determine the SRP exchange capacity of sediment collected in the different periods (A, B, C) of the field manipulation (Fig. 1, Table 1) and a method is presented to allow comparison with whole stream total P exchange dynamics (Section 2.2.).

2.3.1. Batch study, SRP exchange capacity

These experiments were performed using 1 g sediment (oven dried equivalent) to 30 ml of 0, 10, 20 and 50, 100, 500, 1000, 5000 $\mu\text{mol P L}^{-1}$ soluble reactive phosphorus (SRP as KH_2PO_4). This was made up with an ‘artificial river water’ matrix of 0.5 mmol L^{-1} CaCl_2 , with 0.02% azide as a biocide. After 18 h equilibration at 20 °C, solutions were filtered prior to SRP analyses. Triplicate equilibrations had coefficients of variation of final SRP concentrations within 10%. Example isotherms are shown in Supporting Information (Fig. S1). A Langmuir model was fitted describing ΔN_a , the measured P uptake during equilibration ($\mu\text{mol kg}^{-1}$) and C , the remaining SRP concentration after equilibration ($\mu\text{mol L}^{-1}$):

$$\Delta N_a = N_{\max} \frac{KC}{1 + KC} - N_i \quad (3)$$

where K (L kg^{-1}) is a constant, N_i the initial ‘native’ adsorbed P ($\mu\text{mol kg}^{-1}$) and N_{\max} the sorption maximum ($\mu\text{mol kg}^{-1}$). The positive intercept on the x axis (the SRP concentration which gives no change in ΔN_a over the period of equilibration) represents the equilibrium phosphorus concentration (EPC_0 ; Taylor and Kunishi, 1971). Note that only the four lowest batch concentrations were used to determine the EPC_0 (as shown in Fig S1). Readily desorbable ‘native’ P contents of the sediments were determined using the Fe oxide paper strip test according to Chardon et al. (1996).

2.3.2. Batch study, kinetics of SRP sorption

These experiments were performed (same conditions as in Section 2.3.1) at 3.8 $\mu\text{mol SRP L}^{-1}$ (equivalent to river concentrations during effluent discharge) and time points 0, 0.08, 0.25, 0.5, 1, 3, 5, 24 h equilibration. Sorption was converted from $\mu\text{mol kg}^{-1}$ to mmol m^{-2} of river reach by multiplying by the river bed area, specific density of fine sediment <2 mm (1600 kg m^{-3}) and assuming that the first 2 cm depth (as per sampling method) of the river bed sediment layer interacted with overlying waters and a homogeneity of sediment across the river bed. While this depth is rather pragmatic it serves to act as a linear scaling factor in the P uptake modelling and corresponded to previous studies (Fischer and Pusch, 2001).

Phosphate kinetic data was interpreted with the Elovich equation (e.g. Demars, 2008):

$$\frac{dU_{\text{NET}}}{dt} = a \exp(-bU_{\text{NET}}) \quad (4)$$

where U_{NET} is the net uptake (mmol m^{-2}), t time (day), a is the initial uptake rate ($\text{mmol m}^{-2} \text{ day}^{-1}$), and b is the rate constant ($\text{m}^2 \text{ mmol}^{-1}$). With the condition that $U = 0$ at $t = 0$, this integrates (Allen and Scaife, 1966):

$$U_{\text{NET}} = \frac{1}{b} \ln(1 + abt) \quad (5)$$

The nutrient uptake length S_w (m), for comparative purpose with whole stream uptake studies, was calculated as follows (Demars, 2008):

$$S_w = \frac{u}{ab} \quad (6)$$

with u , average water velocity (m day^{-1}).

2.4. Biological P uptake rate

Three independent methods were used to provide estimates of biological uptake from the autotrophs (mostly algae) and heterotrophs (mostly bacteria) at two scales of observation: laboratory or field bioassays and in-situ whole river reach (Fig. 1, Table 1).

2.4.1. Laboratory column experiments

Column sorption experiments were undertaken without biocide to: (i) evaluate any hysteresis in desorption relative to adsorption and (ii) determine any microbial P uptake. Column parameters are described in Supplementary Information Table S1. Briefly, columns (47 g sediment, sieved <2 mm) were equilibrated at 20 °C (flow rate 0.5 mL min^{-1}), until eluant outflow concentrations matched inflows, or became stable following saturation of abiotic P sorption. Columns were equilibrated sequentially to (i) 0.7 $\mu\text{mol L}^{-1}$ SRP, then (ii) elevated SRP (3.8 $\mu\text{mol L}^{-1}$), then (iii) 0.7 $\mu\text{mol L}^{-1}$ SRP again, all using a background electrolyte of 0.5 mmol L^{-1} CaCl_2 . Columns were packed with previously air-dried sediments, but were equilibrated for a fortnight (approximately 1000 pore volumes during phase (i), cf McDowell, 2003). One day of flow equated to 60–75 column pore volumes.

2.4.2. Algal growth rates

Accumulation of benthic algae was determined on artificial clay brick substrates (area 0.04 m^2) placed on the river bed in triplicate at the beginning, and recovered at the end of each experimental period (A, B, C). Bricks were scraped, washings were filtered (Whatman GF/F) and chlorophyll determined by hot extraction of the filter in 3 mL methanol. Chlorophyll *a* concentrations were determined by absorbance at 665 nm (corrected for turbidity at 750 nm) (Talling and Driver, 1963). Algal biomass P accumulation rates ($\text{mmol P m}^{-2} \text{ day}^{-1}$) were calculated from molar ratios chlorophyll *a*:C of 1:35 (Sobczak et al., 2002) and C:P of 158:1 (Kahlert, 1998).

2.4.3. River reach scale studies

Biotic P uptake was based on C:P stoichiometry and gross primary productivity (GPP) and ecosystem respiration (ER) similar to Hall and Tank (2003). Autotrophic production was estimated as 0.5 GPP and heterotrophic respiration (HR) as $\text{ER} - 0.5 \text{ GPP}$. We used the following stoichiometric ratios: algal molar C:P of 158:1 (range 99:1–369:1, Kahlert, 1998), and bacterial molar C:P of 65:1 and C:P of 130:1 for the moderate (0.2) and low (0.05) heterotrophic growth efficiency (HGE) scenarios respectively (Thingstad et al., 1996; Gundersen et al., 2002; Ukpogon, 2005). Heterotrophic production (HP) was calculated from:

$$\text{HGE} = \frac{\text{HP}}{(\text{HP} + \text{HR})} \quad (7)$$

using Solver in Excel. The average heterotrophic production (from low and moderate HGE) was used to calculate the total-biotic uptake and partitioning the relative contribution of algae, bacteria and sediment P uptake. The relative change in

ER (and similarly for total biological uptake) due to the WWTP effluent effect was calculated as follows:

$$\Delta ER_{\text{WWTP}} = \frac{\overline{\Delta ER_D} - \overline{\Delta ER_U}}{\overline{ER_{DA}}} \quad (8)$$

with Δ representing change between period A (before) and C (after) effluent diversion, and subscripts D, U and A denote downstream, upstream and period A, respectively.

Whole stream metabolism (GPP, ER) was calculated at 1 min time steps upstream/downstream the WWTP effluent before, during and after-effluent diversion using the diel oxygen two station approach of Marzolf et al. (1994), with corrections from Young and Huryn (1998). Tracer studies were run with NaCl to determine mean travel time and lateral inflows (here insignificant, <2%) and with propane using diffusion of micro-bubbles across the whole channel to determine the re-aeration coefficient. Due to the trapezoidal shape of the stream channels and relatively fast flowing water, the mixing zones were relatively long (50–70 m), with additional sand bags in the stream to ensure full tracer mixing. Mean travel times were 10 and 17 min for the upstream (140 m) and downstream (177 m) reach 1, respectively. The re-aeration coefficients were 53 ± 4 and $26 \pm 2 \text{ day}^{-1}$ in the upstream and downstream reaches, respectively.

2.5. Data analyses

The design of the experiment was a classic BACI (before and after control experiment) and statistical analyses followed Demars and Harper (2005). Two-way ANOVAs were run (interaction site \times period) to test the effect of the WWTP effluent diversion on ER, GPP, and P uptake. However, the resulting probabilities should only be considered as rough indicators because GPP, ER and P uptake were all highly auto-correlated within the two periods of observations. Strictly, no probabilities should be reported until other similar experiments are reported elsewhere, allowing a meta-analysis. Increase in P uptake, relative to the upstream control, took into account the changes in the upstream control reach as well as the changes in the downstream reach. One-way ANOVAs allowed comparison between periods or sites. Uncertainties based on 1 Standard Deviation (SD) were propagated throughout the calculations as in Demars et al. (2005).

3. Results and discussion

3.1. Changes in-river chemistry

Means of river hydrochemistry for the three experimental periods are given in Table S2. There was some variation in concentrations upstream of the WWTP (control site) so that a one-way ANOVA showed significant ($p \leq 0.05$) differences between treatment periods A, B and C for concentrations of NO_3^- , protons, soluble reactive, unreactive and particulate P (SRP, SUP and Part P, respectively). This was related to changes in discharge between periods (Fig. 2a) and upstream inputs. However, effluent discharge to the river increased concentrations of NH_4^+ , SRP and SUP and, to a lesser extent protons

and DOC, downstream of the WWTP. River SRP was elevated ten-fold by the effluent discharge (Fig. 2b). However, there was no downstream increase in sediment, or particulate P (Part P) related to the effluent discharge. A period of stable summer baseflow preceded our experiment. However, there were several minor storm events when river discharge increased (Fig. 2a), most notably just prior to the commencement of effluent discharge (end of period A), although with no substantial river bed movement.

3.2. Whole river P cycling

Prior to effluent discharge (period A) both upstream and downstream reaches had similar SRP cycling rates (Table 2). The nutrient spiralling studies showed that there was little exchange between the river bed and overlying water suggesting that the river bed was relatively saturated despite low background SRP concentrations. This saturation may be due to SRP pulses from upstream or from the WWTP overflow (especially during high flow and fast transit of water in the river channel (D'Angelo et al., 1991). The uptake velocities ($8\text{--}41 \times 10^{-3} \text{ mm s}^{-1}$) were comparable to other rivers impacted by point sources, e.g. River Erpe ($4.2\text{--}69 \times 10^{-3} \text{ mm s}^{-1}$, Gucker and Pusch, 2006), Spavinaw Creek ($2.3\text{--}7.5 \times 10^{-3} \text{ mm s}^{-1}$, Haggard et al., 2001). However these uptake velocities are 10–100 times slower than those reported in agricultural (Bernot et al., 2006) or pristine streams (Doyle et al., 2003). Hence the Tarland Burn had a small P retention efficiency.

The mass balance approach was adopted to calculate ecosystem (sediment and biological) uptake of SRP. The negative values for period B (Fig. 2c) in both reach 1 and reach 2 indicate SRP mass not accounted for at the downstream site (i.e. uptake of SRP in the reach). Conversely, positive values indicate net SRP release. The positive values during period A were likely to have been related to the storm event. The SRP mass balance is highly sensitive to accurate quantification of the WWTP effluent P inputs and the positive spike around 19th Aug may have occurred due to a period of unaccounted effluent P mass input when the WWTP storage tank capacity was exceeded during the storm.

The SRP load (with relative uncertainties based on 1 SD; $n = 24$ samples) associated with the effluent discharge to the river was variable at $448 \mu\text{mol s}^{-1}$ ($\pm 37\%$). This was derived from effluent flow rates and SRP concentrations measured over several days as 3.4 L s^{-1} ($\pm 13\%$) and $131 \mu\text{mol L}^{-1}$ ($\pm 35\%$). Although this contributes to some uncertainty in the mass balance the general patterns and magnitudes of fluxes hold. River SRP concentrations increased as soon as effluent flow commenced. Variation in-river water SRP concentrations downstream of the WWTP ($\pm 10\text{--}86\%$; Table S2) contributed to variability in the SRP mass balance for the 229 m long reach 1 during period B. Maximum SRP uptake rates of $25\text{--}29 \text{ mmol m}^{-2} \text{ day}^{-1}$ occurred during several days early in period B (Fig. 2c).

Subsequently, on the 24th, 27th and 28th Aug there was a net SRP release indicated. Further variability in the magnitude of the SRP mass balance for reach 1 may relate to inconsistency in effluent quantity and quality for a number of reasons associated with the WWTP operating conditions.

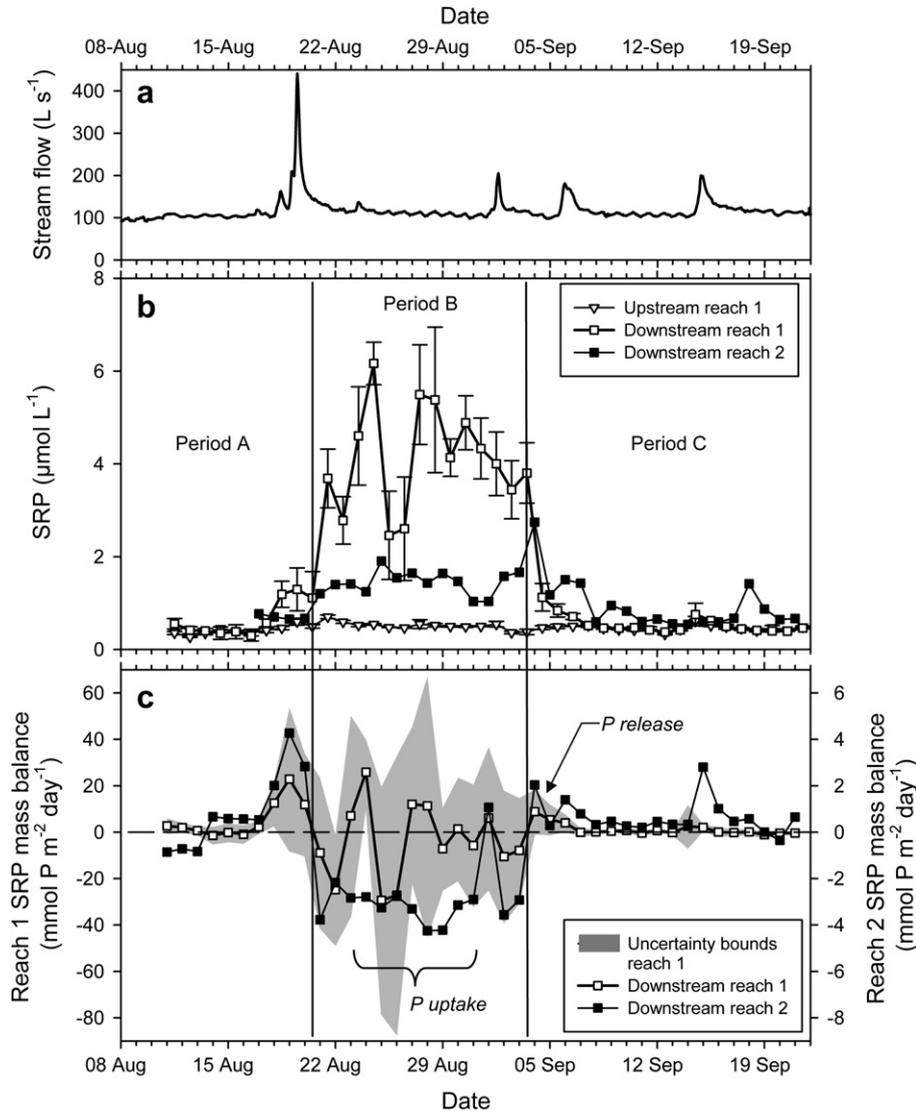


Fig. 2 – Temporal changes during the experimental periods A (prior to-), B (during-) and C (after-effluent discharge) in terms of (a) river flow, (b) SRP concentrations, and (c) SRP mass balance. Symbols are (∇) U, upstream and (\square) D1, downstream reach 1, D2, downstream reach 2 (\blacksquare). Daily mean SRP concentrations (b) include 1 standard error bars for reach 1.

Table 2 – Background SRP concentration (C_{AMB}), added phosphate concentration (C_{ADD}), Phosphate uptake length (S_w), uptake rate (U), and uptake velocity (v_f), upstream and downstream the waste water treatment plant (WWTP) effluent before and during treatment. Negative values represent nutrient release from the stream bed towards the water column. Relative uncertainties (based on 1 SD) are reported within brackets.

	C_{AMB} $\mu\text{mol L}^{-1}$	C_{ADD} $\mu\text{mol L}^{-1}$	S_w (m)	U ($\text{mmol m}^{-2} \text{day}^{-1}$)	v_f (mm s^{-1})
Before diversion					
Upstream WWTP	0.472	0.512	4440 (-2700 to 11 600)	0.35 (-0.22 to 0.90)	0.008 (-0.005 to 0.022)
Downstream WWTP	0.486	0.839	1520 (930–2110)	0.97 (0.58–1.35)	0.023 (0.014–0.032)
During diversion					
Downstream WWTP					
t = 2 h	0.666	4.054	1770 (1140–2400)	1.13 (0.71–1.55)	0.020 (0.013–0.027)
t = 4 h	0.747	4.632	856 (703–1009)	2.65 (2.13–3.16)	0.041 (0.034–0.048)
t = 6 h	0.778	6.518	1218 (979–1460)	1.94 (1.52–2.35)	0.029 (0.023–0.035)
t = 24 h	0.821	7.417	3560 (2200–4920)	0.71 (0.42–0.97)	0.010 (0.006–0.014)
t = 72 h	0.682	4.947	-2300 (-1470 to -3140)	-0.90 (-0.55 to -1.23)	-0.015 (-0.010 to -0.021)

Hence, there were large bounds of uncertainties associated with the SRP mass balance for period B, highlighting the difficulties of working in such systems. The average P mass balance during period B was $-4.4 \text{ mmol m}^{-2} \text{ day}^{-1}$ (ie. uptake).

The magnitude of the SRP cycling rate differed (although within the range of uncertainties) between the mass balance and the nutrient spiralling approaches. However, these techniques showed common patterns; SRP uptake after 24 h of WWTP discharge (22-Aug) and then net release of SRP after 72 h (24-Aug), due to a sudden decrease in SRP concentration coming from the WWTP effluent (Table 2, Haggard et al., 2001). It is only recently that the variability in nutrient flux from the WWTP effluent (a long known fact, e.g. Moss et al., 1988) has been explicitly acknowledged to affect the measured nutrient exchange rate between the river bed sediment and water column (net sorption or release, e.g. Haggard et al., 2005).

As the effluent pipe was switched off the SRP mass balance immediately switched to become positive $+9 \text{ mmol m}^{-2} \text{ day}^{-1}$ (indicating release; even accounting for the degree of uncertainty of -1 to $+18 \text{ mmol m}^{-2} \text{ day}^{-1}$). Over the following four days there was approximately linear decrease in the rate of SRP release from the river towards an equilibrium state with respect to P release.

Patterns in SRP fluxes were similar between the 2440 m reach (reach 2) and the 229 m (reach 1) immediately downstream of the WWTP, notably in the period of SRP release after treated effluent ceased. The principal difference was the greater stability in the mass balance for the longer reach, which maintained near-consistent P uptake ($-2.9 \text{ mmol m}^{-2} \text{ day}^{-1}$) averaged over period B. The slightly smaller average P uptake for the longer reach, than immediately downstream of the WWTP in period B indicated that reach SRP uptake declined with distance as river SRP concentrations driving the uptake decreased.

In terms of the mass balance for the whole reach areas, of a total 542 mol of SRP entering the river during the 14 day period B only 41 mol (8%) was taken up in the 229 m reach immediately downstream, whereas 289 mol (54%) of this input load were taken up in the longer 2440 m reach. Previously reported point source P retention rates, under low flows, have varied greatly: 30% in a 55 km reach in England (House, 2003), 60% over 1–2 km lengths in England (Jarvie et al., 2002) and 63% over 4–8 km reach lengths in the U.S. (Ekka et al., 2006). The amount of P taken up in the 2.4 km downstream reach indicated the river ecosystem nearer the WWTP was unable to protect downstream reaches and buffer the P inputs. Of the P taken up in reach 1 and 2 respectively 39% and 30% was released when effluent flow ceased (period C). Therefore, the majority of the SRP taken up into these reaches was retained; becoming sequestered by sediment sorption, or incorporated into biota and made unavailable for river bed release in the short term.

3.3. Abiotic P sorption capacity of sediments

Langmuir adsorption isotherms showed consistent P saturation of approximately $1000 \text{ } \mu\text{mol kg}^{-1}$ with no significant differences temporally, or spatially (Table S3). Sediment EPC_0 values (the concentration of solution SRP that would cause no net change in sorption) and native adsorbed P (N_i , i.e. P sorbed

in-situ in the channel prior to P exchange capacity experiments) increased for sediments that had been exposed to the greater SRP concentration during the period of effluent discharge to the river. Sediment EPC_0 became greater during period B (Fig. 3) downstream of the WWTP than EPC_0 values for the control (upstream) site. However, the downstream increase was not large in relation to the variability over time at the control site (Table S3).

Sediment EPC_0 values are often compared to river SRP concentrations as an evaluation of the direction and strength of SRP adsorption/desorption (for example Jarvie et al., 2005). Sediments in the upstream control reach remained at approximate equilibrium or showed weak uptake (Fig. 3, close to the 1:1 line). For the downstream reach there was a lag prior to rising sediment EPC_0 after water column SRP had already been elevated for 3 days. Subsequently, downstream sediment EPC_0 increased at the end of the effluent discharge (period B), then decreased as effluent flow ceased. However, a small rise in $\text{EPC}_0 > \text{SRP}$ at the end of period C, which indicated potential for SRP desorption, was corroborated by rising FeO-test P (Table S3). This may be an artefact caused by sampling of the heterogeneous bed sediments.

Kinetic batch equilibrations (using SRP concentrations matching that in the river during effluent discharge) did not show signs of P sorption saturation, i.e. the uptake rate was simply a function of the SRP concentration supplied (Fig S2a), although this was not linear (Fig S2b). There was a large initial P uptake rate with little changes in SRP concentration, and this is reflected in the P uptake velocity (sorption efficiency relative to SRP concentration, Fig S2c). Hence, the uptake rate was fairly independent of change in SRP concentrations within only the first half an hour of the study. The initial uptake rate was 5000 times greater than the averaged sorption measured by the whole river mass balance approach during the diversion of the effluent. The nutrient uptake length calculated from the Elovich parameters and stream velocity was about 0.2 m (that is 10^4 times smaller than what was measured with the nutrient addition experiments; Table 2).

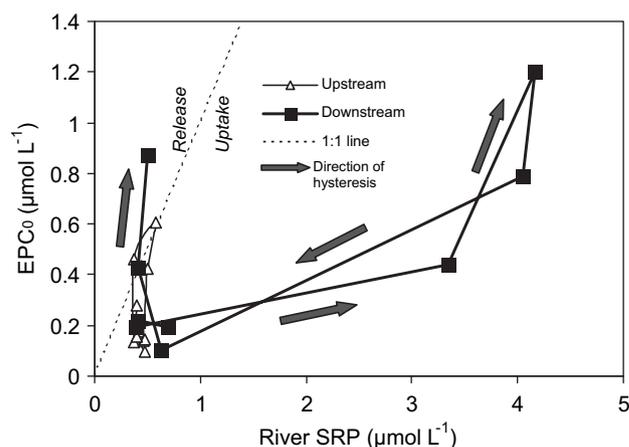


Fig. 3 – Change in sediment EPC_0 and (1 standard error bars), relative to water column SRP during the experimental period for upstream and downstream sites of reach 1.

Of course, we must exercise caution in these batch study results since the redox conditions of these simulations differ from those of in-situ bed sediments. For example, the depth of the sediment exposed to the water SRP, diffusion and pore water concentration gradients that make such simplistic assessments difficult (e.g. House and Denison, 2002). Batch bottle EPC₀ and kinetic isotherms indicate the maximum reaction rate since they describe a system where the sediment is shaken with the water, although this rate could apply if bed sediment became resuspended by a storm event. Consequently, we suggest, batch studies, despite being a common approach adopted in such studies, are inappropriate for upscaling to whole river bed P budgets and modelling (e.g. Wade et al., 2002).

3.4. Biological P cycling

Three methods were used to quantify algal and bacterial P uptake: whole stream metabolism, algal in-situ assay and a laboratory flow-through column sorption experiment. The whole stream metabolism results (Table 3) are compared here between stable low flow periods (under which our tracer studies were conducted; Fig. S3). Uncertainties in daily ER and GPP estimates were about 42% and 2% respectively. The metabolic rates were generally lower downstream from the WWTP effluent at all time ($p < 0.002$), contrary to observations in the River Erpe (Gücker et al., 2006). ER (range 2.2–4.4 g C m⁻² day⁻¹) exceeded GPP (range 0.6–1.6 g C m⁻² day⁻¹) at all times as is generally the case due to allochthonous source of matter and energy (Battin et al., 2008). The metabolic activity of Tarland Burn was relatively low (cf Battin et al., 2008), particularly compared to the highly productive River Erpe (12–22 g C m⁻² day⁻¹), although no uncertainties (which may easily be around 100%) were

reported in Gücker et al. (2006). The metabolic activity of the water column derived from BOD₅ measurements, provided by the Scottish Environmental Protection Agency, represented only about 2% of ER. Therefore, 98% of ER was from the river bed, more than in the larger River Spree (Fischer and Pusch, 2001). GPP increased over time but this was not related to the experiment ($p > 0.5$), unlike from the previous descriptive observations (Gücker et al., 2006). ER however increased by 67% relative to the upstream control (note decrease in ER in the upstream control), and therefore was affected by the effluent diversion ($p < 0.001$). This confirmed previous observations made upstream and downstream of treated effluents (Gücker et al., 2006). Heterotrophic respiration ranged from 2.3 to 3.9 g C m⁻² day⁻¹, similar to the total bacterial respiration estimates of the River Spree (Fischer and Pusch, 2001). Phosphorus uptake rate from autotrophic growth ranged from 0.2 (0.1–0.3) to 0.4 (0.2–0.7) mmol P m⁻² day⁻¹. In the future, uncertainties could be reduced by determining the C:P ratio of the primary producers both upstream and downstream the effluent, rather than relying on published studies (Bowman et al., 2005). Estimates of P uptake rates from heterotrophic growth (using low to moderate bacterial growth efficiencies with different C:P ranges) were between 0.1 and 1.2 mmol P m⁻² day⁻¹ (Table 3). The average total biological uptake rate increased from about 0.51 to 0.70 mmol P m⁻² day⁻¹ downstream of the WWTP effluent, equivalent to 50% increase relative to the upstream control ($p < 0.003$) stable at 0.9 mmol P m⁻² day⁻¹. Hence the biota contributed to the same order of magnitude of P uptake as those measured by whole river mass balance and nutrient addition studies. Note that this method did not quantify the additional uptake by the polysaccharide matrix of the biofilm.

Chlorophyll *a* accumulation rates on artificial substrates, placed in the channel during experimental periods, provided

Table 3 – Gross primary productivity (GPP), ecosystem respiration (ER), in g C m⁻² day⁻¹, and P uptake rates, in mmol P m⁻² day⁻¹, by autotrophs (mostly algae) and heterotrophs (mostly bacteria). Data compare upstream and downstream of the effluent pipe, both before and after-effluent diversion to the stream.

	GPP	ER	Algal P uptake	Low bacterial P uptake	Moderate bacterial P uptake	^a Total biological P uptake
Before diversion						
Upstream						
16 Aug	1.0	4.0	0.25	0.12	1.14	0.88
17 Aug	1.0	4.4	0.26	0.13	1.25	0.95
Downstream						
16 Aug	0.6	2.2	0.16	0.06	0.60	0.49
17 Aug	0.7	2.3	0.18	0.07	0.62	0.52
After diversion						
Upstream						
07 Sep	1.5	3.8	0.41	0.10	0.96	0.94
08 Sep	1.2	3.3	0.33	0.09	0.86	0.80
09 Sep	1.4	3.3	0.36	0.09	0.84	0.83
10 Sep	1.6	3.5	0.41	0.09	0.87	0.89
Downstream						
07 Sep	1.2	3.1	0.31	0.08	0.80	0.75
08 Sep	0.8	2.9	0.22	0.08	0.81	0.67
09 Sep	1.0	2.9	0.26	0.08	0.77	0.69
10 Sep	1.1	2.9	0.30	0.08	0.75	0.71

^a Algal P uptake + average bacterial P uptake.

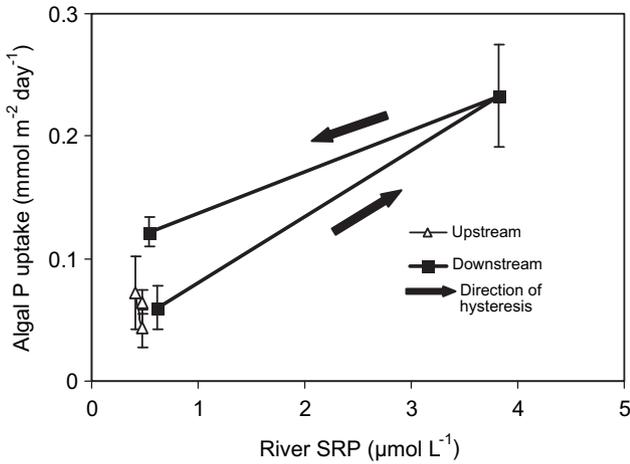


Fig. 4 – Change in benthic algal P uptake (1 standard error bars) as derived from the biofilm accrual experiment, relative to water column SRP during the experimental period for upstream and downstream sites of reach 1.

a direct biological measure. The algal P uptake rate (0.05–0.23 mmol P m⁻² day⁻¹), derived from this chlorophyll *a* accumulation, was remarkably comparable to the whole river GPP measurements. Downstream of the WWTP effluent algal P uptake was greatly enhanced by the elevated water SRP concentrations generated during effluent discharge (Fig. 4). However, algal P uptake continued to be stimulated after-effluent discharge ceased, possibly due to the initial impact of river bed P release during the initial period of algal colonisation on the bricks, and was significantly greater than downstream prior to effluent discharge (*t*-test; *p* = 0.02), or the

upstream control (*p* = 0.04). These results were similar to other short-term bioassays (Francoeur, 2001) but differ somewhat from the whole stream GPP which did not respond to the WWTP effluent. The river bed biofilm was most likely not limited by P as inferred by the nutrient addition studies. Mature biofilms may trap sufficient nutrient in the polysaccharide matrix (Freeman and Lock, 1995; Battin et al., 2003), however this would not be the case in the short-term algal assays.

We also used a flow-through column sediment sorption system to describe uptake and release of sorbed P (Fig. 5). The column SRP uptake at 20 °C (as compared to average in-situ water temperatures of 13 °C) and without biocide describes the combined abiotic sorption and biological (bacterial) uptake of P for the sediment exposed to SRP concentrations equal to in-river concentrations during effluent pipe flow. The two saturated sediment columns, following pre-conditioning (see methods), were exposed to elevated concentrations of 3.8 µmol L⁻¹ SRP (Fig. 5a). The rapid increase in eluant SRP concentration on the sorption period integrates sediment SRP sorption, bacterial uptake and hydraulic interactions between column mobile and immobile flow regions. Column outflow concentrations reached a plateau at an average of 3.0 µmol L⁻¹ within 300–400 pore volumes. The assumption is then applied that the concentration difference (inflows – outflows = 0.8 µmol P L⁻¹) multiplied by the flow rate indicated a biological uptake rate of 1.4 µmol P day⁻¹ per column (48 g sediment). The basis for this was the premise that (i) the concentration plateau represented a state of near maximum abiotic sorption, (ii) that any biotic (bacterial) kinetics of P uptake greatly exceeded the remaining slow kinetic abiotic P sorption, and (iii) that the difference in inflow and outflow concentrations must be due to biotic (bacterial) P uptake (at steady-state).

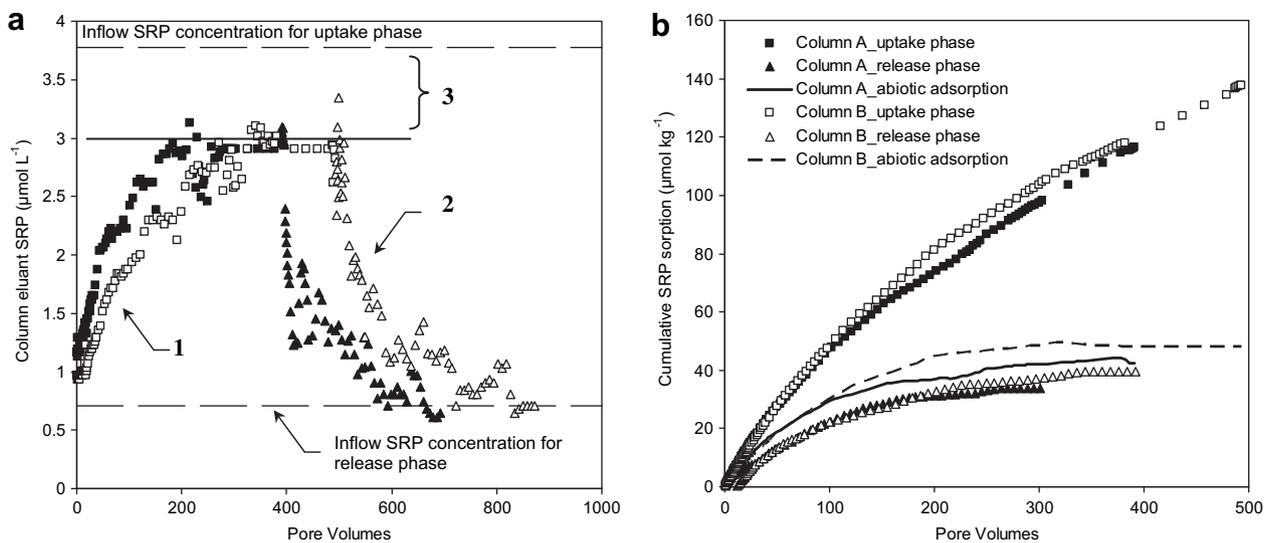


Fig. 5 – Sediment SRP uptake and release phases for duplicate sediment laboratory flow column experiments, expressed in terms of (a) column eluant concentrations (dashed parallel lines indicate the electrolyte SRP concentrations) and (b) cumulative P uptake/release with and without subtraction of the biotic uptake rate. Numbered annotations in part (a) indicate: 1, uptake phase (square symbols); 2, release phase (triangles); 3, observed plateau in outflow concentrations and difference compared to high SRP inflow concentrations used to infer the heterotrophic P uptake rate.

Since the column oxygen supply ($0.4 \text{ mmol O}_2 \text{ day}^{-1}$; flow rates $\times \text{O}_2$ water solubility at 20°C) did not limit biological P consumption, this experimental system provided an estimate of potential heterotrophic P uptake at the surface of oxygenated bed sediments. Using a sediment density (from packed column measurements) of 32 kg per m^2 of river bed for a 2 cm deep layer of sediment (as in Fischer and Pusch, 2001) infers an 'in-river' biological uptake rate of $0.9 \text{ mmol P m}^{-2} \text{ day}^{-1}$ from this column system. This is comparable to P uptake based on ER ($0.1\text{--}1.2 \text{ mmol P m}^{-2} \text{ day}^{-1}$). Using the background electrolyte of $0.5 \text{ mmol L}^{-1} \text{ CaCl}_2$ (matching river Ca concentrations) and with a sediment Ca content of 28 g kg^{-1} there should be no P precipitation in the column. When the P uptake/release is expressed as cumulative mass (Fig. 5b) then only 29% of the total P uptake was released using these flow volumes. However, when we subtracted the biological (bacterial) uptake rate from the cumulative column sorption we found close agreement between the resulting abiotic component of sorptive uptake and release. This indicated that there was limited hysteresis in terms of the abiotic component of adsorption and desorption of P onto the sediment exchange surfaces. In future, a more realistic open column flow experiments could be based on intact sediment cores (e.g. Fischer et al., 2002). The depth of the sediment core could be based on the depth profile of redox or oxygen concentrations (including/excluding the anoxic zone to test the triple zone model; House and Denison, 2002, and monitoring competitive metabolic pathways; Mermillod-Blondin et al., 2005).

3.5. Relative contributions to P cycling during effluent diversion

The most accurate and relevant measurements to calculate the contribution of algae, bacteria and abiotic P uptake were the whole stream metabolism (average of before and after-effluent diversion) and nutrient spiralling studies (average of first 24 h) as measured downstream of the WWTP. Based on these estimates, net P uptake by algae, bacteria and sediment were about $0.2 (\pm 0.1)$, $0.4 (\pm 0.3)$, and $1.0 (\pm 0.9) \text{ mmol m}^{-2} \text{ day}^{-1}$. This assumes that abiotic uptake = total uptake – (algal + bacterial uptake). Note again, however, that some of the abiotic uptake can come from the polysaccharide matrix of the biofilm. Therefore it is likely that biotic and abiotic uptakes were more equivalent than our results suggest as our methodology may have ascribed some biological P uptake in with the in-situ sediment uptake value. This would explain the small amount of P released in the recovery period (most likely due to fast abiotic P release).

4. Conclusions

Whole ecosystem P behaviour as measured in-situ differs markedly from closed system studies. The batch studies showed unrealistic results and their use should probably be limited to the EPC_0 values for suspended sediment or water column studies (rather than bed sediment–water interactions). Where in-situ whole river studies (nutrient additions, stream metabolism) are not practically feasible (e.g. large river) then, open flow-through columns or flumes should be

used. In practice the whole system mass balance study is the simplest approach but it requires continuous monitoring of the WWTP effluent (discharge and pollutant concentration) to reduce the uncertainties reported here. We have shown that biotic and abiotic uptake was of the same order of magnitude with both bioassays and whole river studies based on metabolic activities. Further work should be carried out to reduce our reported uncertainties. Differences in behaviour were probably related to differences in acclimation and history of the systems (e.g. new versus old biofilm, dry versus wet sediment).

Despite some inherent limitations in the present study, it is the first experiment involving a WWTP that has been able to separate the role of the biota from the role of abiotic river bed by combining complementary methods. These independent methods also provided good agreement. We have shown that, even in response to relatively small effluent point sources, ecosystem P retention occurs over several kilometres and requires considerably longer than exposure times for P release under low stable flow conditions. The internal cycling of P involves autotrophic and heterotrophic components. The prolonged period of P retention following even short exposure times to elevated P means (i) limited potential for periods when ecosystem P saturation may decline and (ii) decreasing downstream buffering for cumulative sources. Periods of sediment scour by storms perhaps represent one of few opportunities for reducing internal P status and show the importance of managing and maintaining appropriate fluvial dynamics. However, such entrained sediment P also has implications for downstream water quality.

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Appendix. Supplementary material

Supplementary material can be found, in the online version, at doi:10.1016/j.watres.2010.06.014.

REFERENCES

- Allen, J.A., Scaife, P.H., 1966. The Elovich equation and chemisorption kinetics. *Aust. J. Chem.* 19, 2015–2023.
- Ball, R.C., Hooper, F.F., 1963. Translocation of Phosphorus in a Trout Stream Ecosystem. In: Schultz, V., Klement, A.W.J. (Eds.), *Radioecology*. Reinhold, New York, pp. 217–228.

- Battin, T.J., Kaplan, L.A., Newbold, J.D., Hansen, C.M.E., 2003. Contributions of microbial biofilms to ecosystem processes in stream mesocosms. *Nature* 426, 439–442.
- Battin, T.J., Kaplan, L.A., Findlay, S., Hopkinson, C.S., Marti, E., Packman, A.I., Newbold, J.D., Sabater, F., 2008. Biophysical controls on organic carbon fluxes in fluvial networks. *Nat. Geosci.* 1, 95–100.
- Bernot, M.J., Tank, J.L., Royer, T.V., David, M.B., 2006. Nutrient uptake in streams draining agricultural catchments of the midwestern United States. *Freshwater Biol.* 51, 499–509.
- Bowman, M.F., Chambers, P.A., Schindler, D.W., 2005. Changes in stoichiometric constraints on epilithon and benthic macroinvertebrates in response to slight nutrient enrichment of mountain rivers. *Freshwater Biol.* 50, 1836–1852.
- CEC, 1991. Council of European Communities. Directive concerning urban waste water treatment (91/271/EEC). *Off. J. L135/40*.
- Chardon, W.J., Menon, R.G., Chien, S.H., 1996. Iron oxide impregnated filter paper (Pi test): a review of its development and methodological research. *Nut. Cycling Agroecosyst.* 46, 41–51.
- D'Angelo, D.J., Webster, J.R., Benfield, E.F., 1991. Mechanisms of stream phosphorus retention – an experimental study. *J.N. Am. Benthol. Soc.* 10, 225–237.
- Demars, B.O.L., 2008. Whole-stream phosphorus cycling: testing methods to assess the effect of saturation of sorption capacity on nutrient uptake length measurements. *Water Res.* 42, 2507–2516.
- Demars, B.O.L., Harper, D.M., 2005. Water column and sediment phosphorus in a calcareous lowland river and their differential response to point source control measures. *Water Air. Soil. Pollut.* 167, 273–293.
- Demars, B.O.L., Harper, D.M., Pitt, J.-A., Slaughter, R., 2005. Impact of phosphorus control measures on in-river phosphorus retention associated with point source pollution. *Hydrol. Earth Syst. Sci.* 9, 43–55.
- Dorioz, J.M., Pilleboue, E., Ferhi, A., 1989. Phosphorus dynamics in watersheds: role of trapping processes in sediments. *Water Res.* 23, 147–158.
- Doyle, M.W., Stanley, E.H., Harbor, J.M., 2003. Hydrogeomorphic controls on phosphorus retention in streams. *Water Resour. Res.* 39, 1147.
- Ekka, S.A., Haggard, B.E., Matlock, M.D., Chaubey, I., 2006. Dissolved phosphorus concentrations and sediment interactions in effluent-dominated Ozark streams. *Ecol. Eng.* 26, 375–391.
- Fischer, H., Pusch, M., 2001. Comparison of bacterial production in sediments, epiphyton and the pelagic zone of a lowland river. *Freshwater Biol.* 46, 1335–1348.
- Fischer, H., Sachse, A., Steinberg, C.E.W., Pusch, M., 2002. Differential retention and utilization of dissolved organic carbon by bacteria in river sediments. *Limnol. Oceanogr.* 47, 1702–1711.
- Francoeur, S.N., 2001. Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. *J.N. Am. Benthol. Soc.* 20, 358–368.
- Freeman, C., Lock, M.A., 1995. The biofilm polysaccharide matrix: a buffer against changing organic substrate supply? *Limnol. Oceanogr.* 40, 273–278.
- Gainswin, B.E., House, W.A., Leadbeater, B.S.C., Armitage, P.D., Patten, J., 2006. The effects of sediment size fraction and associated algal biofilms on the kinetics of phosphorus release. *Sci. Total Environ.* 360, 142–157.
- Gücker, B., Brauns, M., Pusch, M.T., 2006. Effects of wastewater treatment plant discharge on ecosystem structure and function of lowland streams. *J.N. Am. Benthol. Soc.* 25, 313–329.
- Gücker, B., Pusch, M.T., 2006. Regulation of nutrient uptake in eutrophic lowland streams. *Limnol. Oceanogr.* 51, 1443–1453.
- Gundersen, K., Heldal, M., Norland, S., Purdie, D.A., Knap, A.H., 2002. Elemental C, N, and P cell content of individual bacteria collected at the Bermuda Atlantic Time-Series Study, (BATS) site. *Limnol. Oceanogr.* 47, 1525–1530.
- Haggard, B.E., Stanley, E.H., Hyler, R., 1999. Sediment-phosphorus relationships in three northcentral Oklahoma streams. *Trans. ASAE.* 42, 1709–1714.
- Haggard, B.E., Storm, D.E., Stanley, E.H., 2001. Effect of a point source input on stream nutrient retention. *J. Am. Water Resour. Ass.* 37, 1291–1299.
- Haggard, B.E., Stanley, E.H., Storm, D.E., 2005. Nutrient retention in a point-source-enriched stream. *J.N. Am. Benthol. Soc.* 24, 29–47.
- Hall, R.O., Tank, J.L., 2003. Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park. *Wyoming. Limnol. Oceanogr.* 48, 1120–1128.
- House, W.A., 2003. Geochemical cycling of phosphorus in rivers. *Appl. Geochem.* 18, 739–748.
- House, W.A., Denison, F.H., Armitage, P.D., 1995. Comparison of the uptake of inorganic phosphorus to a suspended and stream bed-sediment. *Water Res.* 29, 767–779.
- House, W.A., Denison, F.H., 2002. Exchange of inorganic phosphate between river waters and bed-sediments. *Environ. Sci. Technol.* 36, 4295–4301.
- Jarvie, H.P., Jürgens, M.D., Williams, R.J., Neal, C., Davies, J.J.L., Barrett, C., White, J., 2005. Role of river bed sediments as sources and sinks of phosphorus across two major eutrophic UK river basins: the Hampshire Avon and Herefordshire Wye. *J. Hydrol.* 304, 51–74.
- Jarvie, H.P., Neal, C., Williams, R.J., Sutton, E.J., Neal, M., Wickham, H.D., et al., 2002. Phosphorus sources, speciation and dynamics in the lowland eutrophic River Kennet, UK. *Sci. Total Environ.* 282–283, 175–203.
- Jarvie, H.P., Neal, C., Withers, P.J.A., 2006. Sewage effluent phosphorus: a greater risk to river eutrophication than agricultural phosphorus? *Sci. Total Environ.* 360, 246–253.
- Kahlert, M., 1998. C:N:P ratios of freshwater benthic algae. *Arc. Hydrobiol. Adv. Limnol.* 51, 105–114.
- Lottig, N.R., Stanley, E.H., 2007. Benthic sediment influence on dissolved phosphorus concentrations in a headwater stream. *Biogeochemistry* 84, 297–309.
- Marzolf, E.R., Mulholland, P.J., Steinman, A.D., 1994. Improvements to the diurnal upstream–downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. *Can. J. Fish. Aquat. Sci.* 51, 1591–1599.
- McDowell, R.W., 2003. Sediment phosphorus chemistry and microbial biomass along a lowland New Zealand stream. *Aquat. Geochem.* 9, 19–40.
- Mermillod-Blondin, F., Mauclair, L., Montuelle, B., 2005. Use of slow filtration columns to assess oxygen respiration, consumption of dissolved organic carbon, nitrogen transformations and microbial parameters in hyporheic sediments. *Water Res.* 39, 1687–1698.
- Moss, B., Balls, H., Booker, I., Manson, K., Timms, M., 1988. Problems in the construction of a nutrient budget for the River Bure and its Broads (Norfolk) prior to its restoration from eutrophication. In: Round, F.E. (Ed.), *Algae and the Aquatic Environment*. Biopress, Bristol, pp. 327–353.
- Mulholland, P.J., Helton, A.M., Poole, G.C., Hall, R.O., Hamilton, S.K., Peterson, B.J., Tank, J.L., Ashkenas, L.R., Cooper, L.W., Dahm, C.N., Dodds, W.K., Findlay, S.E.G., Gregory, S.V., Grimm, N.B., Johnson, S.L., McDowell, W.H., Meyer, J.L., Valett, H.M., Webster, J.R., Arango, C.P., Beaulieu, J.J., Bernot, M.J., Burgin, A.J., Crenshaw, C.L., Johnson, L.T., Niederlehner, B.R., O'Brien, J.M., Potter, J.D., Sheibley, R.W., Sobota, D.J., Thomas, S.M., 2008. Stream denitrification across

- biomes and its response to anthropogenic nitrate loading. *Nature* 452, 202–205.
- Newbold, J.D., Elwood, J.W., O'Neill, R.V., Sheldon, A.L., 1983. Phosphorus dynamics in a woodland stream ecosystem: a study of nutrient spiralling. *Ecology* 64, 1249–1265.
- Newbold, J.D., Bott, T.L., Kaplan, L.A., Dow, C.L., Jackson, J.K., Aufdenkampe, A.K., Martin, L.A., Van Horn, D.J., de Long, A.A., 2006. Uptake of nutrients and organic C in streams in New York City drinking-water-supply watersheds. *J.N. Am. Benthol. Soc.* 25, 998–1017.
- Rodda, J.C., 2007. Sustaining water resources in South East England. *Atmos. Sci. Lett.* 7, 73–77.
- Slavik, K., Peterson, B.J., Deegan, L.A., Bowden, W.B., Hershey, A.E., Hobbie, J.E., 2004. Long-term responses of the Kuparuk River ecosystem to phosphorus fertilization. *Ecology* 85, 939–954.
- Small, G.E., Helton, A.M., Kazanci, C., 2009. Can consumer stoichiometric regulation control nutrient spiraling in streams? *J.N. Am. Benthol. Soc.* 28, 747–765.
- Sobczak, W.V., Cloern, J.E., Jassby, A.D., Müller-Solger, A.B., 2002. Bioavailability of organic matter in a highly disturbed estuary: the role of algal and detrital sources. *Proc. Natl. Acad. Sci. U S A* 99, 8101–8105.
- Stutter, M.I., Langan, S.J., Cooper, R.J., 2008. Spatial and temporal dynamics of stream water and particulate N, P and C forms along a catchment transect, NE Scotland. *J. Hydrol.* 350, 187–202.
- Talling, J.F., Driver, D., 1963. Some problems in the estimation of chlorophyll-*a* in phytoplankton. TID-7633. In: Proceedings, conference of primary productivity measurement, marine and freshwater, Hawaii, 1961. U.S. Atomic Energy Commission, pp. 142–146.
- Taylor, A.W., Kunishi, H.M., 1971. Phosphate equilibria on stream sediment and soil in a watershed draining an agricultural region. *J. Agric. Food Chem.* 19, 827–831.
- Thingstad, T.F., Riemann, B., Havskum, H., Garde, K., 1996. Incorporation rates and biomass content of C and P in phytoplankton and bacteria in the Bay of Aarhus (Denmark) June 1992. *J. Plankton Res.* 18, 97–121.
- Ukpong, M., 2005. Element homeostasis in bacteria. *McNair Res. J. Summer*, 28–29.
- Van Vliet, M.T.H., Zwolsman, J.J.G., 2008. Impact of summer droughts on the water quality of the Meuse river. *J. Hydrol.* 353, 1–17.
- Wade, A.J., Whitehead, P.G., Hornberger, G.M., Jarvie, H.P., Flynn, N., 2002. On modelling the impact of phosphorus stripping at sewage works on in-stream phosphorus and macrophyte/epiphyte dynamics: a case study for the River Kennet. *Sci. Total. Environ.* 282–283, 395–416.
- Withers, P.J.A., Jarvie, H.P., 2008. Delivery and cycling of phosphorus in rivers: a review. *Sci. Total Environ.* 400, 379–395.
- Webster, J.R., Valett, T.P., 2006. Solute Dynamics. In: Hauer, F.R., Lamberti, G.A. (Eds.), *Methods in Stream Ecology*, second ed. Academic Press, San Diego, pp. 145–160.
- Webster, J.R., Newbold, J.D., Thomas, S.A., Valett, H.M., Mulholland, P.J., 2009. Nutrient uptake and mineralization during leaf decay in streams – a model simulation. *Int. Rev. Hydrobiol.* 94, 372–390.
- Young, R.G., Huryn, A.D., 1998. Comment: improvements to the diurnal upstream–downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. *Can. J. Fish. Aquat. Sci.* 55, 1784–1785.

Supporting Information

Analysis

Water samples were analysed for major cations (ICP emission spectroscopy, Agilent 7500ce, Tokyo, Japan), anions (ion chromatography, Dionex DX600, Sunnyvale, California), and automated colorimetry (San++ analyser, Skalar, Breda, the Netherlands) for $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$ and soluble molybdate reactive P (hereby termed SRP), then for total dissolved P and N (TDP and TDN respectively) and dissolved organic carbon (DOC) using an automated persulphate/UV digestion procedure, all according to the manufacturer's standard methods. The colorimetric detection limits were 0.1, 0.01 and 0.001 mg l^{-1} for C, N and P respectively. Dissolved organic nitrogen (DON) was calculated by difference as $\text{TDN} - (\text{NO}_3\text{-N} + \text{NH}_4\text{-N})$ and soluble unreactive P (SUP; approximating to soluble organic P) as $\text{TDP} - \text{SRP}$.

Sediment pH values were determined at 1g : 30 ml ratios in 0.01 mol l^{-1} CaCl_2 . Total C and N contents were determined on milled subsamples of air-dried sediments (Thermo-Finnigan, Flash EA 1112 CN analyser, Milan, Italy). N contents were below detection limits (<0.03%).

Figure S1. Examples of SRP concentration isotherms with fitted Langmuir models showing the lower concentration region. Sediment EPC_0 values were derived from the intercept on the x axis. The experimental periods (A, B, C) and time of sampling are also indicated (according to Table S3).

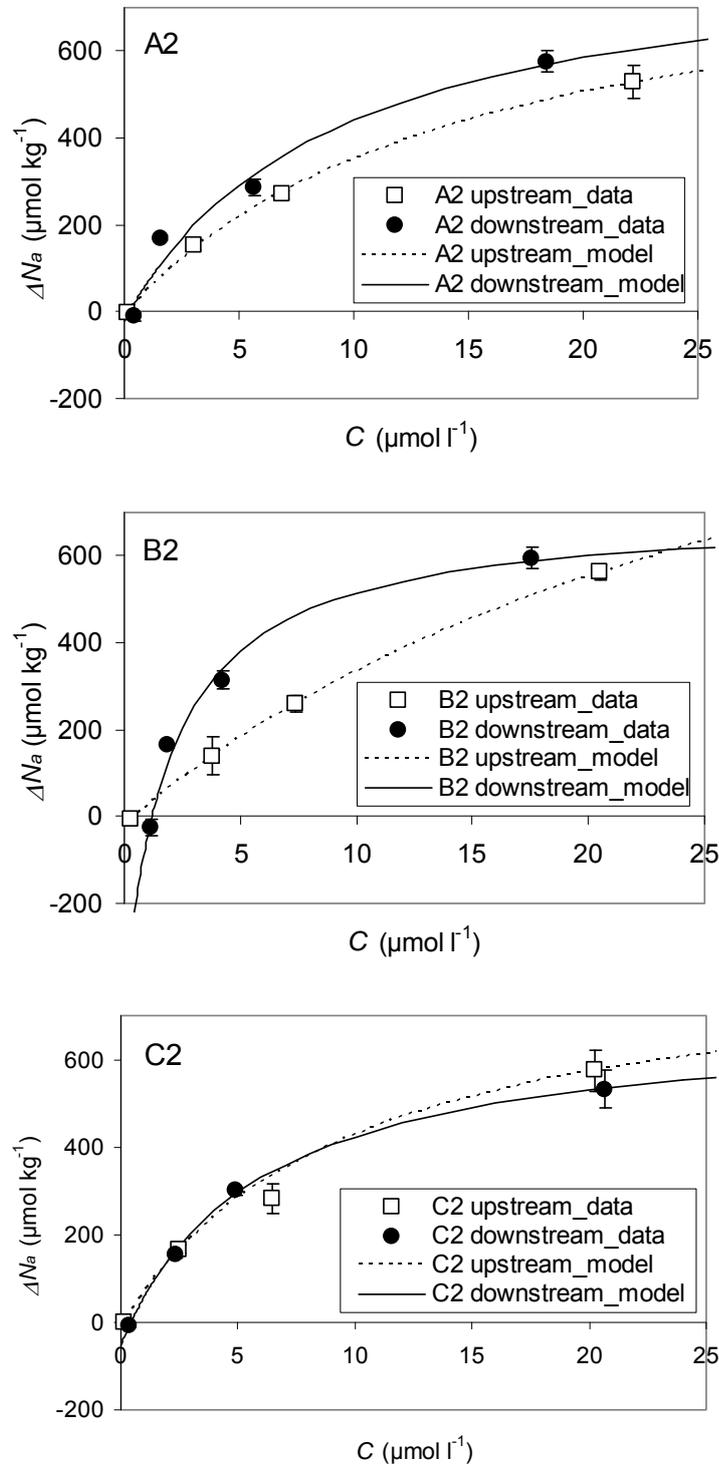


Figure S2. (a) Kinetic isotherm showing rate of SRP uptake by sediments exposed to $3.8 \mu\text{mol L}^{-1}$ SRP (approximating to in-river concentrations during the effluent discharge period) and rate of SRP depletion in the water of the batch. The initial uptake rate $a = 716 \text{ mmol P m}^{-2} \text{ h}^{-1}$, and rate constant $b = 4.08 \text{ m}^2 \text{ mmol}^{-1} \text{ P}$. (b) sediment uptake rate as a function of SRP concentration (see text for conversion of P uptake into areal uptake). (c) sediment uptake velocity (sediment uptake normalised to SRP concentration) as a function of time.

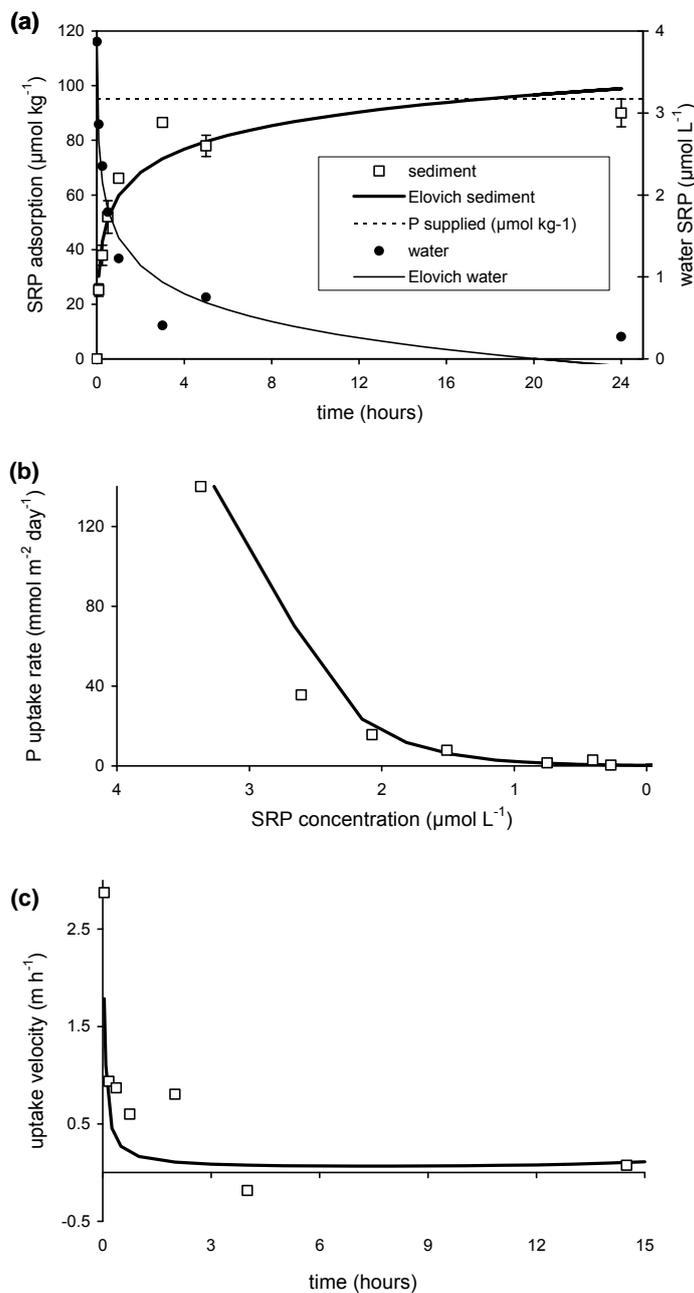


Figure S3. Whole stream metabolism upstream (dark blue) and downstream (black) the WWTP effluent before, during (black bar) and after effluent diversion. Temperature (red), photosynthetic active radiation (PAR, yellow) and discharge (light blue) were also graphed. Note that metabolic activities are not reliable during high flows. Note higher variability in metabolic activity during the first week of effluent diversion. Note that oxygen concentrations were more variable during the first week of effluent diversion due to engineering works.

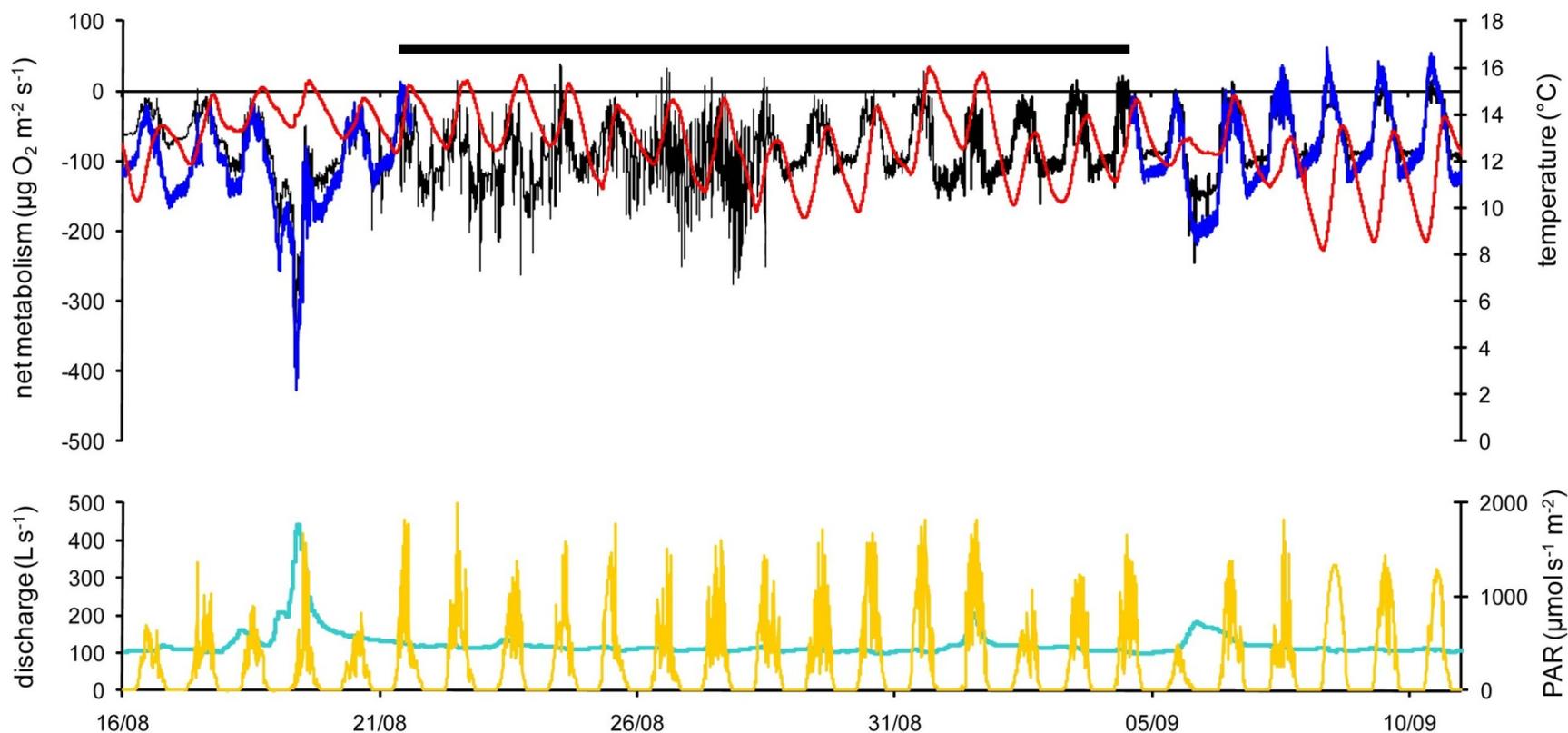


Table S1. Sorption flow column experimental parameters.

Column	A	B
Adsorption phase SRP $\mu\text{mol L}^{-1}$	3.8	3.8
Desorption phase SRP $\mu\text{mol L}^{-1}$	0.7	0.7
Dry sediment weight g	48.1	45.3
Pore volume ml	12.0	9.5
Column flow rate ml min^{-1}	0.5	0.5
Column Peclet number ^a	9.2	10.9

^aAfter SRP sorption experiments, we measured column transport characteristics by recording the breakthrough of a 4.9 ml pulse of $500 \text{ mg Cl}^{-} \text{ dm}^{-3}$ (applied as NaCl and monitored at 5-minute intervals). Chloride concentrations in the effluent fractions were determined by ion chromatography (Dionex 4500i, Sunnyvale, California). The convective-dispersive equation (Chendorain and Ghodrati, 1999) was used to fit parameters D , the dispersion coefficient ($\text{cm}^2 \text{ hour}^{-1}$) and v , the average pore water velocity (cm hour^{-1}) to the breakthrough curves (Figure 3):

$$C_{(L,t)}^f = \frac{(M_R / (Q/1000))L}{2\sqrt{\pi Dt^3}} \exp\left(-\frac{(L-vt)^2}{4Dt}\right), \quad (1)$$

where $C_{(L,t)}^f$ is the flux averaged concentration (mg dm^{-3}), Q is the column flow rate ($\text{cm}^3 \text{ hour}^{-1}$), L is the length of the column (cm), t is the duration until the observation and M_R the recovered tracer mass (mg). The column Péclet number, Pe , was then calculated from:

$$Pe = vL/D. \quad (2)$$

A value of $Pe > 40$ indicates that convective processes dominate and hence our two sediment columns had a similarly moderate degree of dispersive transport. This would act to ‘smear’ the adsorption and desorption phases over time so that column transport was responsible for a small part of the kinetics of sorption depicted for the columns.

Chendorain, M., Ghodrati, M. 1999. Real time continuous sampling and analysis of solutes in soil columns. *Soil Sci. Soc. Am. J.* 63, 464-471.

Table S2. Mean and ranges of river water solutes during periods A (before pipe flow), B (during pipe flow) and C (after pipe flow) at the two locations; Upstream (experimental control) and Downstream (experimental treatment). Concentrations are given as mol l⁻¹ units for P species to correspond with units used for isotherm results, but as mg l⁻¹ for other solutes. Errors for SRP concentrations indicate %relative standard deviations.

Period	River discharge mean (range) L s ⁻¹	NH ₄ -N mg L ⁻¹	NO ₃ -N mg L ⁻¹	DON mg L ⁻¹	DOC mg L ⁻¹	H ⁺ μmol L ⁻¹	SRP μmol L ⁻¹	SUP μmol L ⁻¹	Part P μmol L ⁻¹	SS mg L ⁻¹	River loads mol day ⁻¹	
											SRP	SUP
Upstream												
A		0.04	3.37	0.21	2.49	0.018	0.41 ±28%	0.42	0.26	2.8	4.8	4.8
B		0.03	3.61	0.21	2.15	0.014	0.48 ±22%	0.14	0.64	1.3	5.2	1.3
C		0.03	3.58	0.21	2.27	0.017	0.47 ±20%	0.31	0.14	2.2	4.8	3.2
Downstream												
A	126 (84 - 440)	0.04	3.32	0.21	2.53	0.015	0.62 ±100%	0.44	0.25	1.9	7.7	5.5
B	115 (81 - 204)	0.23	3.84	0.16	2.63	0.023	3.82 ±47%	0.95	0.33	1.7	37.5	9.4
C	118 (88 - 199)	0.05	3.52	0.18	2.53	0.015	0.54 ±56%	0.31	0.19	2.6	5.8	3.2

Table S3. Bed sediment characteristics and Langmuir modelled P sorption properties from batch SRP exchange capacity studies for periods A (before pipe flow), B (during pipe flow) and C (after pipe flow) at the two locations; (a) upstream of the effluent discharge and (b) downstream (reach 1).

Sediment sample	Sampling date	Sediment pH_{CaCl2}	C content g kg⁻¹	FeO test P $\mu\text{mol kg}^{-1}$	N_{max} $\mu\text{mol kg}^{-1}$	N_i $\mu\text{mol kg}^{-1}$	EPC₀ $\mu\text{mol L}^{-1}$	River SRP $\mu\text{mol L}^{-1}$	Uptake/Release^a
(a) Upstream									
A1	11-Aug	6.35	1.9	158	732	13	0.28	0.39	Uptake
A2	15-Aug	6.30	0.9	61	887	8	0.13	0.37	Uptake
A3	17-Aug	6.34	1.2	71	999	39	0.46	0.37	Equilibrium
B1	24-Aug	6.44	1.5	107	1083	49	0.61	0.58	Equilibrium
B2	29-Aug	6.44	1.3	78	1484	20	0.43	0.51	Equilibrium
B3	03-Sep	6.42	2.0	99	1190	7	0.14	0.46	Uptake
C1	11-Sep	6.42	1.9	68	997	11	0.14	0.48	Uptake
C2	14-Sep	6.49	2.0	53	865	14	0.16	0.40	Uptake
C3	21-Sep	6.53	1.6	106	827	8	0.10	0.48	Uptake
(b) Downstream									
A1	11-Aug	6.49	0.8	76	1369	27	0.19	0.69	Uptake
A2	15-Aug	6.46	0.8	78	881	21	0.22	0.41	Uptake
A3	17-Aug	6.48	1.3	142	1243	19	0.20	0.39	Uptake
B1	24-Aug	6.49	0.2	127	925	108	0.44	3.34	Uptake
B2	29-Aug	6.46	1.0	122	1130	429	1.20	4.17	Uptake
B3	03-Sep	6.48	2.1	130	1171	223	0.79	4.05	Uptake
C1	11-Sep	6.45	1.8	105	735	18	0.10	0.63	Uptake
C2	14-Sep	6.53	1.8	146	749	52	0.43	0.41	Equilibrium
C3	21-Sep	6.54	1.7	186	1034	363	0.87	0.50	Release

^aConditions of uptake, equilibrium and release are taken as differences between SRP-EPC₀ of >20%, 20 to -20% and ->20%, respectively (Jarvie et al., 2005).