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Abstract: Human alteration of the global P cycle has led to widespread P loading in freshwater ecosystems. Much research has been devoted to the capacity of wetlands and lakes to serve as long-term sinks for P inputs from the watershed, but we know much less about the potential of headwater streams to serve in this role. We assessed storage and retention of P in biotic and abiotic compartments after an 8-y experimental P addition to a 1st-order stream in a Neotropical wet forest. Sediment P extractions indicated that nearly all P storage was in the form of Fe- and Al-bound P (~700 μg P/g dry sediment), similar to nearby naturally high-P streams. At the end of the enrichment, ~25% of the total P added over the 8-y study was still present in sediments within 200 m of the injection site, consistent with water-column measurements showing sustained levels of high net P uptake throughout the experiment. Sediment P declined to baseline levels (~100 μg P/g dry sediment) over 4 y after the enrichment ended. Leaf-litter P content increased nearly 2× over background levels during P enrichment and was associated with a 3× increase in microbial respiration rates, although these biotic responses were low compared to nearby naturally high-P streams. Biotic storage accounted for <0.03% of retention of the added P. Our results suggest that the high sorption capacity of these sediments dampened the biotic effects of P loading and altered the timing and quantity of P exported downstream.
Key words: phosphorus, sediments, sorption, storage, stream, tropical

Humans mobilize >18 million metric tons of P each year. This mobilization has led to a 75% increase in P stored in freshwater ecosystems compared to pre-industrial conditions (Bennett et al. 2001). P is commonly a limiting nutrient in freshwater ecosystems (Schindler 1977, Elser et al. 2007), but anthropogenic P loading can cause eutrophication in lakes and reservoirs (Carpenter and Bennett 2011). Even in heterotrophic headwater streams, P loading can affect resource availability and alter the structure of the food web (Peterson et al. 1985, Benstead et al. 2009, Davis et al. 2010).

We understand some of the potential ecological consequences of increased P availability in stream ecosystems (Slavik et al. 2004), but the relationship between P loading and ecological effects is complex because it depends on the capacity for P retention through biotic and abiotic mechanisms. Biotic uptake of P by algal cells or heterotrophic microbes depends on a number of factors including the availability of light (in the case of autotrophs) and additional nutrients, and available substrate for algal and microbial growth (Aldridge et al. 2010, Schade et al. 2011, Drake et al. 2012). Some fraction of this P enters slower-turnover consumer biomass compartments (e.g., insects and fish; Small et al. 2009), and the ultimate fate of this consumer biomass could have important consequences for the ecosystem P budget (Vanni et al. 2013).

Abiotic P uptake occurs through sorption, which includes both adsorption to the surfaces of cationic minerals and precipitation with electrolytes (Froelich 1988, Reddy et al. 1999, House and Denison 2000). Sorption of P begins with a rapid ligand exchange reaction that takes place with the reactive surface groups, followed by a slow reaction with exchangeable cations in crystal lattices (Frossard et al. 1995). Sorption capacity increases with particle surface area and with positive surface charge, which depend on both mineralogy and pH of the solution (Chen et al. 1973). Sorption is an equilibrium reaction between P in solid and solution phase, so higher levels of P loading result in net uptake by sediments and decreased P loading leads to net P release from sediments.

The relative importance of biotic and abiotic mechanisms in P retention can vary depending on stream condi-

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tions and on P loading rates. At low levels of P loading, biotic uptake exceeds sorption (Elwood et al. 1981, Newbold et al. 1983), but under elevated P inputs, sorption becomes a dominant retention mechanism (Meyer 1979), buffering downstream P concentrations. Physical characteristics affecting interactions between water and substrate (e.g., stream depth, width, velocity, extent of hyporheic zone, presence of debris dams and other channel complexities) probably influence both biotic and abiotic P uptake and retention (e.g., House et al. 1995, Mulholland et al. 1997). Higher rates of biological P retention have been reported in headwater streams, where potential for biological exchange with water is greatest (Bukavekas 2007). The capacity for sediments to buffer concentrations of dissolved P in streams also is high in small streams where sediment surface area to water volume is greatest and contact time with sediments is relatively long (House and Denison 1998, 2002). However, other investigators have found that abiotic P retention increases in importance downstream, where dissolved P levels are higher (Aldridge et al. 2010).

The time required to achieve equilibrium in response to point-source P loading may be much faster in stream ecosystems than in lakes, wetlands, or floodplains (Sharpley et al. 2014), but could still take months or years for several reasons. First, abiotic retention of P occurs as a 2-step kinetic process, with adsorption of P to the particle surface occurring on the order of minutes to hours and diffusion to the interior of the particle occurring on the order of days to months (Froelich 1988). Second, the downstream transport of fine particulates, under low-flow conditions (Jarvie et al. 2006) or from high-flow events (Dorioz et al. 1989, Drake et al. 2012), could move sediment-bound P downstream and expose new sediment for P sorption. New sediment entering the affected reach from above a point source or from lateral inputs creates new sinks for dissolved P, potentially extending nonequilibrium conditions and dampening biotic effects downstream. In spite of these variables, most studies of P retention in streams rely on short-term experimental P additions (several hours to several days) to quantify biotic P uptake and are based on the implicit assumption that abiotic equilibrium is achieved rapidly (Stream Solute Workshop 1990). The few long-term experimental P additions (or N+P additions) to streams (Peterson et al. 1985, Slavik et al. 2004, Benstead et al. 2009) were focused on biotic responses rather than on quantifying the capacity of both biotic and abiotic processes to retain nutrients over time. Studies of P dynamics downstream of wastewater inputs have shown that retention of P by sediments plays an important role in buffering stream P levels under variable loading conditions (Haggard et al. 2005, Jarvie et al. 2006). However, we have less understanding of the contribution of biotic P storage and of the effect of abiotic P storage on biotic responses to long-term P loading.

In much of the tropics, where wastewater treatment is limited, populations are rapidly urbanizing, and agriculture is intensifying, the ability of streams to buffer the effects of P loading could have important implications for maintaining biodiversity and supporting intact stream ecosystems. Here, we present the results and implications of an 8-y experimental P addition to a headwater stream in a tropical wet forest and examine the subsequent recovery of the stream following the cessation of P addition. We use patterns of spatial and temporal variation in P storage to assess the hypothesis that high P retention by sediments dampens biotic effects of point-source P loading.

METHODS

Study site and description of previous research

We conducted an 8-y experimental P enrichment in a 1st-order stream, the Carapa, at La Selva Biological Station, Costa Rica (lat 10°26′N, long 84°01′W). The stream drains dense secondary forest. Allochthonous leaf-litter input is high (Small et al. 2013), and light availability is low because of the dense multistratal canopy (Pringle et al. 1990). Stream discharge ranges from 2 to 3 L/s, and pH ranges from 5.0 to 5.5 (Small et al. 2012). Strongly weathered residual soils, originating from andesitic lava flows, are characteristic of small watersheds at La Selva including the Carapa. These soils were originally classified as ultisols (Sollins et al. 1994) but have been reclassified as oxisols (Kleber et al. 2007), which are common at tropical latitudes.

From 28 August 1998 to 17 February 2006, phosphoric acid (H_3PO_4) was continuously released from a streamside, gravity-fed carboy. Because of the small size of the stream and a series of riffles below the injection site, complete mixing occurred within 10 m of the release site. Natural background soluble reactive P (SRP) levels for the Carapa are low (mean = 6.5 μg/L). The target P concentration for the study reach during the experiment was 300 μg/L, but the actual concentration decreased longitudinally and was highly variable over time because of variable rates of input and dilution, so the input acted as a dynamic point source. Over 8 y, a total of 53.8 kg P was added to the stream (described by Small et al. 2008). During the enrichment period, stream pH averaged 5.32 ± 0.52 (mean ± SD) 10 m below the injection site, within the range of spatial and temporal variability observed in other small streams at La Selva during this period (Small et al. 2012).

Throughout the 8-y P addition, dissolved P measurements were conducted twice weekly at stations 10, 50, and 100 m downstream from the injection site. From these measurements, net uptake of dissolved P was calculated for 685 different days throughout the 2731-d experiment (Small et al. 2008). An analysis of these data indicate that patterns of net P uptake were consistent with sorption kinetics and that a high net P-uptake rate was maintained over 8 y, but evidence of gradual saturation was found (i.e., net uptake at a given P-loading rate gradually decreased over time; Small et al. 2008). The sustained positive net uptake rate suggests that substantial amounts of P were

retained in the stream and either stored there long-term or transported out of the study reach as sediment or particulate organic matter.

This experiment was originally designed to isolate the effects of P on stream ecosystem processes. Other streams at La Selva receive inputs of groundwater high in P and other solutes (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, HCO_3^{-}, Cl^{-}) (Pringle and Triska 1991). Stream ecosystem processes have been measured at the experimentally P-enriched Carapa and have been reported together with observations from the natural P gradient. The studies showed that P-enrichment in the Carapa stimulated microbial respiration, fungal biomass, leaf P-content, and leaf decomposition rates (Ramírez et al. 2003, 2006, Ardón et al. 2006, Stalcup et al. 2006, Ardón and Pringle 2007). The effects on higher trophic levels were less pronounced because P-enrichment did not stimulate higher growth rates of chironomid larvae (instead, chironomids feeding on high-P leaf litter showed elevated rates of P excretion) (Small et al. 2011).

Desorption

For 2 d before P cutoff at the end of the 8-y P addition, H_3PO_4 inputs were switched from the gravity-fed carboy to a ceramic valveless metering pump (Fluid Metering Incorporated, Syosset, New York) to maintain a constant addition rate. Rhodamine WT (RhWT), a conservative tracer, was added simultaneously using a 2nd metering pump during these 2 d. Water samples were collected at 4-h intervals using ISCO 6712 samplers (Teledyne ISCO, Lincoln, Nebraska) deployed at stations 10, 50, and 100 m downstream from the injection site. After P and RhWT cutoff, which occurred at 1000 h local time on 23 February 2006, water samples were collected by hand at these stations, first at 1-min intervals, and then with decreasing frequency until 1400 h. Samples were then collected every 4 h using ISCO samplers over a 48-h period. Water samples were analyzed at the station laboratory for SRP using the molybdenum blue method (APHA 1988) and RhWT using a benchtop fluorometer (model 10; Turner Designs, Sunnyvale, California).

Short-term P release from sediments after cutoff was estimated as the difference between observed and predicted SRP based on observed RhWT and the SRP:RhWT loading ratio (i.e., the SRP expected if it were to behave conservatively). The mass of P released from sediments passing out of the 50-m study reach in the first 48 h after P cutoff was estimated as the time integral of the excess P (difference between observed and predicted SRP curves) × discharge.

Sediment P extractions

Sediment P extractions were done 5 d before and after P cutoff (18 February 2006, 28 February 2006) at 5 stations along the study reach: 5 m upstream of the P injection (hereafter upstream), and 10, 50, 100, and 200 m downstream. Sediment present in the top 5 cm of the bed was collected with a small trowel and returned to the station laboratory. The bulk sample was gently homogenized with a spatula and passed through a 2-mm sieve to remove large rocks and particulate matter. Most of the bulk sample passed through the sieve. Five replicate aliquots (~1 g wet mass each) from each sieved sample were harvested for P extractions begun the following day. Sediment P was measured using a 5-step sequential chemical fractionation procedure (D'Angelo 2005) that included, in sequence: 1) extraction with 20 mL of deionized water (2 h), 2) extraction with 2 mL of 1 M KCl (2 h), 3) extraction with 20 mL of 0.1 M NaOH (24 h), and 4) extraction with 20 mL of 0.5 M HCl. The respective extracts yielded: 1) water-soluble inorganic and organic P, 2) weakly exchangeable inorganic and organic P, 3) Fe- and Al-bound inorganic P, and 4) organic fulvic- and humic-bound P. Filtrates from each extraction were frozen and later analyzed for SRP as described above. Total P was measured on an acid-digested subsample of the filtrate (APHA 1988). Organic P was calculated as the difference between total and inorganic P.

Sediment P extractions were repeated ~1 y later (5 March 2007) at the upstream and 10-m stations, focusing only on inorganic P (SRP) in filtrates of deionized water and NaOH extractions, based on the results of the original measurements that showed these to be the main components. Four years post-cutoff (12 April 2010), NaOH extractions were repeated at 4 stations along the study stream and at 7 nearby streams that ranged in SRP to provide additional context (Table 1).

Sediment P adsorption isotherms

In April 2010, following the return of sediment P concentrations to background levels, sediment P adsorption isotherms (Axt and Walbridge 1999) were generated for sediments collected from 4 stations along the Carapa study reach (upstream, and 10, 50, and 100 m downstream). Approximately 2 g sediment was equilibrated with solutions at 4 initial P concentrations: 936, 5330, 6727, and 15,950 μg/L. Incubations were done in triplicate and were allowed to equilibrate for 24 h on a shaker table. Samples were centrifuged, and the supernatant was analyzed for SRP as described above. The change in SRP relative to the initial concentration was used to calculate the mass of P adsorbed/g sediment dry mass.

Biotic response

To estimate the amount of P in the biotic compartments, epilithon, coarse particulate organic matter (CPOM), and macroinvertebrate samples were collected and analyzed for P content following methods described by Small and Pringle (2010). Epilithon was collected from unglazed ceramic tiles after 4-wk incubations at 4 stations (upstream, 10, 50, 100 m) along the study stream. Tiles were

Table 1. Mean (SE) sediment P content (μg P/g dry sediment) for sediments collected from up to 5 different stations on the Carapa study stream on 4 different dates using 5 different extraction protocols. Also included are P extractions from 7 nearby streams that naturally vary in ambient soluble reactive P (SRP) concentrations because of inputs of geothermally modified ground water. Dates are formatted mm/dd/yyyy. DI = deionized water.

Stream	Date	Ambient SRP (μg P/L)	DI-extracted	KCl-extracted (undigested)	KCl-extracted (digested)	NaOH-extracted (undigested)	NaOH-extracted (digested)
Carapa-up	2/18/2006	5	0.1 (0)	0.3 (0.1)	0.1 (0)	412.4 (15.5)	458 (37.9)
Carapa-10	2/18/2006	850	0.7 (0)	0.3 (0.1)	0.2 (0.1)	698.8 (18.2)	709.5 (47.6)
Carapa-50	2/18/2006	600	0.4 (0.1)	0.3 (0)		391 (13.1)	510.3 (23.2)
Carapa-100	2/18/2006	400	2.9 (1.5)	0.2 (0)		602.1 (10.3)	643.1 (44.9)
Carapa-200	2/18/2006	300	1.6 (1.4)	0.3 (0.1)		454.8 (19.4)	346.6 (39.1)
Carapa-up	2/28/2006	5	0.6 (0.1)	0.1 (0)	0.1 (0)	339 (2.7)	406.9 (23)
Carapa-10	2/28/2006	5	2 (0)	0.2 (0)	0.2	690.2 (3.4)	761.6 (35.4)
Carapa-50	2/28/2006	5	2.6	0.1 (0)		423.2 (4.5)	396.6 (41.3)
Carapa-100	2/28/2006	5	1.9	0.2 (0)		613.7 (7.6)	580.3 (75.7)
Carapa-200	2/28/2006	5	1.9 (0.4)	0.2 (0)		411.5 (4.3)	434.8 (39.5)
Carapa-up	3/5/2007	5	0.2 (0.1)			176 (32.1)	
Carapa-10	3/5/2007	5	0.1 (0)			425.2 (10.5)	
Carapa-up	4/12/2010	5				155.4 (16.6)	
Carapa-10	4/12/2010	5				126.3 (13.2)	
Carapa-50	4/12/2010	5				131.7 (13.3)	
Carapa-100	4/12/2010	5				129 (10.9)	
Piper-30	3/5/2007	2	0.1			20.7 (7.2)	
Sura-60	3/5/2007	3	0			90.9 (17.1)	
Saltito-100	3/5/2007	3	0.1 (0)			234.3 (12.8)	
Taconazo-30	3/5/2007	3	0.4 (0)			406.7 (70.3)	
Salto-60	3/5/2007	10	0.4 (0.3)			300.8 (106.2)	
Sura-30	3/5/2007	83	0.6 (0.1)			783.6 (33.8)	
Arboleda-30	3/5/2007	135	0.2 (0)			459.4 (130.7)	

collected at 1-mo intervals, immediately before P cutoff and for the subsequent 4 mo. Epilithon was scrubbed from tiles, filtered onto a 0.45-μm glass-fiber filter, and dried. Grab samples of CPOM and macroinvertebrates were collected at these 4 stations on 2 dates prior to P cutoff and on 6 dates over the ensuing 4 months. Biotic samples were dried at 50°C for 48 h, acid-digested (Aqua Regia double acid; Jones et al. 1991), and analyzed spectrophotometrically (ascorbic acid method). Ground pine needles (US National Institute of Standards and Technology, 1575a) were used as an external standard.

On one date before cutoff (19 January 2006) and 2 dates after cutoff (8 March 2006 and 30 June 2006), microbial respiration rates on leaf disks were measured with methods described by Ramírez et al. (2003). Leaves of *Ficus insipida*, a common riparian plant species in Central America, were used as a standardized substrate. Groups of *Ficus* leaves were incubated in the stream for 14 d at stations 10, 50, 100, 200, and 500 m below the site of P addition. Disks were cut from these leaves and incubated at ambient temperature in a chamber with a stirrer and O_2 probe. Rates were expressed as O_2 consumed by microbes per gram ash-free dry mass (AFDM) per hour (mg O_2 g^{-1} AFDM h^{-1}). The P content of the remaining material was measured as described above.

P budget

Storage of dissolved P in the water column within the 50-m study reach during the P-addition experiment was calculated as the mean SRP concentration from the final 48 h of P addition, multiplied by the reach volume, where stream width and depth were estimated as 1.0 m and 0.1 m, respectively. The background level was based on post-experiment sampling over the ensuing months. Groundwater seepage flux (~3.5 μg P/s) was considered to be negligible relative to the stream P flux (>1000 μg P/s). The half-life of dissolved P in this reach was calculated from the decline in SRP measured at the 50-m downstream station after P cutoff.

Areal P concentrations were estimated for the sediment P fractions by applying bulk density of sediments sampled for P extractions and assuming a 5-cm active layer of sediment. Background sediment P storage was based on the 2010 measurements. The half-life of P stored in sediment was calculated by fitting an exponential decay curve to the time-series data.

For P storage in epilithon, the biomass on the ceramic tiles (15 × 7.7 cm) was conservatively extrapolated throughout the wetted stream channel, and epilithon biomass was assumed to be constant over time (i.e., changes in estimated P storage were caused by changing P content of epilithon). The background level was based on the final measurement, and the half-life was calculated by fitting an exponential decay curve to the time-series data.

Biomass of CPOM was estimated from quarterly samples collected with a stovepipe corer during and after the 8-y experiment. The areal density of organic matter in these samples did not change significantly, so the mean was used and applied to CPOM %P measurements to calculate CPOM P storage. Background CPOM P storage was based on the final CPOM %P measurement, and the half-life was calculated by fitting an exponential decay curve to the time-series data.

Insect biomass was based on biomass measurements for chironomid larvae reported in Ramírez and Pringle (2006), and chironomid %P values were taken from Small et al. (2011). Chironomids dominate invertebrate biomass in headwater streams at La Selva and have higher P content than other insect taxa in the stream, so the extent of underestimating the size of the insect P pool caused by excluding other taxa is relatively small. Fish biomass and P content from the study stream were reported by Small et al. (2012).

RESULTS

Desorption

During the final days of P addition, SRP concentrations averaged 819, 549, and 365 µg/L at the 10-, 50- and 100-m stations, respectively (Fig. 1A, B). Based on RhWT concentrations, discharge averaged 1.71, 2.43, and 3.12 L/s at the 10-, 50- and 100-m stations, respectively. Based on mass balance, P uptake averaged 9.5 mg P m^{-2} h^{-1} between 10 and 50 m downstream, and 11.5 mg P m^{-2} h^{-1} between 50 and 100 m downstream during the 48 h before P cutoff.

P concentrations returned to background levels over the 48 h after P cutoff (Fig. 1A, B). P released from sedi-

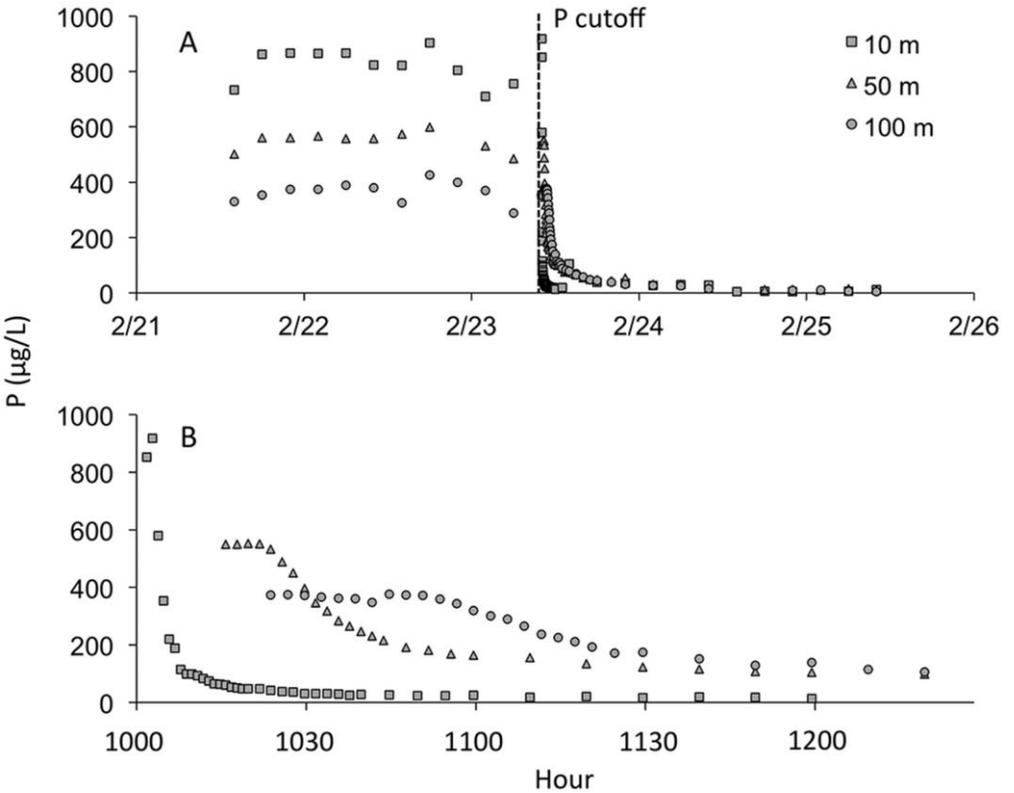

Figure 1. Soluble reactive P (SRP) concentrations at stations 10, 50, and 100 m downstream of the injection site during a 96-h period around the P cutoff in 2006 (A) and details for the 2-h period after the cutoff (B).

ments resulted in SRP concentrations up to 80 μg/L above background initially at the 50- and 100-m stations, tapering off over the ensuing 48 h (Fig. 2). These data suggest P release of ~150 mg/m^2 in the first 2 d at a rate of ~3 mg P m^{-2} h^{-1}.

Sediment P extractions

NaOH extractions yielded P concentrations ~4 orders of magnitude higher than DI and KCl extractions (Table 1), indicating that nearly all stored P was Al- or Fe-bound. No consistent longitudinal pattern was found in sediment P levels 10, 50, 100, and 200 m downstream of the P injection in the Carapa (Table 1). However, a plot of the P levels at the 10-m station vs time shows a clear temporal pattern for P levels in the reach (Fig. 3). At the end of the injection, the average NaOH-extractable P was similar to NaOH-extractable P measured in nearby naturally high-P streams. After 1 y, sediment P at the Carapa 10-m station had declined by 38%, and after 4 y had declined by 82% to a level similar to nearby naturally low-P streams. The station 5 m upstream of the injection site was intended to be a control because it was separated by a riffle from the point of P injection, but elevated SRP levels were observed at this station throughout the experiment, and sediment at this station showed a corresponding 2.3× enrichment in P levels.

Sediment P adsorption isoclines

Sediment P isoclines indicated that Carapa sediments had the capacity to retain ~50 to 100 μg P/g dry sediment at background SRP levels of 100 μg P/L, and 150 to 250 μg P/g dry sediment at background SRP levels of 1000 μg P/L (Fig. 4). Sediments collected from the upstream stations (above the injection site and 10 m below) had a higher sorption capacity than sediments collected at the 50- and 100-m stations.

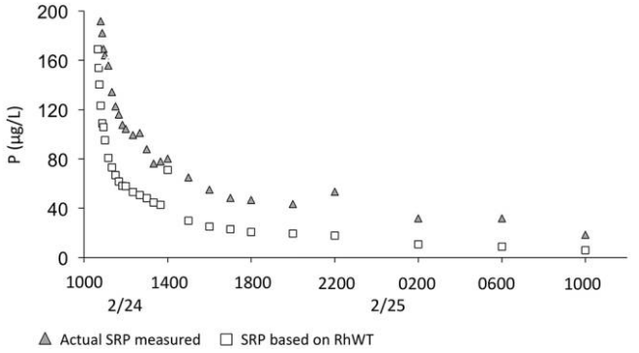

Figure 2. Soluble reactive P (SRP) and calculated SRP (based on Rhodamine WT [RhWT] and SRP:RhWT input ratio) at the 50-m station over a 48-h period after P cutoff in 2006. Difference between the curves was used to estimate short-term P release from sediment.

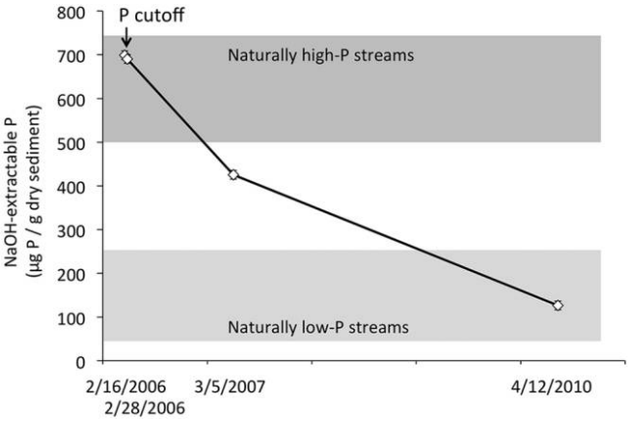

Figure 3. Mean (±1 SE) NaOH-extractable P from sediment at 10-m station shortly before (16 February 2006) and after (28 February 2006) P-cutoff and ~1 (5 March 2007) and 4 y (12 April 2010) after cutoff. Shaded regions indicate the range of NaOH-extractable P measured from nearby naturally high- and low-P streams.

Biotic response

Epilithon P content was initially 0.2–0.3% by dry mass, in the lower range of values measured on tiles in nearby naturally high-P streams (Fig. 5). Epilithon %P declined to 0.1% 4 mo after P cutoff. Leaf litter %P was ~0.10% before P cutoff, lower than values in naturally high-P streams, and gradually returned to background levels of ~0.06% over the next 4 mo (Fig. 6). P content of *Ficus* leaf packs incubated in the stream declined after P cutoff that was similar to the decline in P content of CPOM grab samples. Both P content and microbial respiration rates on the *Ficus*

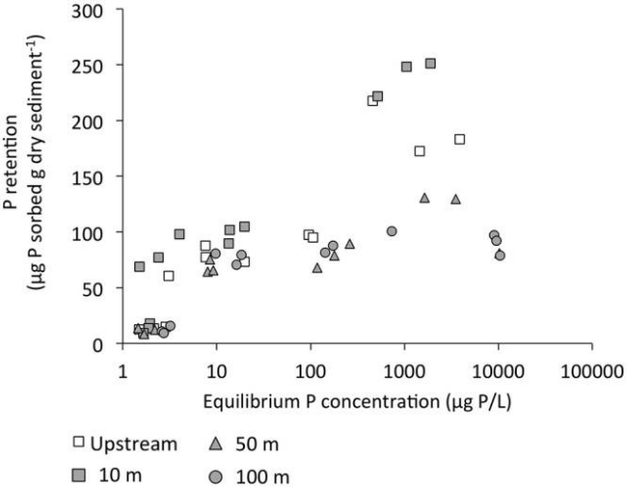

Figure 4. P sorption isoclines for sediment collected from 4 stations. Measurements were done 4 y after the P cutoff and sediment P content had returned to baseline levels.

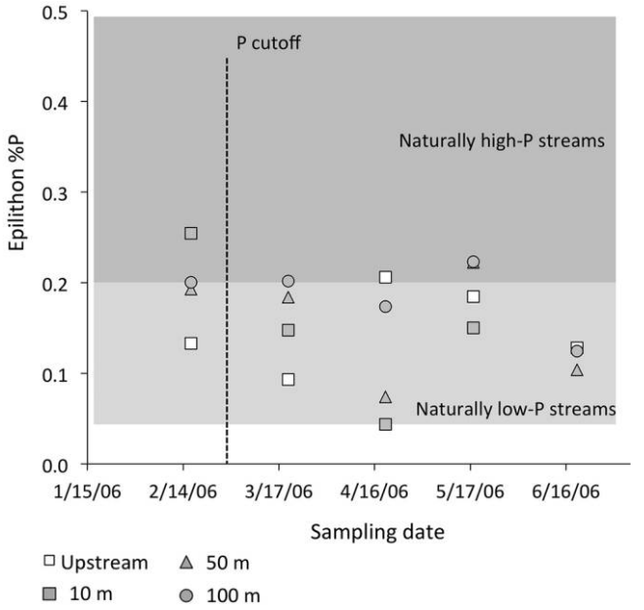

Figure 5. P content of epilithon collected from ceramic tiles at 4 stations in the study stream over 5 sampling dates in 2006 after P cutoff. Shaded regions depict epilithon P content from naturally high- and low-P streams in the region (Small and Pringle 2010).

leaves were ~3× higher before the cutoff than 4 mo after cutoff (Figs 6, 7).

P budget

During the P-addition experiment, water showed the greatest relative increase, ~36× background levels, with a half-life of 0.1 d (Table 2). Fe- and Al-bound sediment P was by far the greatest P reservoir, accounting for 99% of stored P in the stream ecosystem, with an estimated 88,000 mg P/m^2 at the end of P addition, compared to an estimated 15,800 mg P/m^2 background at this site (a 5.5× increase). This large sediment P pool also had the longest half-life of any measured compartment at 235 d. Based on these numbers, at the time of P cutoff, the 200-m reach below the P-addition point contained ~13 kg P above background levels, nearly 25% of the total P added (53.8 kg) over the 8-y study. In comparison, P storage in biotic compartments was negligible. We estimated ~11 mg excess P/m^2 stored in epilithon and ~7 mg excess P/m^2 stored in CPOM (Table 2). These biotic compartments had relatively long half-lives (epilithon: 63 d, CPOM: 91 d). No increase in P content was observed in insects in the study reach.

DISCUSSION

After 8 y of nearly constant P loading designed to simulate conditions in nearby naturally high-P streams, we found that sediment, but not biotic compartments, reached P levels comparable to those measured in naturally high-P streams. More than 99% of excess P in the study reach was stored as Al- and Fe-bound sediment P, accounting for 10% of the total P added over the 8-y experiment. This finding is consistent with observed patterns of sustained P uptake based on longitudinal SRP measurements throughout the 8-y experiment (Small et al. 2008).

The observed dynamics of P release from sediments after the end of the enrichment—an initial pulse followed by a sustained slow release—is consistent with the 2-step kinetic model described by Froelich (1988). The sorption of SRP onto precipitated Al or Fe hydroxides is relatively slow and occurs over hundreds of hours (Bolan et al. 1985). In streams where suspended sediment concentrations are low, diffusion of SRP into the sediments becomes a rate-limiting step (House et al. 1995). The P stored on the interior of particles would have been relatively resistant to short-timescale fluctuations in SRP concentration that occurred during the experiment, and the P stored on the exterior of particles would have acted to stabilize SRP concentrations over the course of the experiment.

In contrast to sediment, biotic compartments showed a low level of P enrichment and were a negligible contributor to reach-scale P storage. Detritus (CPOM) and epilithon were enriched 1.7 and 3.1×, respectively, but these levels represented <½ the P content of detritus and epilithon in a nearby naturally high-P stream (Small and Pringle 2010). The reason for this muted response is not entirely clear, given the relatively high SRP concentrations

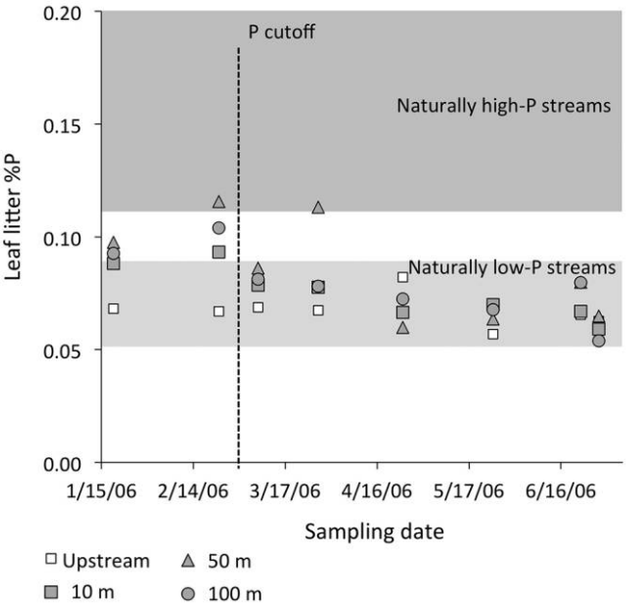

Figure 6. P content of *Ficus* leaves incubated in stream water at 4 stations in the study stream over 5 sampling dates in 2006 after P cutoff. Shaded regions depict *Ficus* leaf P content from incubations in naturally high- and low-P streams (Small et al. 2011).

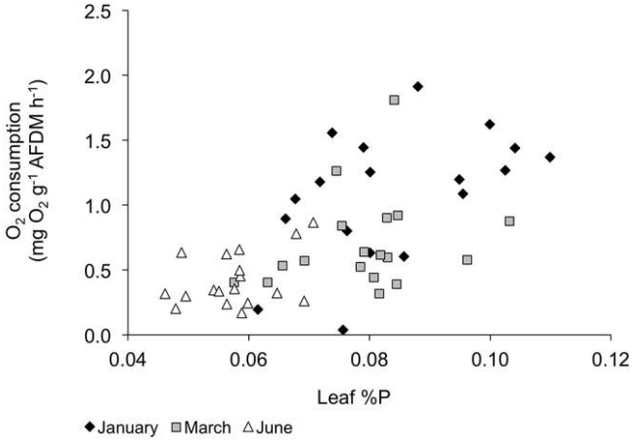

Figure 7. *Ficus* leaf-disk O_2 consumption rates vs leaf P content during P addition (January 2007) and after P cutoff (March and June 2007). AFDM = ash-free dry mass.

documented at the downstream stations throughout the experiment (Small et al. 2008) and the long turnover time of P in these biotic compartments (Table 2) relative to fluctuations in P inputs during the experiment. One factor that may have suppressed biotic P storage in the study reach was the downstream transport of materials. Throughout the experiment, sediment and leaf litter were transported into the study reach laterally and from upstream of the injection site, effectively diluting the pool of P-enriched substrate. Likewise, an unknown but potentially significant amount of P probably was exported from the study reach in the form of suspended organic matter and sediments. The half-life values estimated in Table 2 represent a combination of actual P removal from substrate (via desorption, mineralization, etc.) with the turnover of substrate resulting from new inputs and downstream transport. The fact that saturation of net P uptake was observed over the course of the experiment (Small et al. 2008) suggests that the time scale of P retention is faster than the time scale of sediment turnover. It seems possible that turnover of organic material caused by downstream transport out of the study reach may have occurred faster relative to time scales of P retention (e.g., Cushing et al. 1993).

The most pronounced biotic response to P addition was microbial respiration, a result illustrating the important linkage between P and C dynamics in this detritus-based stream. These results were consistent with respiration rates reported in the Carapa early in the P-enrichment experiment and from nearby streams that naturally range in P (Ramírez et al. 2003). Our results expand on the results of a study by Ramírez et al. (2003) by showing the relationship between leaf P and respiration rates and documenting the return to baseline conditions over a 4-mo period (Fig. 7). A 3× increase in microbial respiration rates could have potentially large implications for turnover rates of organic C. However, changes in the standing stock of detritus were not observed over the course of our study, suggesting that other factors, such as consumption by invertebrates and downstream transport, may be more important. Nutrient additions in a detritus-based headwater stream in the southeastern USA also resulted in elevated microbial respiration rates (Benstead et al. 2009). Benstead et al. (2009) also documented an even larger response in the export of fine particulate organic matter (FPOM), mediated by shifts in the invertebrate community (Davis et al. 2010). We did not measure export of FPOM in the Carapa, but the lack of observed response by either detritus or insect standing stocks in our study may indicate that invertebrates played a limited role in mediating the ecosystem response to P addition. In contrast to the southeastern USA stream, in which nutrient enrichment led to increased production of primary consumers (Davis et al. 2010), the dominant invertebrate consumers in the Carapa, chironomid larvae,

Table 2. Estimated P budget at the end of the 8-y P-addition experiment compared to background levels. The lower value for water-soluble sediment P (86 mg P/m^2) came from P extractions in deionized water. The higher value (150 mg P/m^2) was calculated by measuring excess soluble reactive P (SRP) relative to the conservative tracer at the end of the experiment (Fig. 2). Half-life values were calculated by fitting an exponential decay model to time-series measurements after the end of the P addition. CPOM = coarse particulate organic matter.

Category	Description	Enrichment (mg P/m^2)	Background (mg P/m^2)	Relative increase	Half-life (d)
Dissolved	Water	73	2	36	0.1
Sediment	Water soluble	86–300	18	5–17	0.5
Sediment	Fe- and Al-bound	88,000	15,800	5.5	235
Sediment	Organic fulvic and humic-bound	1340			
Biotic	Epilithon	17	6	3.1	63
Biotic	CPOM	17	10	1.7	91
Biotic	Insects	1	1	0	
Biotic	Fish	15	15	0	

were not able to respond to P-enriched food with increased growth rates or P storage, but instead excreted the additional P they consumed (Small et al. 2011), indicating that these consumers were adapted to low-P food resources.

Our results reflect the response of a single stream to P addition, and whether the patterns of P retention observed here would apply to streams in other regions is not clear. The clay-rich soils and acidic streams of La Selva Biological Station probably provide conditions that are favorable for sediment P retention (Fig. 4), and the dense forest canopy precluded a strong algal response to nutrient addition. P exported from our study reach into higher-order streams with increased light availability could have led to increased algal production there. Studies in several other rivers have documented patterns in P retention similar to what we observed. For example, sediments in a 3rd-order stream in the southeastern USA buffered stream SRP concentrations against variable SRP loading from an upstream wastewater treatment plant (Haggard et al. 2005). In the UK, sediments in river basins receiving sewage effluent discharges consistently acted as SRP sinks, whereas sediments in other river basins acted as SRP sources (Jarvie et al. 2005). Reductions in point-source P loading in the River Lambourn led to an abrupt shift from net uptake to net release of P from sediments for the subsequent 9 mo (Jarvie et al. 2006). Legacy P in terrestrial soils, floodplains, and lake sediments can persist on time scales from decades to centuries (Sharpley et al. 2014), but the time scale of recovery for our study reach is consistent with other reported values for in-channel P storage (Owens et al. 2001, Jarvie et al. 2006, Collins and Walling 2007a, b, Ballantine et al. 2009).

P is of central importance in the management of aquatic ecosystems. Thus, improved understanding is needed of the relationships among P loading, availability, and the capacity for P storage in stream ecosystems. These relationships control ecological responses to P loading in streams and the magnitude and timing of P exported downstream. In future nutrient-addition experiments (e.g., experiments associated with the Stream Experimental Observatory Network), researchers should take advantage of opportunities to follow the fate of P added to streams, in addition to measuring biotic responses that result from this P. Such measurements will allow us to better understand how stream ecosystems respond to, and recover from, P loading.

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