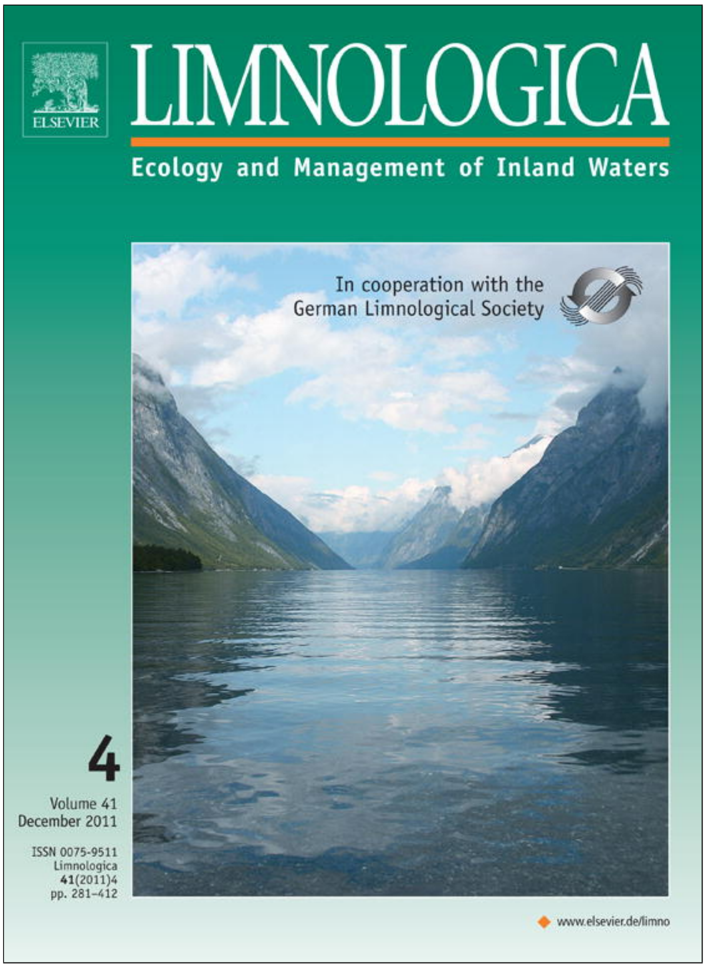
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Phosphate uptake in a macrophyte-rich Pampean stream

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ABSTRACT

We estimated phosphate uptake in an eutrophic and macrophyte-rich Pampean stream, in a basin where dominant land use was agricultural activity. Our hypothesis was that phosphate retention may be linked to macrophyte abundance, especially that of submerged plants. Four short-term constant-rate phosphate addition experiments were carried out in late spring, summer, autumn and winter under different discharge, background phosphate concentration and macrophyte abundance. Changes in added soluble reactive phosphorus (SRP) concentration were monitored along the studied reach and corrected for dilution to estimate phosphate uptake length (S_w) and other spiraling metrics (V_f or mass transfer coefficient, and U or nutrient uptake rate). All spiraling metrics indicated a higher SRP retention in spring than in autumn, despite macrophyte cover was similar in both sampling dates (39% and 51%, respectively). Phosphate retention was not detected neither in summer (when macrophyte biomass and especially that of submerged plants was the highest one) nor in winter. Ambient SRP concentrations in the stream were an order of magnitude higher than concentrations observed in pristine streams; nevertheless, under the varying conditions of discharge and phosphate levels observed in our study, we found no retention in summer and winter, a phosphate uptake comparable with those measured in impaired streams in autumn, and with those measured in pristine streams in late spring.

Introduction

Phosphorus content in surface waters has dramatically increased in the last years in response to widespread urbanization and agriculture intensification (Mainstone and Parr 2002). Rivers and streams are particularly vulnerable to eutrophication due to their proximity to population centers, sensitivity to land use change and ubiquitous exploitation (Withers and Jarvie 2008). In addition, fluvial systems are characterized by their high capacity to transform and retain nutrients (Peterson et al. 2001), and understanding these processes is of paramount importance for eutrophication management.

Pampean streams are situated in Buenos Aires province, a region which supports 48% of total Argentine population and where 56% of country industries are established (Indec 1994). In recent years, a process of agriculture intensification and land transformation in the region has begun, changing the traditional low external-input farming system to an intensive farming scheme (Viglizzo et al. 2001). Despite the increase of human impact on Pampean lotic bodies, there is not information on their ability to process and retain nutrients, modulating nutrient export downstream.

According Ensign and Doyle (2006), nutrient uptake is influenced by two kinds of factors. Biochemical factors include biotic uptake by bacteria, fungi, algae and macrophytes, while geomorphological factors as channel size and transient storage determine the residence time of water and, consequently, the exposure of dissolved nutrients to biochemically reactive substrates. Channel size affects the ratio of surface area to channel volume, determining the proportion of solute in contact with the benthos, while transient storage describes the temporal retention of solutes in pools, backwater and hyporheic zone where water moves slower than in the main channel (Ensign and Doyle 2006; Webster and Valett 2007).

Most research on phosphate retention has been performed in pristine forested streams of the northern hemisphere, characterized by turbulent flows, low nutrient levels and biotic communities dominated by consumers. On the other hand, some authors have evaluated nutrient retention in impaired streams affected by wastewater treatment plant inputs (Haggard et al. 2005; Merseburger et al. 2005). Pampean streams situated in central Argentina show intermediate features among both types of lotic systems. The gentle slope of the Pampean region determines laminar flow and low discharge (<50 L/s), and high irradiance levels are found even in the upper stream reaches due to the lack of a forested riparian zone. These features allow the development of dense macrophyte communities in headwaters and medium-sized streams, with the autochthonous primary production relying on algal and macrophytic communities and autotrophic P/R ratios

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(Vilches and Giorgi 2010). As streambeds are composed by hard and homogeneous substrata with fine sediments (primarily silt and clay) and they generally lack of stones and pebbles, habitat heterogeneity depends on aquatic vegetation that plays a paramount role in structuring the biological community (Giorgi et al. 2005). High nutrient levels have been reported in these streams, but the eutrophic status of Pampean waterbodies seems to be a common feature from long time ago, very much before the introduction of cattle by the Spaniards during the Colonial period and the rise of agriculture in the 19th century (Feijoó and Lombardo 2007). Therefore they cannot be considered 'impaired' streams in a strict sense, even though they are eutrophic.

In this study, we estimate phosphorus retention in a Pampean stream that shows different macrophyte abundance along the year. Aquatic vegetation may enhance nutrient retention in several ways: incorporating nutrients into their biomass, increasing available surface for epiphytic algae that absorb nutrients, creating zones of dead waters for solute storage, and promoting stream-groundwater exchange (by increasing streambed roughness and altering sediment porosity and permeability) (Salehin et al. 2003; Gücker and Boëchat 2004). In Pampean streams, macrophytes represent a variable substrate that changes along the time (Giorgi et al. 2005), and this may affects not only the relative importance of the biological uptake but the size of the transient storage zone at a reach scale.

Methods

The study was conducted in the Las Flores stream, a third-order stream of the Luján river basin (NE of Buenos Aires province) that is considered representative of many Pampean streams. Mean stream discharge is 30 L/s and current velocity is low (~20 cm/s), while mean nutrient concentrations are high (0.77 mg $P–PO_4$/L and 4.14 mg $N–NO_3$/L). High phosphate levels in Pampean streams cannot only be attributed to agricultural land use and they may be also related to the phosphorus-rich volcanic material deposited in the region during the Quaternary (Morrás 1993). Comparatively, nitrogen levels are not as high as expected from the extend of agricultural activities in the region (Feijoó and Lombardo 2007; Mugni et al. 2005), and it has been suggested that this may result from low fertilization loads in the Pampean region in comparison with those from North America and Europe (Viglizzo et al. 2001, 2003).

Other physico-chemical and biological characteristics of the stream are described elsewhere (Feijoó et al. 1999; Giorgi et al. 2005). Predominant land use within the sub-catchment located upstream of the study reach was agricultural activity (Amuchástegui 2006).

Four phosphate enrichment experiments were carried out in December 2007 (late spring), February (summer), April (autumn), and August 2008 (winter) in a 160-m reach (mean width = 4.40 m; mean depth = 0.30 m). Short-term nutrient additions were performed with a conservative (NaCl) and a nonconservative solute (phosphorus as HK_2PO_4, amended with nitrate and ammonia to avoid an excessive change in the N/P ratio of water). The solute solution was prepared in the field with filtered stream water and added at a constant flow rate (100 mL/min) using a peristaltic pump (Etratron DS) placed at the head of the reach, just before a channel narrowing to enhance solute mixing. Phosphate concentration in the solution was adjusted for each addition to result in a target stream concentration that was within the natural concentration range (0.1–2.1 mg $P–PO_4$/L; Giorgi et al. 2005) and always lower than 1 mg/L. Additions lasted between 60 and 150 min, and total added phosphorus varied from 54 to 135 g. Triplicate water samples were collected in polyethylene bottles every 20 m before the beginning of the addition to determine background conductivity and phosphate concentration in the reach. Samples of the solute solution were also taken to check on that its phosphate concentration was within the expected values. During the addition, triplicate water samples were taken when tracer concentration reached a plateau in the farthest downstream station, as determined by continuous measurement of conductivity at this station. Conductivity was measured in the water samples. Then, samples were filtered through Whatman GF/F membranes and stored in an ice chest during approximately 2 h until they were transported to the laboratory. Soluble reactive phosphorus (SRP) was determined in the samples within one day from collection using the ascorbic acid method (APHA 1992). Added phosphate concentration (estimated as the difference between plateau and background phosphate concentrations) was corrected for dilution, dividing it by conductivity (represented as the difference between plateau and background conductivities). Nutrient uptake length (S_w) was calculated as the negative inverse of the slope (k) of the regression between the natural logarithm of the dilution-corrected concentration of injected phosphorus and the distance downstream (Stream Solute Workshop 1990):

$$S_w = -\frac{1}{k}$$

S_w depends on water velocity and nutrient load; so, it cannot be used when comparing solute dynamics across different streams or in the same stream under varying environmental conditions. For this reason, we also calculated two other retention metrics that correct the effect of these factors on nutrient retention (Stream Solute Workshop 1990; Webster and Valett 2007). The mass transfer coefficient, V_f (mm/min), can be conceptualized as the velocity at which a nutrient moves through the water column towards the sediment, and thus as an index of nutrient uptake demand (Doyle et al. 2003; Webster and Valett 2007):

$$V_f = \frac{uh}{S_w}$$

where u is water velocity and h is the mean stream depth. This metric is appropriate for comparison across sites when water depth and velocity vary, but is highly dependent on nutrient concentration (Fellows et al. 2006).

The second metric is the nutrient uptake rate, U ($\mu g/(m^2\, min)$), that reflects the magnitude of flux of nutrient from water column to the biota expressed on the basis of stream bottom area (Stream Solute Workshop 1990; Webster and Valett 2007):

$$U = \frac{C_b Q}{w S_w}$$

where C_b is the background nutrient concentration and w is the mean stream width. U may be a more suitable metric for comparing streams with very different nutrient concentrations (Fellows et al. 2006).

Discharge was estimated by the constant-injection method (Gordon et al. 1992), from the equation:

$$Q = 1000\frac{(c_t - c_1)}{(c_1 - c_0)}Q_t$$

where c_1 is the plateau conductivity at the end of the reach, c_0 is the background conductivity, c_t is the conductivity of the injected solute solution, and Q_t is the injection rate of the tracer.

In two sampling occasions (autumn and winter), conductivity was registered at the farthest downstream station until it returned to background values. These data were used to estimate hydraulic parameters using OTIS, a one-dimensional solute transport model (Runkel 1998). The governing equation underlying the model is the advection–dispersion equation with additional terms to account for transient storage and lateral inflow. Model parameters are

Table 1
Hydrological and biological parameters and spiraling metrics estimated in Las Flores stream at each sampling date.

	Hydraulic variables			Background SRP concentration (mg/L)	Spiraling metrics			Macrophytes	
	Discharge (L/s)	A_S (m^2)	A_S/A (m)		S_w (m)	V_f (mm/min)	U (μg/(m^2 min))	Cover (%)	Biomass (kg)
Late spring	31.2	–	–	0.88	250	3.67	1.49	38.7	–
Summer	35.7	–	–	0.35	–	–	–	83.5	187.7
Autumn	5.5	0.05	0.455	0.22	833	0.50	0.02	51.0	94.9
Winter	48.6	0.02	0.015	0.23	–	–	–	43.5	1.3

manipulated iteratively until the model-generated concentration curve provides the best-fit to empirical tracer data as determined by visual inspection. OTIS allows the estimation of the transient storage zone size (A_S; i.e., the cross-sectional area of the storage zones), the relative transient storage zone size (A_S/A; i.e., transient storage zone size divided by main channel cross-sectional area), and the storage zone exchange coefficient (α; i.e. the fraction of water entering the storage zones per unit of time) (Runkel 1998).

At each sampling date, wetted width was measured in 17 equidistant transects to determine surface area of the study reach. Macrophyte abundance was mapped by visually estimating percentage of the streambed covered by each plant species in 5-m subreaches. These data were used to calculate stream surface covered by different life forms (filamentous algae and emergent, submerged, and floating macrophytes). In addition, 10 macrophyte samples were taken with a 0.04-m^2 quadrat along the reach at each sampling date (except in late spring) to estimate plant biomass discriminated among functional groups. Plants were transported to the laboratory, sorted into life forms, dried at 60 °C until constant weight, and weighed. Biomass and cover data were combined to determine biomass of the different life forms at a reach scale (Feijoó and Menéndez 2009).

Results

Environmental conditions at the stream varied greatly among the experiments. Background SRP concentration in late spring triplicated those of the other sampling dates, while discharge was an order of magnitude lower in autumn (5.5 L/s) and varied from 31.1 to 48.6 L/s in the other sampling dates. Macrophyte cover (expressed as percentage of the stream reach surface) also changed, ranging from 38.7% in late spring to 83.5% in summer (Table 1).

In the experiments of summer and winter, SRP concentration did not decrease downstream after accounting for background concentration and dilution and S_w could not be estimated (Fig. 1), indicating that there may be no net phosphate retention at the reach. In spring and autumn, regression of natural logarithm of the dilution-corrected concentration of injected SRP vs. downstream distance was significant ($p < 0.01$ and $p < 0.05$, respectively). S_w was lower in spring (250 m) than in autumn (833 m) (Fig. 1 and Table 1). Given that discharge and background phosphate level were very different at both sampling dates, the other spiraling metrics should be more appropriated to characterize nutrient demand in these situations. The mass transfer coefficient (V_f), which accounts for differences in discharge, was higher in spring than in autumn as did the nutrient uptake rate (U) (Table 1). Consequently, despite

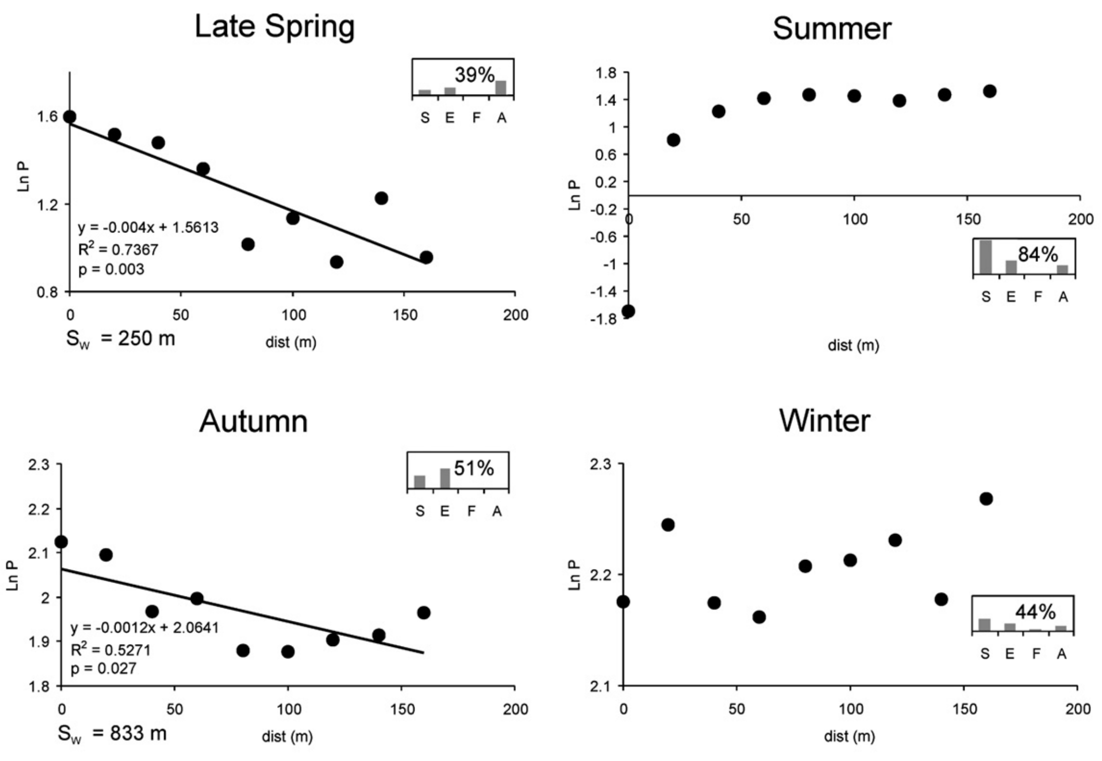

Fig. 1. Semi-log plot of dilution-corrected concentration of phosphate (in (mg SRP/L)/(μS/cm)) and distance. Regression equations (and significance) and S_w values for negative relations are indicated. Insert: cover (in m^2) of the different functional groups (S: submerged, E: emergent, F: floating macrophytes, A: filamentous algae) at the studied reach. The percentage indicated total macrophyte cover as percent of the stream reach surface.

Table 2
Macrophyte biomass (g/m^2) of the different life forms at the different sampling dates in the stream.

	Submerged	Emergent	Floating	Algae	Total
Summer	131.0	119.7	0	2.0	252.7
Autumn	34.6	95.3	0	0	129.9
Winter	1.3	0.6	0	0	1.9

the difference in discharge and phosphate concentration all spiraling metrics gave the same result: a higher SRP retention in spring than in autumn.

Macrophyte cover in summer doubled those from the other sampling dates (Table 1). Dominance of macrophyte life forms also changed in the different experiments: submerged plants dominated in summer and winter and emergent plants in autumn, while algal cover was higher in late spring (Fig. 1). A similar trend in dominance of life forms among seasons was observed for macrophyte biomass, nevertheless some differences emerged. Total biomass was also higher in summer but it was very low in winter, despite the relatively high plant cover (44%). Submerged macrophytes also dominated total biomass in summer, but the relative contribution of emergent plants to plant community was higher when considering its biomass than when considering its cover (Table 2).

Transient storage zone size (A_S) and relative transient storage zone size (A_S/A) were estimated for autumn and winter adjusting conductivity curves generated by OTIS to empirical data obtained at the farthest downstream station. A_S/A was an order of magnitude higher in autumn than in winter (Table 1).

Discussion

S_W measured in spring was within the interquartile range of values reported for pristine streams, while in autumn it was rather long (Table 3). Although background phosphate concentration at the stream was an order of magnitude higher than concentrations observed in pristine streams (0.001–0.03 mg/L) (Marcé and Armengol 2009), S_W (and thus overall uptake velocity) in spring and autumn was shorter than typical values for impaired streams (Haggard et al. 2005; Merseburger et al. 2005; Marcé and Armengol 2009). In spring, the mass transfer coefficient V_f that accounts for the differences in discharge was also within the range reported for pristine streams, while in autumn it was similar to those observed in impaired streams (Ensign and Doyle 2006; Marcé and Armengol 2009) (Table 3). Nutrient uptake rate U was much higher in spring than in autumn, indicating a higher areal phosphate uptake in the former season. Thus, under the varying conditions of discharge and SRP levels observed in our study, we found no retention in summer and winter, a phosphate uptake comparable with those measured in impaired streams in autumn, and with those measured in pristine streams in late spring.

It has long been accepted that aquatic vegetation affects solute transport and retention in lotic environments. However, there are few studies where nutrient uptake length was measured in vegetated streams as most research on solute retention has been performed in forested streams of the northern hemisphere, where macrophyte communities are poorly represented. In general, the presence of macrophyte communities has been associated to a higher nutrient retention. On one hand, this has been attributed to the lower current velocity produced by the luxurious development of submerged macrophytes (Wilcock et al. 2002), and to the enlargement of the transient storage zone in vegetated streams where nutrient retention is enhanced by increasing both residence time and contact of solute with biogeochemically active surfaces (Gücker and Boëchat 2004; Ensign and Doyle 2005; Gücker and Pusch 2006). On the other hand, greater uptake of inorganic nutrients has been related to higher rates of metabolism due to assimilatory demand by macrophytes and other autotrophs. In particular, phosphate uptake rates were positively associated to ecosystem metabolic rates (especially gross primary production) in eutrophic lowland streams with well-developed macrophyte communities (Gücker and Pusch 2006). Hence, nutrient retention could depend not only on plant biomass but on the metabolic activity of the whole autotrophic community, particularly in open streams dominated by autochthonous carbon contributions (Fellows et al. 2006).

In this study, the relative transient storage zone size decreased from a 46% in autumn to a 1.5% in winter, and this should be associated to the decline of macrophyte biomass from 95 to 1.3 kg of dry weight. These punctual estimations suggest that extensive in-channel vegetation increased the surface transient storage zone in the stream. The solute transport model used here cannot distinguish true hyporheic exchange from in-stream transient storage in vegetated reaches (Salehin et al. 2003), but in Las Flores stream the hyporheic compartment would be poorly represented. Sedimentary catchments with fine sediments (like Pampean catchments) may have low alluvial hydraulic conductivities (and thus reduced A_S/A) and high residence time of water in the stream channel (Valett et al. 1996; Morrice et al. 1997). The small relative transient storage zone estimated in Las Flores stream in winter, when macrophyte abundance was low, also supports the hypothesis that most transient storage should be related to the aquatic vegetation in this stream.

Macrophyte biomass increased and dominated in spring-summer in Las Flores stream (Giorgi et al. 2005; Giorgi and Feijoó 2010), reaching its highest productivity in summer (Vilches and Giorgi 2010). But, despite the great productivity and the enlarged transient storage zone that may occur in summer, no SRP retention was detected in that season. Algal communities are also well developed in the stream. The contribution of phytobenthos to total autotrophic biomass is not important except when floods wash the other communities downstream (Vilches and Giorgi 2010), but a dense epiphytic community can develop attached to the aquatic vegetation. During our study, epiphytic biomass was higher in spring (1.3 mg Chl-*a*/g plant dry weight), decreased in summer possibly due to the shading produced by macrophyte growth (0.35 mg Chl-*a*/g plant dry weight) (Giorgi and Feijoó 2010), and increased again in autumn (0.87 mg Chl-*a*/g plant dry weight) (Ferreiro, pers.

Table 3
Comparison of phosphate spiraling metrics obtained in this study with those reported by other authors in pristine and impaired streams. In some cases, total or interquartile ranges are indicated.

Stream	S_w (m)	V_f (mm/min)	U (μg/(m²min))	Source
Las Flores (spring)	250	3.67	1491.3	This study
Las Flores (autumn)	833	0.5	19.0	This study
21 streams of 3rd order	99–743 (2)	1.4–7.1 (2)	9.8–88 (2)	Ensign and Doyle (2006)
46 pristine streams	31–283 (2)	1.73–7.14 (2)	–	Marcé and Armengol (2009)
20 impaired streams	2398–6042 (2)	0.25–1.20 (2)	–	

(1) Total range.
(2) Interquartile range.

communication 2010). In addition, abundance of filamentous algae was higher in spring than in the other seasons, covering a 21% of the total surface of the studied reach. We detected phosphate retention in spring when filamentous and epiphytic algal abundance were the highest ones, and in autumn when epiphyton biomass was elevated. Our results suggest that phosphate uptake could rely more on the biomass and metabolic activity of the algal community, while macrophytes could play an important role giving structure to the physical environment and generating habitat heterogeneity. Consequently, our hypothesis is that macrophytes may not enhance nutrient retention principally by direct assimilation, but by enlarging the transient storage zone and giving a substrate for attachment to micro- and filamentous algae that should control nutrient retention in Las Flores stream. However, additional data are needed to investigate this hypothesis, especially on the metabolic activity of the different autotrophic communities.

To summarize, we observed different situations at Las Flores stream along the year: we did not detect net phosphate retention in summer and winter, and found low retention relative to nutrient supply in autumn and high retention (within the range reported for pristine streams) in spring. Increased macrophyte abundance should not always be reflected in a higher SRP uptake. In particular, in summer when macrophyte biomass (and especially that of submerged plants) was higher, no retention was detected. Overall, our results indicate that phosphate uptake is highly variable along the year and that spiraling metrics should be estimated under different environmental conditions and seasons to evaluate the whole potential of the stream for nutrient processing.

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