



The biotic contribution to the benthic stream sediment phosphorus buffer

Z. P. Simpson · R. W. McDowell · L. M. Condon

Received: 7 May 2020 / Accepted: 8 October 2020 / Published online: 17 October 2020
© Springer Nature Switzerland AG 2020

Abstract Benthic stream sediments interact strongly with phosphorus (P) and can buffer dissolved reactive P (DRP) concentrations. The sediment P buffer can be measured with the sediment equilibrium phosphate concentration at net zero sorption (EPC_0), which often correlates well with DRP. Yet, it is unclear how much of this P affinity in sediments is attributable to biotic (microbial P demand) or abiotic (sorption) processes. To clarify the role of biotic processes on EPC_0 , we used two experiments with benthic sediment from 12 streams. First, sediments sterilized by γ -irradiation increased in EPC_0 compared to fresh sediments by a median of 83%. This increase in EPC_0 was likely a result of cell lysis, where microbial biomass P (2.4 to 22.6 mg P kg⁻¹) was re-adsorbed to sediment surfaces. This data also shows that the sediment microbial biomass is a significant, yet under-reported biotic

stock of P in streams compared to their photic zone counterpart (i.e., periphyton). In a second experiment, fresh sediment EPC_0 was measured after alleviating potential limitation of carbon (C) and nitrogen (N) for microbial growth. Sediment EPC_0 did not change with C addition and decreased slightly (0.5 $\mu\text{g P L}^{-1}$ or $\sim 5\%$ decrease) with N addition, suggesting these sediments strongly buffered DRP towards the EPC_0 in spite of biotic demand. Together, these experiments suggest that sediment EPC_0 was primarily abiotic in nature but that sediments may subsidize biotic P requirements through desorption. Further work is needed on whether this relation holds for streams with different substrate, geology, and nutrient inputs.

Keywords Phosphorus · Sediment · Streams · Microbial biomass · Buffer · EPC_0

Responsible Editor: Amy M. Marcarelli.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10533-020-00709-z>) contains supplementary material, which is available to authorized users.

Z. P. Simpson (✉) · R. W. McDowell · L. M. Condon
Department of Soil and Physical Sciences, Lincoln
University, Lincoln, New Zealand
e-mail: zpsimpso@gmail.com

R. W. McDowell
AgResearch, Lincoln Science Centre, Lincoln, New
Zealand

Introduction

Benthic sediments provide much of the phosphorus (P) attenuation observed in streams (Haggard and Sharpley 2007; Hamilton 2012). Sediments contain inorganic particles of varying sizes and geochemical characteristics derived from the parent material, which determine the sites available for P sorption (House 2003). Sediments also contain allochthonous and autochthonous stocks of organic matter (OM; Tank

et al. 2010; Kaplan and Cory 2016), providing fuel for the metabolism of sediment microbial biofilms (Battin et al. 2016). These characteristics make the sediment matrix a hotspot of biogeochemical cycling for P as well as for carbon (C) and nitrogen (N). Hence, when P is removed from the water column at baseflow, it is difficult to pinpoint which biotic and abiotic mechanisms are responsible. Such mechanisms, when known, could inform better nutrient pollution modelling (Macintosh et al. 2018) and subsequent strategies to mitigate the effects of P enrichment (Meals et al. 2010; McDowell et al. 2018; Drohan et al. 2019).

The range of abiotic and biotic mechanisms that remove dissolved reactive P (DRP) from solution and into sediments is diverse. For example, reactive surfaces on particles, such as highly sorptive hydrous metal oxides (Dzombak and Morel 1987; Sposito 2004), often constitute much of the abiotic P sorption capacity and P storage in soils and sediments (Small et al. 2016; Audette et al. 2018; Herndon et al. 2019). Meanwhile, microorganisms entrained in the sediment matrix also require P for their growth and likely supply much of the biotic P uptake for stream sediments (Sinsabaugh et al. 2009; Van Horn et al. 2011; Hill et al. 2012). This biotic P uptake is dependent on stoichiometric demand (Cross et al. 2005; Maranger et al. 2018). While microorganisms beneath the benthic zone (primarily heterotrophs due to lack of light; Battin et al. 2016) can vary in stoichiometric P requirements (Cross et al. 2005; Scott et al. 2012), they approximate a molar C:N:P ratio of 60:7:1 (Cleveland and Liptzin 2007; Hill et al. 2010; Sinsabaugh et al. 2012). This ratio is P-rich relative to that of other lotic biota (Cross et al. 2005) and indicates the potential for sediment microorganisms to moderate stream P cycling if sufficient C and N are available. The distinction between abiotic and biotic sediment P removal is important, however, as not only are their rates and capacities different, they also differ in their responses to other processes within the stream (e.g., varying C and N resource supplies or changes in pH).

Sediments can strongly buffer solution DRP towards a concentration termed the equilibrium phosphate concentration at net zero sorption (EPC_0 ; Froelich 1988). The EPC_0 is measured from a series of batch sediment incubations with solutions of varying initial phosphate concentrations; the concentration where neither net removal nor release of P by the sediment occurs (i.e., the x -intercept from a P

sorption plot) is the EPC_0 (Taylor and Kunishi 1971; Froelich 1988). As such, sediment EPC_0 relates to the potential of a sediment to buffer variable DRP concentrations in the water column. For example, Ekka et al. (2006) showed increases of several $mg\ P\ L^{-1}$ in sediment EPC_0 downstream from point source inputs of DRP relative to sediments immediately upstream, showing how the EPC_0 is elevated with greater loadings of P. In large stream survey studies, McDaniel et al. (2009) and McDowell (2015) found that EPC_0 not only related to P loading (e.g., relatively greater when influenced by point sources or agricultural land use) but also correlated with particle size and geochemical characteristics. That is, given a similar P loading, more (chemically) sorptive sediments will have a lower EPC_0 and thus a greater potential to buffer in-stream DRP concentrations.

EPC_0 does not differentiate between abiotic or biotic mechanisms that control sediment P flux. The sediments used for EPC_0 likely harbor both abiotic and biotic P exchange processes. So, a recurring question for stream P cycling has been: What is the relative contribution of abiotic and biotic processes towards the EPC_0 ? By sterilizing the sediments via varying methods, EPC_0 has been observed to increase dramatically or by very little, with similarly inconsistent changes in P sorption capacity (Meyer 1979; Klotz 1985; Munn and Meyer 1990; Haggard et al. 1999; Lottig and Stanley 2007; McDaniel et al. 2009; Griffiths and Johnson 2018). Unfortunately, such comparisons can suffer from the choice of sterilization method, as several methods cause severe physicochemical changes to the sediment. For example, autoclaving can disrupt soil OM and surface chemistry (Trevors 1996; Buessecker et al. 2019). Measurements of abiotic P sorption will not be representative unless sediment physicochemistry is undisturbed. Further, few EPC_0 comparisons have included corrections for cell lysis following sterilization, where microbial biomass P can release into solution and may adsorb onto the sediment surface (e.g., Klotz 1985; McDaniel et al. 2009).

This study seeks to distinguish biotic and abiotic contributions to stream benthic sediment EPC_0 while minimally disrupting sediment physicochemistry. We account for P in the microbial biomass and determine if removing C- and N-limitation on biotic P demand affects the EPC_0 . Our two hypotheses were that (1) abiotic processes determine EPC_0 and so EPC_0 would

be similar for fresh and sterilized sediments if microbial biomass P were negligible and that (2) microbial P demand, and so the biotic contribution to sediment EPC_0 , would be most pronounced under P-limitation versus C- or N-limitation. For the first experiment, we measured EPC_0 in sediments both fresh and following the use of γ -irradiation to sterilize sediments. For the second experiment, we measured sediment EPC_0 with and without addition of C and N to remove microbial C- and/or N-limitation.

Methods

Sites and sampling

We sampled 12 streams across Banks Peninsula, New Zealand (Fig. 1), thus targeting streams draining a consistent geology (volcanic-basic; Snelder and Biggs 2002) but a variety of land uses, and therefore, variable C, N, and P inputs (Supplementary Table S1). The streams were 2nd to 4th order, with catchment areas

ranging from 3.7 to 51 km². Benthic substrates were generally gravel to gravel/cobble. Catchment land uses for 2018, derived from the New Zealand Land Cover database (v5.0; <https://iris.scinfo.org.nz/>), varied from predominantly native forest and shrub (83%) to predominantly intensive grassland (82%).

Our sampling was designed to accommodate an immediate delivery of sediments to be γ -irradiated with minimal storage time. Hence, we sampled 7 sites one day prior to shipping and five sites on the day of shipping samples for γ -irradiation.

All streams were sampled at summer baseflow conditions (November 2019) with no recent disturbances. Benthic sediments were collected in actively flowing zones, primarily in riffles (i.e., avoiding depositional pools and lateral storage areas). To collect enough mass and to capture the spatial variability in sediments across the channel, we sampled \sim 10 to 30 m of stream length by sampling in a zig-zag pattern. We collected the top 1–5 cm of substrate with a shovel, wet-sieved the material (< 2 mm), decanted excess water after settling, and

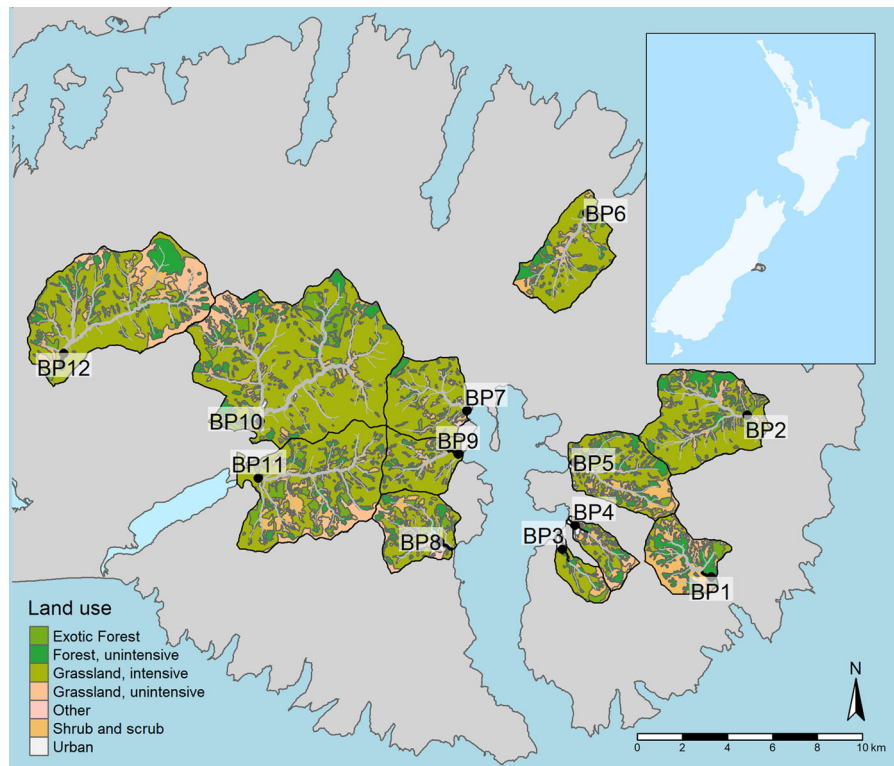


Fig. 1 Study streams on Banks Peninsula, New Zealand. Detailed site information is given in Table S1. Land uses for 2018 were simplified from the LCDB v5.0 database

kept the composite, fine sediment sample (approximately 0.5 to 1 kg per site). A water sample was collected from the thalweg and filtered (0.45 μm) into vials with minimal headspace. Both sediment and water samples were kept on ice and in the dark until they were refrigerated (4 $^{\circ}\text{C}$) in the laboratory and a set of subsamples for stream water were frozen (-20 $^{\circ}\text{C}$). Additionally, we used a HACH HQ40D meter to measure stream temperature, dissolved oxygen (via an optical probe), pH, and specific conductivity in situ; all probes were calibrated on the day of sampling according to manufacturer instructions.

Sterilization via γ -irradiation

To control for potential microbial P uptake, we sterilized sediment subsamples via γ -irradiation, as this method non-invasively kills or damages cells with minimal effects on sediment physicochemistry (McLaren 1969; Trevors 1996; Buessecker et al. 2019). Subsamples from all 12 sediments were γ -irradiated with a nominal 25 kGy dose from a ^{60}Co source (MSD Animal Health, Upper Hutt, New Zealand). The actual dose delivered was later confirmed to be 26 kGy (Dave Harris, MSD Animal Health, pers. comm.). This dose is comparable to that used in previous studies and represents a favorable tradeoff between potential physicochemical disruption of the sediment and effective sterilization (Meyer 1979; Östlund et al. 1989; Qiu and McComb 1995; McNamara et al. 2003; Buessecker et al. 2019).

We managed the logistics of sampling, sterilization, and analyses so that (1) sediments were most reflective of in situ conditions for EPC_0 , (2) sterilized sediments had minimal time for microbes to re-proliferate, and (3) both fresh and sterilized sediments were held under similar conditions throughout prior to analyses. Sediment samples were immediately shipped to be γ -irradiated following collection. Samples were kept cool throughout all handling via freeze-packs (with replacement as needed), thus maintaining temperature comparable to that of the fresh sediments (kept refrigerated in the laboratory at 4 $^{\circ}\text{C}$). Once γ -irradiated, sterilized sediments were returned to the laboratory within 24 h (4 days since sampling). Using aseptic handling for the sterilized sediments, analyses for dehydrogenase activity (DHA; both γ -irradiated and fresh sediments) and for EPC_0 (γ -irradiated)

began immediately (see below). All batch incubations for γ -irradiated sediment EPC_0 were completed within 48 h of sterilization.

Water analyses

Dissolved reactive P was measured on filtered stream samples within 24 h. We used the molybdenum-blue method (Murphy and Riley 1962) with a 5 cm quartz cell (method detection limit of ~ 2 $\mu\text{g P L}^{-1}$). Replicate measurements and external quality-control P standards established relative error at $< 5\%$. Frozen water samples were thawed and immediately analyzed for dissolved organic C (DOC) via a TOC analyzer and for mineral N via flow-injection analysis. Two samples were below detection for $\text{NO}_3\text{-N}$ (0.1 mg N L^{-1}) while all but one sample (0.13 mg N L^{-1}) were below detection for $\text{NH}_4\text{-N}$ (0.1 mg N L^{-1}).

Sediment physicochemical analyses

Throughout, we refer to wet, un-sterilized sediments as ‘fresh’ and wet, sterilized sediments as γ -irradiated. Except where noted, analyses on these sediments are for wet sediments but all values are given on a dry-weight (d.w.) mass basis.

A subsample of sediment was oven-dried (105 $^{\circ}\text{C}$) overnight to measure moisture content. Another subsample was freeze-dried for total elemental and particle size analyses (below) as well as for storage. For the following sediment analyses, we used either duplicate or triplicate measurements. Sediment pH was measured on fresh sediments in DI H_2O with a 1:5 g g^{-1} (sediment:solution) after 30 min equilibration. Water extractable P was measured by shaking fresh sediments with DI H_2O (1:10 g g^{-1}) for one hour, centrifuging (2400 $\times g$ for 10 min), filtering the supernatant (0.45 μm) and analyzing for DRP as above. Anion storage capacity (ASC; Saunders 1965) of sediments was measured by shaking 1 g d.w. of fresh sediment in 5 mL of a 1000 mg P L^{-1} solution (as KH_2PO_4) in an acetate buffer (pH adjusted to 4.60) for 24 h. The remaining DRP in this extract was measured and ASC was expressed as % of the original concentration removed. Sediment particle sizes (on percent volume basis; Eshel et al. 2004) were determined with laser-diffraction (< 1 mm fraction only) with a Malvern Mastersizer 2000 particle size analyzer according to Sperazza et al. (2004). To meet

instrumental constraints for particle size analyses, freeze-dried sediments (un-sterilized only) were sieved to < 1 mm, which represents the majority of the fine sediments collected here. Sediment total C and N were measured on freeze-dried sediments via dry-combustion (Carter and Gregorich 2007) with an Elementar Vario-Max CN elemental analyzer. Sediment total P and metals (Al, Ca, Fe, and others) were measured on freeze-dried sediments via ICP-OES (Varian 720-ES) following a microwave digestion with nitric acid and hydrogen peroxide (USEPA method 3050B; Campisano et al. 2017).

Sediment microbial enzymes and biomass P

We measured dehydrogenase activities (DHA) on both fresh and γ -irradiated sediments as an indicator for (1) microbial metabolic activity in fresh sediments and (2) sterile conditions for γ -irradiated sediments prior to EPC₀ incubations. DHA relates to the breakdown of organic compounds during microbial respiration in soils and sediments (Hill et al. 2002, 2012; Prosser et al. 2011). DHA should be minimal for the γ -irradiated sediments since γ -irradiation eliminates or inactivates most microbes and enzymes (McLaren 1969; Tabatabai 1994). However, we note that some enzymes may persist after γ -irradiation (Powelson and Jenkinson 1976; Blankinship et al. 2014) and the DHA method used here (below) may give false positive readings for the sterilized samples since γ -irradiation can reduce minor amounts of redox-sensitive species (Östlund et al. 1989; Buessecker et al. 2019). Similarly, preliminary tests with autoclaved sediments yielded only a $\sim 75\%$ decrease in DHA relative to fresh sediment DHA. While not perfect, we expected DHA to be greatly reduced for sterilized sediments as a means to ensure that negligible sediment microbial activity occurred during EPC₀ experiments.

For the DHA assay, we used 2,3,5-triphenyltetrazolium chloride (TTC) as the substrate, which is reduced enzymatically to triphenylformazan (TPF) as the product (Tabatabai 1994; Öhlinger and Von Mersi 1996). Two g d.w. of fresh and γ -irradiated sediments were incubated with two mL of a 0.5% (w/v) TTC solution—as recommended for coarse sediments with relatively low organic matter—in a 0.1 M TRIS buffer (pH adjusted to 7.6) for 24 h at 25 °C. The incubation was terminated by adding 10 mL of methanol (AR grade; $\geq 99.8\%$) and vortexing. After centrifuging,

we analyzed the supernatants by measuring absorbances at 485 nm within 1 h with a 1 cm light path. Standards of TPF were prepared in methanol and analyzed in the same fashion. Method blanks were included throughout and sediments were analyzed in triplicate or duplicate (if sample amount was limited). We took care to minimize exposure to light during handling (Öhlinger and Von Mersi 1996). DHA is expressed here as mg TPF kg⁻¹ h⁻¹.

We estimated microbial biomass P in the fresh sediments following the methodology of Brookes et al. (1982) and McLaughlin et al. (1986) as outlined for sediments by McDowell (2003). Notably, we analyzed sediments wet rather than dry, as recommended for soil microbial biomass P analysis by Brookes et al. (1982). Additionally, while clear guidance is lacking on storage times, microbial biomass measurements for soils kept cool are stable for several weeks following initial disturbance effects from sampling (Kouno et al. 1995; Turner and Romero 2010). Hence, our analyses began ~ 3 weeks after sampling. Briefly, 1 g d.w. of fresh sediment was weighed into three centrifuge tubes, in duplicate. To one (P_{killed}), 0.5 mL of liquid chloroform stabilized in amylene was added (rather than as vapor; McLaughlin et al. 1986), capped, and mixed while the other two treatments (P_{fresh} and P_{spike}) were capped—all tubes then incubated at room temperature for 24 h. Each treatment was then extracted with 0.5 M NaHCO₃ (pH adjusted to 8.5) for 30 min, with the P_{spike} treatment receiving an additional P spike in the extraction equivalent to 25 mg P kg⁻¹ sediment. The reactive P in the extracts was measured with a modified molybdenum-blue method suitable for alkaline extracts (Dick and Tabatabai 1977; He and Honeycutt 2005). We note that the P_{fresh} treatment is equivalent to Olsen P, an indicator for bioavailable P. Microbial biomass P (mg P kg⁻¹) was calculated by:

$$\frac{(P_{killed} - P_{fresh})}{K_P} \times \frac{spike}{(P_{spike} - P_{fresh})}$$

where all P values (P_{killed}, P_{fresh}, P_{spike}, and *spike*) are given on a mg P kg⁻¹ basis and K_P is a coefficient for the recovery of microbial biomass P with chloroform killing. Here, we assumed K_P to be 40% (Brookes et al. 1982; McLaughlin et al. 1986; Jenkinson et al. 2004).

Equilibrium phosphate concentrations at net zero sorption (EPC_0) and nutrient treatments

The equilibrium phosphate concentration at net zero sorption (EPC_0) was determined for γ -irradiated sediments and then fresh sediments in close succession (completed within 48 and 96 h of sterilization, respectively). We weighed 0.5 g d.w. of wet sediment (Simpson et al. 2019) into 15 mL centrifuge tubes and added 10 mL of solution (DI water with background of 3 mM $CaCl_2$; Lucci et al. 2010) adjusted to four P concentrations (as KH_2PO_4): 0, 30, 100, and 250 $\mu\text{g P L}^{-1}$. Up to three replicates were used at each concentration depending on the amount of sample available. The tubes were shaken (end over end) for 16 h, centrifuged, and supernatants filtered (0.45 μm). Extracts were then refrigerated until analyzed for DRP within 24 h. Standards for DRP were prepared in the same background matrix (3 mM $CaCl_2$).

For the nutrient addition experiment, we alleviated C- and/or N-limitation during the fresh sediment EPC_0 incubations by adding labile C and/or N to the solutions in a factorial experimental design. We targeted a molar C:N:P ratio of 200:20:1 to create an environment enriched in C and N but limiting in P. Nutrient treatments were + C (C:P of 200:1), + N (N:P of 20:1), C + N (C:N:P of 200:20:1), or 'none' (only P). We added C as D-(+)-glucose and N as KNO_3 to the solutions used for EPC_0 . We also applied the C + N treatment to the γ -irradiated sediment as a check but these data yielded no discernable difference from the standard EPC_0 treatment and so are not discussed for sake of brevity.

Each sediment EPC_0 was then determined as the x -intercept from regressing P sorption (mg P kg^{-1}) against initial P concentration (Simpson et al. 2019). We excluded the points from the 250 $\mu\text{g P L}^{-1}$ treatment, as 100 $\mu\text{g P L}^{-1}$ already provided suitable adsorption points and greater P concentrations only diminishes the linearity of the observed P sorption near the EPC_0 . We estimated the uncertainty in the measured EPC_0 with a 95% confidence interval (Simpson et al. 2019).

Further, under our alternative hypothesis that microbial biomass P can shift EPC_0 following sterilization, we predicted changes in EPC_0 post-sterilization assuming that all microbial biomass P would be re-adsorbed upon lysis. To this end, we calculated the linear sorption slopes on an equilibrium P

concentration basis and projected these slopes according to the microbial biomass P; i.e., we divided microbial biomass P by the equilibrium sorption slope to get the expected change in EPC_0 . This result would be the expected increase in EPC_0 if 100% of microbial biomass P were adsorbed by the sediments while assuming nearly linear adsorption rates. We compared these predictions to the observed changes in EPC_0 following γ -irradiation.

Statistical analyses

Statistical summaries reported here are generally the median (mean \pm SD). However, we do not summarize NH_4 -N data (too many missing values) and summarize NO_3 -N data (two out of 12 values below detection limit) via methods for censored data (Helsel 2005). For NO_3 -N summaries, we apply the Kaplan–Meier method as implemented in the 'NADA' R package (Lee 2020).

For the sterilization experiment (a paired treatment design), we analyzed the changes in EPC_0 ($n = 12$) with a paired Wilcoxon signed rank test. The null hypothesis is that the distribution of pairs has a median of 0 (Hollander et al. 2013). For the alternative case, we estimated the shift in the median with the pseudo-median and its 95% confidence interval. We also examined changes in EPC_0 following γ -irradiation with Spearman correlations versus catchment, sediment, and stream variables.

For the nutrient addition experiment, we sought to test for the effect of nutrients (+ C and + N) and their interaction (C + N) on EPC_0 via a two-way layout ($n = 48$). The facts that (1) EPC_0 were not estimated with the same certainty (i.e., varying confidence intervals) and (2) that the EPC_0 were dependent upon the sediment analyzed (i.e., there are 12 'clusters' in the data) meant that this information needed to be incorporated in our test. Thus, we analyzed this experiment with a mixed-effects model, where sediment could be a grouping variable (random effect). We used nonlinear mixed-effects (R package "nlme"; Pinheiro et al. 2020) so that we could re-write the linear sorption model to have the x -intercept (the EPC_0) as a parameter to be estimated and to test for nutrient effects on EPC_0 directly, making greater use of the data. More details on this approach are in the Supplementary Information.

All analyses were conducted in R, ver. 4.0.2 (R Core Team 2020).

Results

Stream and sediment characteristics

At time of sampling, the streams reflected typical summer baseflow conditions (Table 1). While the streams had appreciable DOC (median, 1.73 mg L⁻¹) and DRP concentrations (49 µg L⁻¹), NH₄-N concentrations were mostly below detection (one stream, BP9, measured 0.13 mg L⁻¹) and NO₃-N concentrations were also low (0.21 mg L⁻¹). Using DOC and NO₃-N, stream water molar C:N had a median of 10.4. The fine (< 2 mm) benthic sediments (Table 2) were sandy, but quite sorptive (median ASC of 56% in a range of 0–100%), as expected for sediments with volcanic geology. Sediment organic matter content varied considerably across the sediment from 12 streams, with mean (± SD) sediment total C and total N concentrations of 12.8 (± 7.1) and 0.86 (± 0.51) g kg⁻¹, respectively. Median sediment C:N was 16.9. Correlation matrices and Spearman correlations for select variables are shown in Supplementary Figs. S1–S3. No strong correlations between catchment land use and stream or sediment physicochemical characteristics were noted.

Table 1 Stream water (*n* = 12) physicochemistry (at time of sampling), dissolved organic C (DOC), nitrate-N (NO₃-N), and dissolved reactive P (DRP)

Variable	Unit	Summary
Temperature	°C	13.7 (13.8 ± 1.5)
DO	% saturation	99.4 (99.6 ± 3.3)
Specific conductivity	µS cm ⁻¹	148 (147 ± 16)
pH	S.U	7.58 (7.54 ± 0.18)
DOC	mg L ⁻¹	1.73 (1.73 ± 0.67)
NO ₃ -N	mg L ⁻¹	0.195 (0.200 ± 0.078)
DRP	µg L ⁻¹	48.6 (48.9 ± 8.7)

Summary values are given as the median (mean ± standard deviation). DO is dissolved oxygen

Table 2 Benthic sediment (*n* = 12 streams) characteristics, total elemental concentrations, extractable P, and sorption as anion storage capacity

Variable	Unit	Summary
pH	S.U	7.03 (6.99 ± 0.149)
Sand	%	79.9 (77.3 ± 9.4)
Silt	%	17.8 (20.2 ± 8.11)
Clay	%	1.95 (2.49 ± 1.32)
Total C	g kg ⁻¹	11.2 (12.8 ± 7.05)
Total N	g kg ⁻¹	0.833 (0.861 ± 0.513)
Total P	g kg ⁻¹	2.18 (2.17 ± 0.48)
Total Fe	g kg ⁻¹	58.4 (61.9 ± 8.6)
Total Al	g kg ⁻¹	41.8 (43.0 ± 9.6)
Total Ca	g kg ⁻¹	8.90 (9.01 ± 1.57)
Water extractable P	mg kg ⁻¹	4.15 (4.38 ± 1.22)
Olsen P	mg kg ⁻¹	26 (27.6 ± 5.1)
Anion storage capacity	%	55.6 (53.5 ± 8.06)

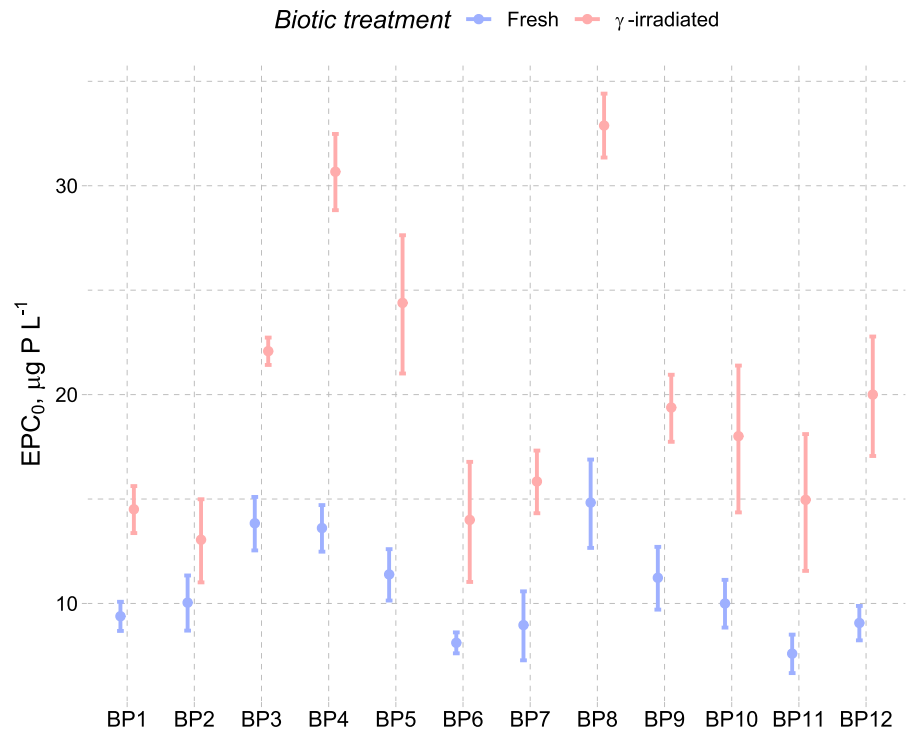
Summary values are given as the median (mean ± standard deviation)

Gamma irradiation experiment

Fresh sediment dehydrogenase activities (DHA) varied widely (Supplementary Fig. S4), with mean activities of 1.89 (± 0.82) mg TPF kg⁻¹ h⁻¹. Fresh sediment DHA was closely associated with sediment total C ($\rho = 0.91$, $p < 0.001$) and total N ($\rho = 0.93$, $p < 0.001$). However, DHA decreased for all samples following γ -irradiation (62 to 86% decrease), commensurate with previous experimental work for soils (McNamara et al. 2003; Gebremikael et al. 2015). We consider the γ -irradiated samples here to have had negligible microbial activity throughout the EPC₀ measurements given that (1) the decreasing trend in DHA with sterilization was consistent and (2) the irradiation dose given (26 kGy) was sufficient to eliminate the majority of microbes and inhibit growth for the timeframe of our experiment (48 h post sterilization; McLaren 1969; Östlund et al. 1989; McNamara et al. 2003).

Fresh sediment EPC₀ ranged from 7.6 to 14.8 µg P L⁻¹ (Fig. 2) and correlated with in-stream DRP concentrations ($\rho = 0.65$, $p = 0.026$). Sediment microbial biomass P averaged 11.5 (± 5.6) mg P kg⁻¹ and correlated closely with fresh EPC₀ ($\rho = 0.87$, $p = 3.1e-4$).

Fig. 2 Effect of γ -irradiation on sediment equilibrium phosphate concentrations at net zero sorption (EPC_0). Bars indicate the 95% confidence interval



Following γ -irradiation, EPC_0 increased for all sediments studied, though 95% confidence intervals for the calculated EPC_0 overlapped for one sediment (BP2; Fig. 2). A paired Wilcoxon signed rank test estimated a median increase in EPC_0 of 8.32 (6.38 to 12.6) $\mu\text{g P L}^{-1}$ following γ -irradiation, an 83% increase relative to the median fresh EPC_0 . Notably, this consistent increase in EPC_0 with sterilization relative to fresh EPC_0 was related to microbial biomass P (Fig. 3a; $\rho = 0.63$, $p = 0.032$), with a regression slope of 0.635 (0.268 to 1.00) $\mu\text{g P L}^{-1}$ per mg P kg^{-1} (the intercept was not different from zero). We note no other clear effect of γ -irradiation on P sorption relative to that of fresh sediments (e.g., changes in sorption slopes or variability in sorption).

We examined the relationship between changes in EPC_0 post-sterilization and microbial biomass P under the hypothesis that shifts in EPC_0 may be due to adsorption of the P flush following cell lysis. The predicted increases in EPC_0 based on the adsorption of the microbial biomass P are plotted against actual changes in EPC_0 following γ -irradiation in Fig. 3b. These independent estimates were consistent with the measured changes in EPC_0 post-sterilization (root

mean square error (RMSE) of 5.22 $\mu\text{g P L}^{-1}$) but were positively biased for larger magnitude changes.

Nutrient amendment experiment

Fresh sediments were analyzed for EPC_0 with additions of glucose and/or KNO_3 solutions to remove C- and/or N-limitation on microbial growth. When considering the measurement uncertainties within a given sediment, EPC_0 did not appear to vary significantly between the nutrient treatments for any one sediment (Fig. 4). However, when modeling the full data set with nonlinear mixed effects, we found a statistically significant decrease in EPC_0 with N addition but no significant change with C addition (Table 3; more modeling details in Supplementary Information). The likelihood-ratio tests and Akaike Information Criterion showed that more complex models with an effect for + C ($p = 0.07$) and the interaction effect (C + N; $p = 0.62$) on EPC_0 did not out-perform the simpler model with only the + N effect. Though statistically significant, the + N effect on EPC_0 was relatively small, with a change in EPC_0 of -0.56 (95% CI -0.81 to -0.31) $\mu\text{g P L}^{-1}$. Higher order effects (e.g., + N effect on EPC_0

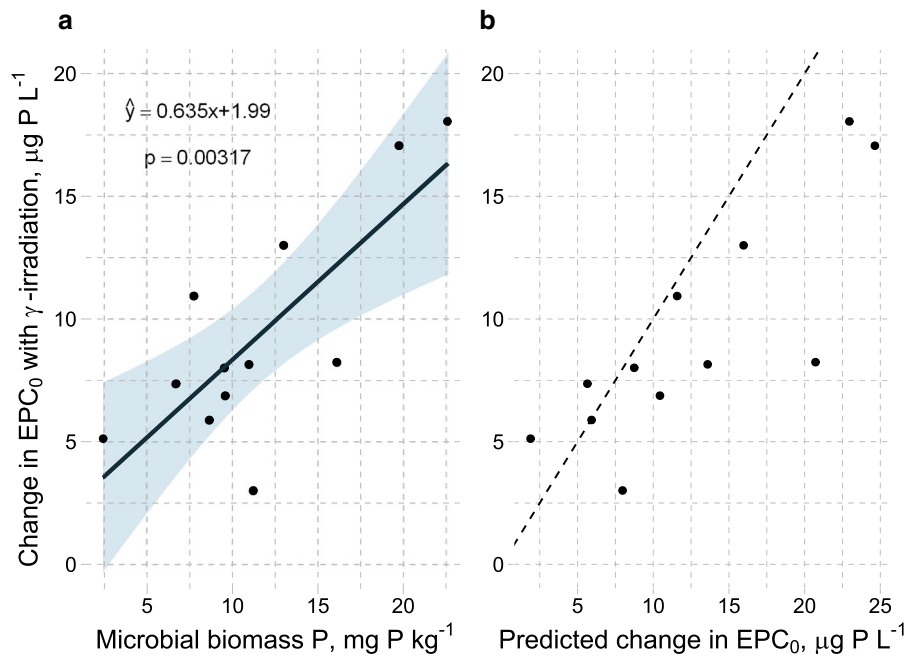


Fig. 3 The increase in sediment EPC₀ due to γ -irradiation (sterilized EPC₀ minus fresh EPC₀) plotted (a) as a function of sediment microbial biomass P. A linear regression with standard error about the fit is shown. This change in EPC₀ with sterilization is also plotted (b) against the predicted change in

EPC₀ based on the adsorption data and the microbial biomass P assuming 100% adsorption of the lysed P (dashed line is the 1:1 line). Note that these estimates could be negatively biased since we extrapolated a linear sorption curve to equilibrium concentrations past the likely range of linear sorption

Fig. 4 Sediment EPC₀ and its 95% confidence interval (via individual linear fits) for sediments from all 12 study streams and for four nutrient treatments: none (i.e., the original EPC₀ method, denoted here as -), + C (C:P of 200:1), + N (N:P of 20:1), and C + N (C:N:P of 200:20:1). Note that γ -irradiated sediments were also analyzed for the C + N treatment but this data did not differ from the original EPC₀ data

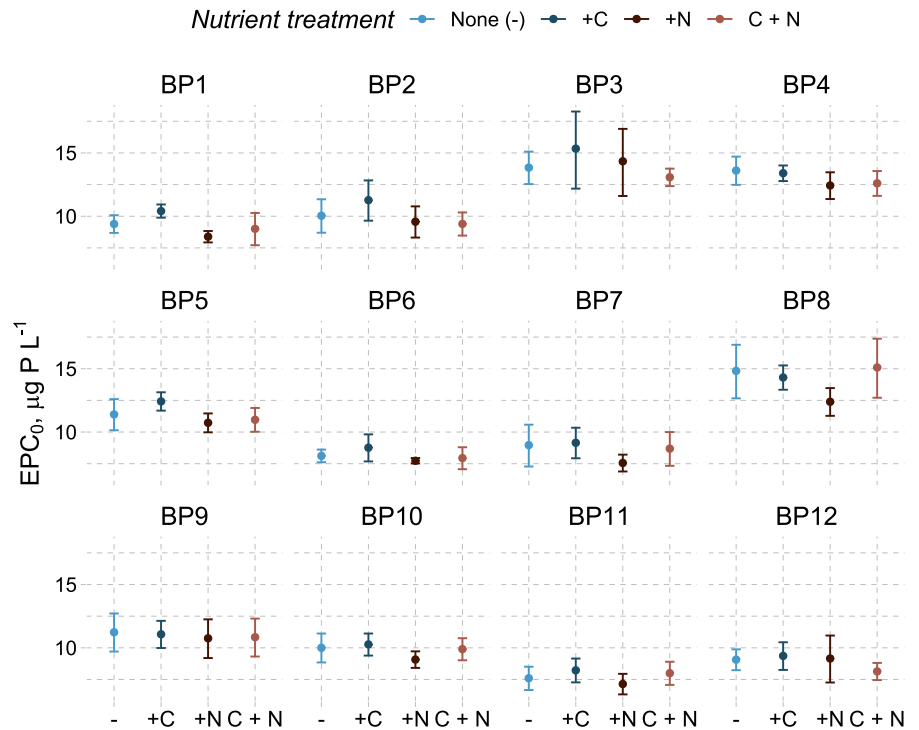


Table 3 Model summary for the nonlinear mixed effects fit for the nutrient amendment experiment

Term	Units	Estimate	95% C.I.
Fixed effects			
EPC ₀	μg P L ⁻¹	10.8	9.58–12.0
N effect on EPC ₀	μg P L ⁻¹	– 0.560	– 0.808 to – 0.312
B	mg P kg ⁻¹ per μg P L ⁻¹	0.01982	0.01976–0.01989
Random effects			
EPC ₀ by sediment	μg P L ⁻¹	2.15	1.43–3.22
Within sediment group error	mg P kg ⁻¹	0.0259	0.0242–0.0277

Fixed effects include the two parameters, EPC₀ (x -intercept) and β (slope), plus a term for the + N effect on EPC₀—other nutrient effects were not significant. Random effects include deviations in EPC₀ per sediment and the remaining within-group error. C.I. is confidence interval

varying per individual sediment) were not warranted by the data, neither were nutrient effects on sorption slopes.

Discussion

Microbial biomass as a stock of phosphorus: lysed P accounts for changes in EPC₀

Upon γ -irradiation, the flush of P from the microbial biomass (median of 10 mg P kg⁻¹) was free to adsorb onto the sediment, increasing EPC₀ (Figs. 2, 3). While this result does not speak to the direct comparison of biotic and abiotic contributions towards sediment P uptake, it does show the potential influence of the sediment microbial biomass as a stock of P in streams. Similar to their contribution towards total P cycling in terrestrial ecosystems (Cleveland and Liptzin 2007; Turner et al. 2013), the microbial biomass represents a significant fraction of the total P in stream ecosystems. Importantly, sediment microbial biomass P is likely a very transient P pool, therefore contributing to P dynamics throughout the stream network (Mulholland et al. 1997; McDowell 2003; McDowell and Sharpley 2003; Cross et al. 2005).

Few data exist on microbial biomass P in stream and river sediments, with ranges (using the same method from Brookes et al. (1982)) varying from: 0.5 to 10 mg P kg⁻¹ in a 3rd to 4th order catchment having intensive forestry and pastoral land use (McDowell 2003), 2.4 to 23 mg P kg⁻¹ in the present study, and 10 to 45 mg P kg⁻¹ in a large river subject to

considerable agricultural and urban pollution sources (Jaiswal and Pandey 2019). Streams with less P inputs will likely have much less sediment microbial biomass P (e.g., site BP1 in this study) though this also depends partly on the sediment (see below). For comparison, soil microbial biomass P concentrations can be an order of magnitude greater, varying from 3 to 430 mg P kg⁻¹ (Cleveland and Liptzin 2007), likely due to the relatively greater organic matter content (Sinsabaugh et al. 2012). Considering that biomass C:P ratios are often more P-rich in sediment microbes (about 60:1; Cleveland and Liptzin 2007; Sinsabaugh et al. 2009) compared to periphyton (roughly on the order of 10² to 10³; Cross et al. 2005), future work could investigate sediment microbial biomass as an important and perhaps variable stock of P in streams.

We assumed all of sediment microbial biomass P would be adsorbed by sediment surfaces and at a linear rate. However, we urge caution in applying this assumption to other sediments, owing to potential nonlinear P sorption (Froelich 1988; House 2003) and that amounts of microbial biomass P likely differ. Nonetheless, the consistency with actual changes in EPC₀ following γ -irradiation corroborates the hypothesis that the microbial biomass P stock can re-adsorb onto sediment surfaces, thereby increasing EPC₀ following sterilization. Since γ -irradiation is the least likely among sterilization methods to alter sediment physicochemical properties (Eno and Popenoe 1964; Berns et al. 2008; Buessecker et al. 2019), we can reasonably assert that the re-distribution of P—and the resulting increase in EPC₀—was primarily due to cell lysis (Meyer 1979; Klotz 1985).

Reconciling past experiments on biotic vs. abiotic sediment P uptake

We suspect that our results may explain two of the inconsistencies in past attempts to separate biotic and abiotic P uptake in sediments: sterilization techniques and a lack of microbial biomass P data. Firstly, more disruptive sterilization techniques than γ -irradiation have generally been used, namely, autoclaving and biocides. For example, when analyzing autoclaved sediments, several studies observed impressive differences between P sorption metrics for fresh and autoclaved sediments, concluding that up to $\sim 40\%$ of the total sediment sorption capacity was biotic (Haggard et al. 1999; Ryan et al. 2007; McDaniel et al. 2009). However, autoclaving likely changed the physicochemistry of these sediments since, in addition to cell lysis, autoclaving can disrupt sediment organic matter, alter surface chemistry, and increase some extractable elements such as Al, Fe and Mn (Meyer 1979; Wolf and Skipper 1994; Trevors 1996; Buessecker et al. 2019). In two comparison studies, Meyer (1979) and Klotz (1985) assessed potential biotic influence on sediment P uptake by comparing sediments analyzed fresh, autoclaved, and either γ -irradiated or with a phosphorylation inhibitor (cabonyl-cyanide *m*-chlorophenylhydrazone; CCCP), respectively. They found that autoclaving sediments released more P but also changed P sorption kinetics and capacity. In contrast, γ -irradiated sediments had sorption kinetics comparable to that of fresh sediments but had an increase in EPC_0 (attributed to cell lysis), while CCCP-treated sediments had no change in sorption capacity relative to fresh sediments. Similarly, Triska et al. (2006) found autoclaved sediments released more than $10 \times$ more P than fresh sediments in desorption assays and retained $\sim 40\%$ less P in sorption assays whereas a CCCP-treated sediment was comparable to fresh sediment for both sorption and desorption.

Aside from autoclaving, various biocides (e.g., $HgCl_2$, azide) have been applied to separate biotic and abiotic P uptake in stream sediments. Results with biocides have ranged from small increases in EPC_0 ($2 \mu g P L^{-1}$; Griffiths and Johnson 2018) to large or unrealistic changes in EPC_0 and sorption capacities (Lottig and Stanley 2007; Stutter et al. 2010). However, little is known about the potential unintended chemical interactions these biocides have with

sediment surfaces. For example, $HgCl_2$ can react with organic matter, altering solution pH in the process (Buessecker et al. 2019), as noted by Griffiths and Johnson (2018) in their fresh vs. sterilized EPC_0 comparison. Buessecker et al. (2019) compared multiple methods to remove only the biotic mechanisms for soil N_2O production and concluded γ -irradiation to be the most suitable, as other methods (autoclaving, azide, $HgCl_2$, Zn, and chloroform) produced more physicochemical changes relative to fresh soil. We argue that such effects also apply to stream sediments. Neither autoclaving nor biocides will likely be accurate in removing solely the biotic reactions responsible for sediment P uptake. Thus, while not always acknowledged, conclusions for biotic and abiotic sediment P uptake reliant on autoclaving or biocides are most likely inaccurate.

In addition to disruptive sterilization methods, a second source for error in past estimates of biotic contributions to sediment P uptake was a lack of microbial biomass P data. As discussed, the microbial biomass can release a substantial flush of P upon lysis (Fig. 3a) that can shift the equilibrium P concentration considerably. If our alternative hypothesis is correct—that the increase in EPC_0 with non-disruptive sterilization is due to re-adsorption of the microbial biomass P following cell lysis (Fig. 3b)—then we predict that the effect of sterilization would be even more pronounced for sediments with comparable microbial biomass P but weaker sorption capacities. Owing to their volcanic geology and high metal-oxide content (Dahlgren et al. 2004), our study sediments were relatively reactive and had high P sorption capacities (mean ASC of 54%); for comparison, the mean ASC from a national survey of sediments from 76 large New Zealand rivers was 9% (McDowell 2015). Interestingly, Munn and Meyer (1990) compared fresh and γ -irradiated sediment EPC_0 for two streams: one from a volcanic geology and one from a weathered granite geology. While both sediments had similarly low EPC_0 (5.4 and $1.0 \mu g P L^{-1}$, respectively), the increases post-sterilization were respectively 3.2 and $9.7 \mu g P L^{-1}$, consistent with a greater P sorption capacity in the volcanic geology. This increase was attributed to a predominance in biotic P uptake with no acknowledgement of possible bias due to the microbial biomass P.

Does carbon and nitrogen addition promote biotic P uptake and lower EPC₀?

Microorganisms in sediments demand nutrients according to the elemental stoichiometry of their biomass (Sterner and Elser 2002; Sinsabaugh et al. 2009). In contrast to lotic biofilm communities towards the top of the benthic zone (i.e., where light promotes autotrophic growth; Battin et al. 2016), and unlike for soil, sediment microbial communities (primarily heterotrophic) are more often C- or N-limited rather than P-limited (Hill et al. 2010, 2012; Sinsabaugh et al. 2012). Hence, we would only expect significant sediment biotic P uptake when C- and N-limitation is relieved. For sediments, microbial P-limitation is likely when C:P (molar basis) exceeds 60:1 and when N:P exceeds 7:1 (Cleveland and Liptzin 2007; Sinsabaugh et al. 2012). Under P-limitation, we expect that sediment microbes would sequester P for growth, thus potentially lowering the sediment EPC₀ through desorption. In other words, under P-limitation, sediment microbes may deplete P concentrations in solution below the sediment EPC₀, promoting P desorption from sediment surfaces and potentially lowering sediment EPC₀.

In this study, EPC₀ did not respond to C addition but decreased with N addition in both the + N and C + N treatments ($-0.5 \mu\text{g P L}^{-1}$; Fig. 4; Table 3). The + N effect here may have been dampened due to the large P (de)sorption capacity of these sediments (Small et al. 2016; Griffiths and Johnson 2018), as sediments display the greatest P buffering when near the EPC₀ (Froelich 1988). Indeed, the literature notes varied responses in P uptake to N supply. Using sediments in flume experiments at natural stream temperature, McDowell et al. (2017) observed relatively greater DRP uptake with N additions, a likely biotic effect since there was also a consistent increase in sediment microbial biomass P for the treatments with additional N. In contrast, Griffiths and Johnson (2018) measured stream P uptake rates while varying the background NO₃-N concentration: despite evidence for strong N- and P-colimitation, in-stream P uptake was unaffected by background N concentration, suggesting that stream P uptake was predominantly controlled by abiotic sediment P sorption rather than biotic P uptake. In a stream with plentiful N supply (mean of $1.1 \text{ mg NO}_3\text{-N L}^{-1}$), Oviedo-Vargas et al. (2013) measured P spiraling and sediment EPC₀

during an experimental amendment of labile C (acetate) $\sim 1 \text{ mg C L}^{-1}$ above ambient conditions to stimulate sediment heterotrophic P demand. Despite greater C uptake during the experiment, P uptake was insensitive to the C addition (as whole-stream P uptake velocities and as sediment EPC₀). In their study, Oviedo-Vargas et al. (2013) suggested that the predominantly heterotrophic microorganisms within the sediments were meeting their P demands through organic P mineralization or from sediment P desorption rather than from the water column. Similarly, the sediments in the present study buffered solution P towards the EPC₀ through abiotic sorption processes in spite of biotic P uptake.

A reason for the response to N but not C may lie in the fact that labile C can increase microbial respiration (Roberts et al. 2007; Oviedo-Vargas et al. 2013; Demars 2019) but will not directly affect P concentrations. It is microbial production, not respiration, that carries a P demand (Sterner and Elser 2002). Sediment microorganisms here may have had enough C supply available to meet their demands for production, thereby not influencing EPC₀ for the + C treatment. For example, sediment biofilms can hold over C in their extracellular polymeric substances (Battin et al. 2016) or enzymatically degrade organic substrates (Findlay et al. 2003; Sinsabaugh et al. 2009; Kaplan and Cory 2016) to meet C requirements. Most of the catchments in our study had some amount of pasture (35 to 82% for sites BP2 through BP12), which may provide an ample source of C when soil pore waters hydrologically connect to the streams (Kaplan and Cory 2016; McNally et al. 2017). Additionally, these pasture-dominated catchments had diminished stream canopy cover, meaning likely greater autochthonous C supplies for the stream ecosystem due to increased primary productivity (Dodds 2007; Finlay 2011). This may have supplied sufficient high-quality C resources (e.g., from algal biomass turnover; Meyer 1994; Kaplan and Cory 2016) to the sediments to prevent any C-limitation before EPC₀ measurements.

Our results from labile C and N additions to EPC₀ measurements suggest little to no influence of biotic P demand on EPC₀ for these streams, with only a small overall effect arising from the removal of potential N-limitation (Table 3). Coupled with the results of the first experiment—that much of the apparent differences in P sorption between fresh and sterilized sediments is due to the microbial biomass P flush—

this experiment suggests that sediment biotic P demand may not be a significant part of the EPC_0 as it is typically measured, i.e., with only P added. Rather, the benthic sediment EPC_0 may primarily reflect the sediment's abiotic sorption characteristics and prior P loading to that sediment (Froelich 1988; McDaniel et al. 2009; McDowell 2015). Our results come from streams that are likely N-limited and that drain a naturally P-rich geology. For other streams, however, if sediments are poorly sorptive (e.g., little Al/Fe oxides or coarse particle sizes) and P availability is low, then biota may have a relatively stronger influence on P uptake in sediments (Lottig and Stanley 2007). The dynamic between P sorbed to sediments and P immobilized in microbial biomass should be explored in other systems that vary in stream geomorphology, sediment characteristics, and nutrient inputs.

Improving our understanding of the role of microbes in stream P attenuation

The results from this study help to separate some of the processes usually lumped together in sediment P sorption and, at the greater scale, P attenuation in streams. Characterizing these processes more clearly will help target future research on sediment P reactions, for example, in determining the role of microbial biomass as a stock of P and its relationship with the abiotic sediment P compartment. When one measures EPC_0 , the EPC_0 is unlikely to be affected by biotic uptake partly since P is often not the limiting nutrient for sediment heterotrophic microbes. Rather, EPC_0 is largely a function of the sediment reactive surfaces and the previous exposure of P to that sediment. Integrating this information and more biota-specific P reactions with hydrological fluxes will improve our understanding of stream and catchment-scale P biogeochemistry (Manzoni and Porporato 2011).

Sediment EPC_0 provides information on how sediments subsidize available P for microbial growth and so influence stream P biogeochemistry. Sediments may either maintain or accumulate more sorbed P ($DRP \geq EPC_0$) but, when local DRP concentrations are depleted through *inter alia* microbial P uptake ($DRP < EPC_0$), sediments may desorb P so nearby microorganisms can meet their stoichiometric constraints. If sediments drip feed P—according to the

EPC_0 —to 'P-hungry' sediment biofilms (particularly heterotrophic bacteria), then we could expect a positive relationship between EPC_0 and microbial biomass P. Indeed, the two variables had a strong positive correlation ($\rho = 0.87$, $p = 3.1e-4$), suggesting that sediment microorganisms play a role in regulating P desorption from sediments back into the water column. This coincides with what we know on the coupled biogeochemical cycling of C, N, and P in streams (Oviedo-Vargas et al. 2013; Maranger et al. 2018) and has important consequences. For example, the P subsidy provided by stream sediments (as approximated by EPC_0) may promote greater C and N processing in streams (Stelzer et al. 2003; Findlay and Sinsabaugh 2006; Tank et al. 2010). Another consequence, however, is that this P supply could support a large sediment biofilm, meaning the biofilm may clog hyporheic flowpaths (Hartwig and Borchardt 2015; Battin et al. 2016) thus limiting the interaction between reactive sediment surfaces and P in the water-column (Boano et al. 2014; Weigelhofer et al. 2018). Further exploring the sediment P buffer will likely aid us in better characterizing stream P biogeochemistry and its consequences (Hamilton 2012).

Overall, considering the dynamic between the sediments and their microbial biofilms will help in understanding the stream sediment P buffer and hence stream P attenuation. This understanding can sharpen our view of stream P biogeochemistry but also help in understanding the effects of our efforts in mitigating nutrient pollution, e.g., from changing wastewater inputs (Scott et al. 2011; Wilcock et al. 2020) or from management on-land (Meals et al. 2010; McDowell et al. 2018). This recognition of both biotic and abiotic mechanisms can help avoid unrealistic expectations by policy agencies in the speed or efficacy of such efforts to mitigate P pollution.

Acknowledgements We thank Dave Harris (MSD Animal Health) for accommodating our samples for γ -irradiation and ensuring a quick turn-around; Roger Creswell for helping with logistics; Andrea Leptin and Phuong Nguyen for providing valuable help in sampling and lab work and commenting on earlier drafts; and the landowners for site access. This manuscript was greatly improved by constructive comments from two anonymous reviewers and Associate Editor Dr. Marcarelli. We thank the Our Land and Water National Science Challenge for funding the study (Contract C10X1507 from the New Zealand Ministry of Business, Innovation and Employment).

Author contributions ZPS and RWM conceived the study. ZPS performed the research, analyzed the data, and wrote the manuscript. All authors contributed to the design of the study and reviewed the manuscript.

Funding Funding for this study was provided by the Our Land and Water National Science Challenge, contract C10X1507 from the New Zealand Ministry of Business, Innovation and Employment.

Data availability Land use data (LCDB v5; <https://lris.scinfo.org.nz/>) and stream information (REC; <https://niwa.co.nz/freshwater-and-estuaries/management-tools/river-environment-classification-0>) are available from LRIS Portal and NIWA, respectively. The experimental data generated in this study are available on Figshare at: <https://doi.org/10.6084/m9.figshare.12253328.v3>.

Code availability R code used in analyses is available on Figshare at: <https://doi.org/10.6084/m9.figshare.12253328.v3>

Compliance with ethical standards

Conflict of interest All the authors declared that they have no conflict of interest.

References

- Audette Y, O'Halloran IP, Nowell PM et al (2018) Speciation of phosphorus from agricultural muck soils to stream and lake sediments. *J Environ Qual* 47:884. <https://doi.org/10.2134/jeq2018.02.0068>
- Battin TJ, Besemer K, Bengtsson MM et al (2016) The ecology and biogeochemistry of stream biofilms. *Nat Rev Microbiol* 14:251–263. <https://doi.org/10.1038/nrmicro.2016.15>
- Berns AE, Philipp H, Narres H-D et al (2008) Effect of gamma-sterilization and autoclaving on soil organic matter structure as studied by solid state NMR, UV and fluorescence spectroscopy. *Eur J Soil Sci* 59:540–550. <https://doi.org/10.1111/j.1365-2389.2008.01016.x>
- Blankinship JC, Becerra CA, Schaeffer SM, Schimel JP (2014) Separating cellular metabolism from exoenzyme activity in soil organic matter decomposition. *Soil Biol Biochem* 71:68–75. <https://doi.org/10.1016/j.soilbio.2014.01.010>
- Boano F, Harvey JW, Marion A et al (2014) Hyporheic flow and transport processes: mechanisms, models, and biogeochemical implications. *Rev Geophys* 52:603–679. <https://doi.org/10.1002/2012RG000417>. Received
- Brookes PC, Powlson DS, Jenkinson DS (1982) Measurement of microbial biomass phosphorus in soil. *Soil Biol Biochem* 14:319–329
- Buessecker S, Tylor K, Nye J et al (2019) Effects of sterilization techniques on chemodenitrification and N₂O production in tropical peat soil microcosms. *Biogeosciences* 16:4601–4612. <https://doi.org/10.5194/bg-16-4601-2019>
- Campisano R, Hall K, Griggs J et al (2017) Selected analytical methods for environmental remediation and recovery (SAM) 2017. U.S Environmental Protection Agency, Washington, D.C.
- Carter MR, Gregorich EG (2007) Soil sampling and methods of analysis. CRC Press, Boca Raton
- Cleveland CC, Liptzin D (2007) C:N:P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry* 85:235–252. <https://doi.org/10.1007/s10533-007-9132-0>
- Cross WF, Benstead JP, Frost PC, Thomas SA (2005) Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. *Freshw Biol* 50:1895–1912. <https://doi.org/10.1111/j.1365-2427.2005.01458.x>
- Dahlgren RA, Saigusa M, Ugolini FC (2004) The nature, properties and management of volcanic soils. Advances in agronomy. Academic Press, Cambridge, pp 113–182
- Demars BOL (2019) Hydrological pulses and burning of dissolved organic carbon by stream respiration. *Limnol Oceanogr* 64:406–421. <https://doi.org/10.1002/lno.11048>
- Dick WA, Tabatabai MA (1977) Determination of orthophosphate in aqueous solutions containing labile organic and inorganic phosphorus compounds. *J Environ Qual* 6:82–85. <https://doi.org/10.2134/jeq1977.00472425000600010018x>
- Dodds WK (2007) Trophic state, eutrophication and nutrient criteria in streams. *Trends Ecol Evol* 22:669–676. <https://doi.org/10.1016/j.tree.2007.07.010>
- Drohan PJ, Bechmann M, Buda A et al (2019) A global perspective on phosphorus management decision support in agriculture: lessons learned and future directions. *J Environ Qual* 48:1218–1233. <https://doi.org/10.2134/jeq2019.03.0107>
- Dzombak DA, Morel FMM (1987) Adsorption of inorganic pollutants in aquatic systems. *J Hydraul Eng* 113:430–475. [https://doi.org/10.1061/\(ASCE\)0733-9429\(1987\)113:4\(430\)](https://doi.org/10.1061/(ASCE)0733-9429(1987)113:4(430))
- Ekka SA, Haggard BE, Matlock MD, Chaubey I (2006) Dissolved phosphorus concentrations and sediment interactions in effluent-dominated Ozark streams. *Ecol Eng* 26:375–391. <https://doi.org/10.1016/j.ecoleng.2006.01.002>
- Eno CF, Popenoe H (1964) Gamma radiation compared with steam and methyl bromide as a soil sterilizing agent. *Soil Sci Soc Am J* 28:533–535. <https://doi.org/10.2136/sssaj1964.03615995002800040024x>
- Eshel G, Levy GJ, Mingelgrin U, Singer MJ (2004) Critical evaluation of the use of laser diffraction for particle-size distribution analysis. *Soil Sci Soc Am J* 68:736–743. <https://doi.org/10.2136/sssaj2004.7360>
- Findlay S, Sinsabaugh RL (2006) Large-scale variation in subsurface stream biofilms: a cross-regional comparison of metabolic function and community similarity. *Microb Ecol* 52:491–500. <https://doi.org/10.1007/s00248-006-9095-z>
- Findlay SEG, Sinsabaugh RL, Sobczak WV, Hoostal M (2003) Metabolic and structural response of hyporheic microbial communities to variations in supply of dissolved organic matter. *Limnol Oceanogr* 48:1608–1617. <https://doi.org/10.4319/lo.2003.48.4.1608>
- Finlay JC (2011) Stream size and human influences on ecosystem production in river networks. *Ecosphere* 2:art87. <https://doi.org/10.1890/ES11-00071.1>

- Froelich PN (1988) Kinetic control of dissolved phosphate in natural rivers and estuaries: a primer on the phosphate buffer mechanism. *Limnol Oceanogr* 33:649–668
- Gebremikael MT, De Waele J, Buchan D et al (2015) The effect of varying gamma irradiation doses and soil moisture content on nematodes, the microbial communities and mineral nitrogen. *Appl Soil Ecol* 92:1–13. <https://doi.org/10.1016/j.apsoil.2015.03.003>
- Griffiths NA, Johnson LT (2018) Influence of dual nitrogen and phosphorus additions on nutrient uptake and saturation kinetics in a forested headwater stream. *Freshw Sci*. <https://doi.org/10.1086/700700>
- Haggard BE, Sharpley AN (2007) Phosphorus transport in streams: processes and modeling considerations. In: Radcliffe DE (ed) *Modeling phosphorus in the environment*, 1st edn. CRC Press, Boca Raton
- Haggard BE, Stanley EH, Hyler R (1999) Sediment-phosphorus relationships in three northcentral Oklahoma streams. *Trans ASAE* 42:1709–1714
- Hamilton SK (2012) Biogeochemical time lags may delay responses of streams to ecological restoration. *Freshw Biol* 57:43–57. <https://doi.org/10.1111/j.1365-2427.2011.02685.x>
- Hartwig M, Borchardt D (2015) Alteration of key hyporheic functions through biological and physical clogging along a nutrient and fine-sediment gradient. *Ecohydrology* 8:961–975. <https://doi.org/10.1002/eco.1571>
- He Z, Honeycutt CW (2005) A modified molybdenum blue method for orthophosphate determination suitable for investigating enzymatic hydrolysis of organic phosphates. *Commun Soil Sci Plant Anal* 36:1373–1383. <https://doi.org/10.1081/CSS-200056954>
- Helsel DR (2005) More than obvious: better methods for interpreting nondetect data. *Environ Sci Technol* 39:419A–423A. <https://doi.org/10.1021/es053368a>
- Herndon EM, Kinsman-Costello L, Duroe KA et al (2019) Iron (oxyhydr)oxides serve as phosphate traps in Tundra and boreal peat soils. *J Geophys Res Biogeosci*. <https://doi.org/10.1029/2018JG004776>
- Hill BH, Elonen CM, Jicha TM et al (2010) Sediment microbial enzyme activity as an indicator of nutrient limitation in the great rivers of the Upper Mississippi River basin. *Biogeochemistry* 97:195–209. <https://doi.org/10.1007/s10533-009-9366-0>
- Hill BH, Elonen CM, Seifert LR et al (2012) Microbial enzyme stoichiometry and nutrient limitation in US streams and rivers. *Ecol Indic* 18:540–551. <https://doi.org/10.1016/j.ecolind.2012.01.007>
- Hill BH, Herlihy AT, Kaufmann PR (2002) Benthic microbial respiration in Appalachian Mountain, Piedmont, and Coastal Plains streams of the eastern U.S.A. *Freshw Biol* 47:185–194. <https://doi.org/10.1046/j.1365-2427.2002.00791.x>
- Hollander M, Wolfe DA, Chicken E (2013) *The one-sample location problem. Nonparametric statistical methods*, 3rd edn. Wiley, Hoboken, pp 39–114
- House WA (2003) Geochemical cycling of phosphorus in rivers. *Appl Geochem* 18:739–748. [https://doi.org/10.1016/S0883-2927\(02\)00158-0](https://doi.org/10.1016/S0883-2927(02)00158-0)
- Jaiswal D, Pandey J (2019) Investigations on peculiarities of land-water interface and its use as a stable testbed for accurately predicting changes in ecosystem responses to human perturbations: a sub-watershed scale study with the Ganga River. *J Environ Manag* 238:178–193. <https://doi.org/10.1016/j.jenvman.2019.02.126>
- Jenkinson DS, Brookes PC, Powlson DS (2004) Measuring soil microbial biomass. *Soil Biol Biochem* 36:5–7. <https://doi.org/10.1016/j.soilbio.2003.10.002>
- Kaplan LA, Cory RM (2016) Chapter 6 - Dissolved organic matter in stream ecosystems: forms, functions, and fluxes of watershed tea. In: Jones JB, Stanley EH (eds) *Stream ecosystems in a changing environment*. Academic Press, Boston, pp 241–320
- Klotz RL (1985) Factors controlling phosphorus limitation in stream sediments. *Limnol Oceanogr* 30:543–553. <https://doi.org/10.4319/lo.1985.30.3.0543>
- Kouno K, Tuchiya Y, Ando T (1995) Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. *Soil Biol Biochem* 27:1353–1357. [https://doi.org/10.1016/0038-0717\(95\)00057-L](https://doi.org/10.1016/0038-0717(95)00057-L)
- Lee L (2020) NADA: nondetects and data analysis for environmental data. Version 1.6-1.1. <https://CRAN.R-project.org/package=NADA>
- Lottig NR, Stanley EH (2007) Benthic sediment influence on dissolved phosphorus concentrations in a headwater stream. *Biogeochemistry* 84:297–309. <https://doi.org/10.1007/s10533-007-9116-0>
- Lucci GM, McDowell RW, Condron LM (2010) Evaluation of base solutions to determine equilibrium phosphorus concentrations (EPC₀) in stream sediments. *Int Agrophys* 24:157–163
- Macintosh KA, Mayer BK, McDowell RW et al (2018) Managing diffuse phosphorus at the source versus at the sink. *Environ Sci Technol*. <https://doi.org/10.1021/acs.est.8b01143>
- Manzoni S, Porporato A (2011) Common hydrologic and biogeochemical controls along the soil–stream continuum. *Hydrol Process* 25:1355–1360. <https://doi.org/10.1002/hyp.7938>
- Maranger R, Jones SE, Cotner JB (2018) Stoichiometry of carbon, nitrogen, and phosphorus through the freshwater pipe. *Limnol Oceanogr Lett* 3:89–101. <https://doi.org/10.1002/lo2.10080>
- McDaniel MD, David MB, Royer TV (2009) Relationships between benthic sediments and water column phosphorus in Illinois streams. *J Environ Qual* 38:607–617. <https://doi.org/10.2134/jeq2008.0094>
- McDowell RW (2015) Relationship between sediment chemistry, equilibrium phosphorus concentrations, and phosphorus concentrations at baseflow in rivers of the New Zealand National River Water Quality Network. *J Environ Qual* 44:921–929. <https://doi.org/10.2134/jeq2014.08.0362>
- McDowell RW (2003) Sediment phosphorus chemistry and microbial biomass along a lowland New Zealand stream. *Aquat Geochem* 9:19–40. <https://doi.org/10.1023/B:AQUA.0000005620.15485.6d>
- McDowell RW, Elkin KR, Kleinman PJA (2017) Temperature and nitrogen effects on phosphorus uptake by agricultural stream-bed sediments. *J Environ Qual* 46:295–301. <https://doi.org/10.2134/jeq2016.09.0352>

- McDowell RW, Schallenberg M, Larned S (2018) A strategy for optimizing catchment management actions to stressor–response relationships in freshwaters. *Ecosphere* 9:e02482. <https://doi.org/10.1002/ecs2.2482>
- McDowell RW, Sharpley AN (2003) Uptake and release of phosphorus from overland flow in a stream environment. *J Environ Qual* 32:937–948. <https://doi.org/10.2134/jeq2003.9370>
- McLaren AD (1969) Radiation as a technique in soil biology and biochemistry. *Soil Biol Biochem* 1:63–73. [https://doi.org/10.1016/0038-0717\(69\)90035-2](https://doi.org/10.1016/0038-0717(69)90035-2)
- McLaughlin MJ, Alston AM, Martin JK (1986) Measurement of phosphorus in the soil microbial biomass: a modified procedure for field soils. *Soil Biol Biochem* 18:437–443. [https://doi.org/10.1016/0038-0717\(86\)90050-7](https://doi.org/10.1016/0038-0717(86)90050-7)
- McNally SR, Beare MH, Curtin D et al (2017) Soil carbon sequestration potential of permanent pasture and continuous cropping soils in New Zealand. *Glob Change Biol* 23:4544–4555. <https://doi.org/10.1111/gcb.13720>
- McNamara NP, Black HJJ, Beresford NA, Parekh NR (2003) Effects of acute gamma irradiation on chemical, physical and biological properties of soils. *Appl Soil Ecol* 24:117–132. [https://doi.org/10.1016/S0929-1393\(03\)00073-8](https://doi.org/10.1016/S0929-1393(03)00073-8)
- Meals DW, Dressing SA, Davenport TE (2010) Lag time in water quality response to best management practices: a review. *J Environ Qual* 39:85–96. <https://doi.org/10.2134/jeq2009.0108>
- Meyer JL (1994) The microbial loop in flowing waters. *Microb Ecol* 28:195–199. <https://doi.org/10.1007/BF00166808>
- Meyer JL (1979) The role of sediments and bryophytes in phosphorus dynamics in a head water stream ecosystem. *Limnol Oceanogr* 24:365–375. <https://doi.org/10.4319/lo.1979.24.2.0364>
- Mulholland PJ, Marzolf ER, Webster JR et al (1997) Evidence that hyporheic zones increase heterotrophic metabolism and phosphorus uptake in forest streams. *Limnol Oceanogr* 42:443–451. <https://doi.org/10.4319/lo.1997.42.3.0443>
- Munn NL, Meyer JL (1990) Habitat-specific solute retention in two small streams: an intersite comparison. *Ecology* 71:2069–2082. <https://doi.org/10.2307/1938621>
- Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 27:31–36. [https://doi.org/10.1016/S0003-2670\(00\)88444-5](https://doi.org/10.1016/S0003-2670(00)88444-5)
- Öhlinger H, Von Mersi W (1996) Enzymes involved in intracellular metabolism. In: Schinner F, Öhlinger R, Kandeler E, Margesin R (eds) *Methods in soil biology*. Springer, Berlin, Heidelberg, pp 235–245
- Östlund P, Carman R, Edvardsson UG, Hallstadius L (1989) Sterilization of sediments by ionizing radiation. *Appl Geochem* 4:99–103. [https://doi.org/10.1016/0883-2927\(89\)90062-0](https://doi.org/10.1016/0883-2927(89)90062-0)
- Oviedo-Vargas D, Royer TV, Johnson LT (2013) Dissolved organic carbon manipulation reveals coupled cycling of carbon, nitrogen, and phosphorus in a nitrogen-rich stream. *Limnol Oceanogr* 58:1196–1206. <https://doi.org/10.4319/lo.2013.58.4.1196>
- Pinheiro JC, Bates DM, Debroy S, Sarkar D (2020) nlme: linear and nonlinear mixed effects models. Version R package version 3.1–144. <https://CRAN.R-project.org/package=nlme>
- Powlson DS, Jenkinson DS (1976) The effects of biocidal treatments on metabolism in soil—II. Gamma irradiation, autoclaving, air-drying and fumigation. *Soil Biol Biochem* 8:179–188. [https://doi.org/10.1016/0038-0717\(76\)90002-X](https://doi.org/10.1016/0038-0717(76)90002-X)
- Prosser JA, Speir TW, Stott DE (2011) Soil oxidoreductases and FDA hydrolysis. *Methods of soil enzymology*. Soil Science Society of America, Madison, pp 103–124
- Qiu S, McComb AJ (1995) Planktonic and microbial contributions to phosphorus release from fresh and air-dried sediments. *Mar Freshwater Res* 46:1039–1045. <https://doi.org/10.1071/mf9951039>
- R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
- Roberts BJ, Mulholland PJ, Hill WR (2007) Multiple scales of temporal variability in ecosystem metabolism rates: results from 2 years of continuous monitoring in a forested headwater stream. *Ecosystems* 10:588–606. <https://doi.org/10.1007/s10021-007-9059-2>
- Ryan RJ, Packman AI, Kilham SS (2007) Relating phosphorus uptake to changes in transient storage and streambed sediment characteristics in headwater tributaries of Valley Creek, an urbanizing watershed. *J Hydrol* 336:444–457. <https://doi.org/10.1016/j.jhydrol.2007.01.021>
- Saunders WMH (1965) Phosphate retention by New Zealand soils and its relationship to free sesquioxides, organic matter, and other soil properties. *N Z J Agric Res* 8:30–57. <https://doi.org/10.1080/00288233.1965.10420021>
- Scott JT, Haggard BE, Sharpley AN, Romeis JJ (2011) Change point analysis of phosphorus trends in the Illinois river (Oklahoma) demonstrates the effects of watershed management. *J Environ Qual* 40:1249–1256. <https://doi.org/10.2134/jeq2010.0476>
- Scott JT, Cotner J, LaPara T (2012) Variable stoichiometry and homeostatic regulation of bacterial biomass elemental composition. *Front Microbiol*. <https://doi.org/10.3389/fmicb.2012.00042>
- Simpson ZP, McDowell RW, Condon LM (2019) The error in stream sediment phosphorus fractionation and sorption properties effected by drying pretreatments. *J Soils Sedim* 19:1587–1597. <https://doi.org/10.1007/s11368-018-2180-3>
- Sinsabaugh RL, Follstad Shah JJ, Hill BH, Elonen CM (2012) Ecoenzymatic stoichiometry of stream sediments with comparison to terrestrial soils. *Biogeochemistry* 111:455–467. <https://doi.org/10.1007/s10533-011-9676-x>
- Sinsabaugh RL, Hill BH, Shah JJF (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature* 462:795–798. <https://doi.org/10.1038/nature08632>
- Small GE, Ardón M, Duff JH et al (2016) Phosphorus retention in a lowland neotropical stream following an eight-year enrichment experiment. *Freshw Sci* 35:1–11. <https://doi.org/10.1086/684491>
- Snelder TH, Biggs BJB (2002) Multiscale river environment classification for water resources management. *J Am Water Resour Assoc* 38:1225–1239. <https://doi.org/10.1111/j.1752-1688.2002.tb04344.x>

- Sperazza M, Moore JN, Hendrix MS (2004) High-resolution particle size analysis of naturally occurring very fine-grained sediment through laser diffractometry. *J Sedim Res* 74:736–743. <https://doi.org/10.1306/031104740736>
- Sposito G (2004) *The surface chemistry of natural particles*. Oxford University Press, Oxford
- Stelzer RS, Heffernan J, Likens GE (2003) The influence of dissolved nutrients and particulate organic matter quality on microbial respiration and biomass in a forest stream. *Freshw Biol* 48:1925–1937. <https://doi.org/10.1046/j.1365-2427.2003.01141.x>
- Sterner RW, Elser JJ (2002) *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press, Princeton
- Stutter MI, Demars BOLL, Langan SJ (2010) River phosphorus cycling: separating biotic and abiotic uptake during short-term changes in sewage effluent loading. *Water Res* 44:4425–4436. <https://doi.org/10.1016/j.watres.2010.06.014>
- Tabatabai MA (1994) Soil enzymes. In: Weaver RW (ed) *Methods of soil analysis: Part 2—Microbiological and biochemical properties*. Soil Science Society of America, Madison, pp 775–833
- Tank JL, Rosi-Marshall EJ, Griffiths NA et al (2010) A review of allochthonous organic matter dynamics and metabolism in streams. *J N Am Benthol Soc* 29:118–146. <https://doi.org/10.1899/08-170.1>
- Taylor AW, Kunishi HM (1971) Phosphate equilibria on stream sediment and soil in a watershed draining an agricultural region. *J Agric Food Chem* 19:827–831
- Trevors JT (1996) Sterilization and inhibition of microbial activity in soil. *J Microbiol Methods* 26:53–59. [https://doi.org/10.1016/0167-7012\(96\)00843-3](https://doi.org/10.1016/0167-7012(96)00843-3)
- Triska F, Pringle CM, Duff JH et al (2006) Soluble reactive phosphorus (SRP) transport and retention in tropical, rain forest streams draining a volcanic landscape in Costa Rica: in situ SRP amendment to streams and laboratory studies. *Biogeochemistry* 81:145–157. <https://doi.org/10.1007/s10533-006-9048-0>
- Turner BL, Lambers H, Condron LM et al (2013) Soil microbial biomass and the fate of phosphorus during long-term ecosystem development. *Plant Soil* 367:225–234. <https://doi.org/10.1007/s11104-012-1493-z>
- Turner BL, Romero TE (2010) Stability of hydrolytic enzyme activity and microbial phosphorus during storage of tropical rain forest soils. *Soil Biol Biochem* 42:459–465. <https://doi.org/10.1016/j.soilbio.2009.11.029>
- Van Horn DJ, Sinsabaugh RL, Takacs-Vesbach CD et al (2011) Response of heterotrophic stream biofilm communities to a gradient of resources. *Aquat Microb Ecol* 64:149–161. <https://doi.org/10.3354/ame01515>
- Weigelhofer G, Ramião JP, Pitzl B et al (2018) Decoupled water-sediment interactions restrict the phosphorus buffer mechanism in agricultural streams. *Sci Total Environ* 628–629:44–52. <https://doi.org/10.1016/j.scitotenv.2018.02.030>
- Wilcock RJ, McDowell RW, Quinn JM et al (2020) Dynamics of phosphorus exchange between sediment and water in a gravel-bed river. *N Z J Mar Freshw Res*. <https://doi.org/10.1080/00288330.2020.1741402>
- Wolf DC, Skipper HD (1994) Soil sterilization methods of soil analysis. Part 2 *Microbiol Biochem Prop* 5:41–51. <https://doi.org/10.2136/sssabookser5.2.c3>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.