

## Dissolved phosphorus uptake in subtropical and temperate streams of Argentina

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### ABSTRACT

#### Phosphate uptake in subtropical and temperate streams of Argentina

Phosphate retention processes in streams of a subtropical and a temperate region were studied. Our hypothesis was that retention differs in the two regions. We expect more retention in the subtropical region than in the temperate one. If that were confirmed the differences in stream phosphate uptake may be driven by an interaction between phosphate concentration and algal biomass, associated with differences in temperature. Phosphate retention was studied by carrying out constant rate additions. Nutrient uptake rate ( $U$ ) and mass transfer coefficient ( $V_f$ : 2.67 E-04 y 9.27 E-05 m/s to subtropical streams and temperate one respectively) were associated with background phosphate, macrophyte biomass and transient zone storage according to PCA analysis. Thus, streams of temperate regions respond more to macrophyte biomass than streams of subtropical regions which were associated principally to benthic algae. These results are novel for South America and they reflect the relative importance of benthic algal or macrophyte biomass and production in relation to the P uptake rate at the different environments.

**Key words:** P-retention; streams; subtropical and temperate regions; spiralling metrics

### RESUMEN

#### Captación de fósforo disuelto en ríos subtropicales y templados de Argentina

Se estudió el proceso de retención de fosfato en ríos de regiones subtropicales y templadas. Nuestra hipótesis fue que la retención difiere en las dos regiones. Esperamos más retención en la región subtropical que en la templada. Confirmado esto; las diferencias podrían deberse a una interacción entre la concentración de fosfato y la biomasa de algas, asociada a las diferencias de temperatura. La retención de fosfato se estudió realizando adiciones en continuo de P. La tasa de asimilación ( $U$ ) y la velocidad de asimilación ( $V_f$ : 2.67 E-04 y 9.27 E-05 m/s para los ríos subtropicales y templados respectivamente) fueron asociadas con la concentración basal de fósforo, la biomasa de macrófitas y la zona de almacenamiento transitorio de acuerdo con un análisis de PCA. Así, los ríos de las regiones templadas responden más a la biomasa de las macrófitas que los ríos de las regiones subtropicales que se asociaron principalmente a las algas bentónicas. Estos resultados son novedosos para América del Sur y reflejan la importancia de la biomasa y producción de algas bentónicas o macrófitas en relación con la tasa de captación de P en diferentes ambientes.

**Palabras clave:** retención de fósforo, ríos, regiones subtropical y templada, métricas de retención

## INTRODUCTION

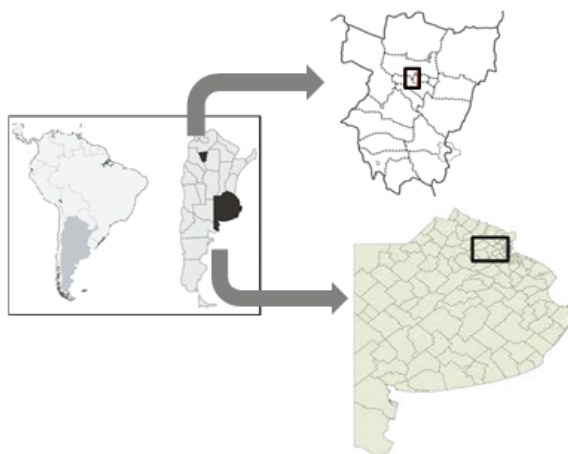
Nutrient uptake is an important functional feature of lotic ecosystems. In streams, nutrients are continuously transformed from dissolved inorganic forms to particulate organic matter and eventually back to dissolved inorganic forms as they are transported downstream. This coupling of nutrient cycling and downstream transport was defined as spiralling (Webster & Patten, 1979). Nutrient spiralling describes the process of nutrient cycling between various in-stream compartments, which are coupled with advective transport. Nutrient addition experiments are widely used to assess the ability of stream ecosystems to retain solutes and they allow estimation of the uptake length ( $S_w$ ), the uptake velocity ( $V_f$ ), and nutrient uptake rate ( $U$ ).  $S_w$  is the average distance that a molecule travels in the water column before being removed by the benthic compartment,  $V_f$  is the velocity at which a molecule moves through the water column toward the sediment, and  $U$  reflects the magnitude of molecule flux from water column to the biota under background conditions (D'Angelo *et al.*, 1991; Webster & Valett, 2007; Álvarez *et al.*, 2010).

Nutrient retention is related to hydrology, nutrient availability, and biological community. There are other factors that have an indirect influence on nutrient retention. It is well known that higher temperatures may lead to higher algal growth (Godwin & Carrick, 2006). Thus, an increase in temperature would influence nutrient dynamics. Light is one of the most important factors affecting primary production at streams. Therefore the photoautotrophic community is important in nutrient uptake rates. Among them, it has been shown that gross primary productivity (GPP) may have a main role in controlling uptake rates in streams of Europe and North America. Indeed, GPP has been related to both daily variations in stream nutrient concentrations as well as to regional gross uptake rates. Many streams have low benthic irradiances because of high loads of suspended sediment or because of shading by riparian vegetation, which are a significant constraint on lotic primary production. Stream-side trees are very effective at intercepting solar

radiation, reducing benthic irradiances to as low as  $10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  or less in streams flowing through undisturbed forests. Low irradiances are a powerful constraint on benthic algal photosynthesis (Hill & Dimick, 2002).

South America is a continent that covers all latitudes and climates. Because of the development of cattle farming and other activities, subtropical and temperate regions have a significant anthropogenic impact that produces erosion and nutrient enrichment in water bodies. Nutrient retention has been widely studied in temperate streams from North America and Europe (Martí & Sabater, 1996; Payn *et al.*, 2005; Sabater *et al.*, 2005; Álvarez *et al.*, 2010), but there are few studies from South America under different types of climates. There is no information about what factors are controlling nutrient uptake in temperate and subtropical streams of South America. The two regions clearly differ in hydrological regime, temperature, and climate. Moreover, these two regions have different in-stream photoautotrophic community, but are affected by similar anthropogenic pressures at the closest zone at least. Under this context the present study attempt to fill a gap in our knowledge on nutrient retention processes in two dissimilar regions to understand temperate and subtropical streams with the same anthropogenic impact but different climate.

The aim of this study was to characterize phosphorus retention in two groups of streams of two different regions of Argentina under different climate conditions and with similar intensity of rural activities. We hypothesized that retention metrics are different in both temperate and subtropical streams despite other differences in structure and function but that the algae are principally the responsible of the dissolved P retention capability at the stream. We predicted that climate differences jointly with phosphorous concentration at each stream drive algal growth. Furthermore, an increase in benthic algal biomass would be reflected in an increase in phosphorus retention. Hence, phosphorus uptake rate would be higher (with a higher  $V_f$ ) in subtropical streams than in temperate streams except when local conditions reduce algal development.



**Figure 1.** Location of the Subtropical and temperate streams. Location of the Argentina in South America (Left). Center map shows the provinces chosen as examples of subtropical (northern) and temperate regions (southern). In the right squares enclose studied area in Tucuman and Buenos Aires provinces. *Ubicación de los ríos subtropicales y templados. Ubicación de Argentina en Sudamérica (izquierda). El mapa del centro muestra las provincias elegidas como ejemplos de regiones subtropicales (norte) y templadas (sur). Dentro de los recuadros se incluye el área estudiada en las provincias de Tucumán y Buenos Aires.*

## MATERIAL AND METHODS

### Study Site

We selected six streams: La Choza, Haras, and Las Flores streams in a temperate region (Pampas) and Potrerillo, San Javier, and Noque in a subtropical region of Argentina (Yungas) (Fig. 1).

The Pampean biome lies in the eastern plains of Argentina between 30-39° South, Uruguay and the southern end of the state of Rio Grande do Sul, in Brazil. It comprises an extremely flat region with a warm-temperate climate, rainfall distributed throughout the year ranging from 600 to 1200 mm and an average annual temperature between 13 and 17 °C (Cabrera & Willink 1980). The annual radiation value of photosynthetic active radiation (PAR) is 365  $\mu\text{mol}/\text{m}^2 \text{ s}$  while radiation reaches 292  $\mu\text{mol}/\text{m}^2 \text{ s}$  in spring (Murphy, 2008). Under natural conditions, vegetation in the region is that of the grassland, with annual grasses adapted to the occurrence of fires

in summer and frost in winter. Pampean streams are also characterized by low gradients, lack of riparian tree vegetation, and diverse macrophyte communities with abundant development of epiphytic algae. Streambeds are formed by hard and homogeneous substrata of fine sediments (primarily silt and clay), with high content in calcium carbonate, and no stones or pebbles (Giorgi *et al.*, 2005). The flow regime of streams in this area can be classified as mesic groundwater *sensu* LeRoy Poff & Ward (1989) with a mean flow of 20 L/s. These streams have approximately 70 % of rangeland while 30 % is considered residential. This area is characterized by extensive rural activities (cattle farming and agriculture). Selected streams are considered to be representative of many Pampean streams of low orders.

On the other hand, the mountainous area of North-western Argentina is formed by two orographic units: Pampean and Subandinean ranges. The central area of these mountain ranges comprises a wide extension of mountain rainforest called Yungas. They contain the headwaters of important basins and include many intermountain valleys used for agriculture and pastures, with slopes clearly higher than those in Pampean regions (Izquierdo & Grau 2009). In this region the average annual rainfall is 1200 mm and average annual temperatures are between 18 and 20 °C. The annual radiation values are similar to those of the temperate zone, 365  $\mu\text{mol}/\text{m}^2 \text{ s}$ , being 342  $\mu\text{mol}/\text{m}^2 \text{ s}$  in spring (Murphy, 2008) but there is forest vegetation in nearly all the basin while in temperate streams grassland is the dominant coverture. San Javier, Potrerillo, and Noque are second order streams and they are part of the San Javier Basin. The three have high slopes that generate torrential rivers. These streams have a crystalline base-ment composed of metamorphic rock and igneous intrusions (Guido, 2011). These subtropical watercourses are characterized by abundant development of epilithon. San Javier Basin is characterized by extensive rural activities (cattle farming and agriculture) and human housing that promote systems open to light irradiation. Although stream slopes and the bottom substrates can be different in the two zones, we selected three low order streams at both regions

**Table 1.** Hydraulic and biological variables at the studied streams of subtropical and temperate regions. Q: water flow; w: mean width; u: mean velocity; h: mean depth; %T: percent of transparency; As: Transient zone storage. Benthic biomass: expressed as chlorophyll-*a*; Macrophyte biomass: grams dry mass per unit area. *Variables hidráulicas y biológicas de los ríos estudiados en las regiones subtropical y templada.* Q: caudal; w: ancho medio; u: velocidad media; h: profundidad media; %T: porcentaje de transparencia; As: zona de almacenamiento transitorio. *Biomasa bentónica: expresada como clorofila-a; Biomasa de macrófitas: gramos de materia seca por unidad de área.*

Stream	Conductivity ( $\mu\text{S}/\text{cm}$ )	pH	Q (L/s)	w (m)	u (m/s)	h (m)	%T	As ( $\text{m}^2$ )	T ( $^{\circ}\text{C}$ )	Background P- $\text{PO}_4^{3-}$ concentration (mg/L)	Benthic biomass ( $\text{mg}/\text{m}^2$ )	Macrophyte biomass ( $\text{g}/\text{m}^2$ )
Potreriillo	1180	8.3	47	2.33	0.16	0.09	75	0.001	18	0.012	28.85	0.02
San Javier	325	8.5	60	2.43	0.17	0.11	100	0.001	25	0.06	2.14	0.05
Noque	320	8.9	14	2.87	0.04	0.08	96	0.004	23.5	0.09	35.5	0.03
Las Flores	873	8.3	21	1	0.05	0.38	100	0.006	16	0.878	12.27	130.6
Choza	1408	7.8	38	2.6	0.04	0.29	75	0.003	17	0.160	8.80	0.7
Haras	942	7.5	55	1.5	0.23	0.16	60	0.004	13	0.25	0.77	63.9

with reaches with similar slopes and considered that the benthic algae grown on pebbles (epilithon) or macrophytes (epiphyton) were functionally similar communities.

The study was performed in spring because this season has the highest algae growth. A 100 m reach was selected at each stream in an area with homogeneous riparian vegetation and hydraulic characteristics like lack of lateral inflow or outflow.

### Sampling

Stream mean width was calculated measuring three times along 100 m reach. Mean depth was calculated using measures of three different transect along the reach with a graduate rod. Water velocity was determined in the streams using a Flo-Mate 2000 digital speedometer. Discharge values were calculated from flow velocity data (Global Flow Probe; accuracy 0.03 meters per second), and the width and depth of the river. The percent of transparency was measured using the Transparency Turbidity Tube (AQUAlogger 210TY).

### Benthic algal biomass

Epilithic samples were collected using a 5 by 5 cm frame attached to the surface of the rocks that it could be scraped without loss of material. The scraped material was collected into a vial and then filtered (Whatman GF/F), extracted in 90 % acetone for 24 h, and analyzed spectrophotometrically for chlorophyll-*a* (APHA, 1998). This estimation was expressed as chlorophyll *a* mass per area of scraped rock. Epiphyton samples were obtained by collecting macrophytes in a 20 by 20 cm frame. Samples were carried to the laboratory where they were identified, washed and cleaned to obtain a suspension that was filtered through Whatman GF/C glass fiber membranes. Chlorophyll-*a* determination was carried out in the same way as described previously. In addition to this, ten macrophyte samples of similar size were subtracted from along the reach at each sampling date to estimate plant biomass per square meter. Plants were dried in the laboratory at 60  $^{\circ}\text{C}$  until constant weight, and weighed. An aliquot of the scraped material

from rocks and plants was preserved in formaldehyde 1 % to identify the epilithic or epiphytic algae and to determine percent of representation of the different algal divisions.

### P- additions

Short-term phosphorous additions were performed at each reach with a conservative (NaCl) and a non-conservative tracer (phosphorus as  $K_2HPO_4 \cdot H_2O$  amended with nitrate and ammonia to avoid an excessive change in the N/P ratio of water). The solution was prepared in the field with filtered stream water and then added at constant flow rate from the head of the reach just before a channel narrowing, to enhance solute mixing. Phosphorus concentration in the solution was adjusted for each addition to obtain an increase of two times over the natural concentration. Triplicate water samples were collected in polyethylene bottles before the beginning of the addition to determine background conductivity and phosphorus concentration in the reach. Before and during the addition, triplicate water samples were taken every 20 m in a 100 m long reach, until tracer concentration reached a plateau in the farthest downstream station, as determined by continuous conductivity measurements (conductivity sensor HACH Sension 5). Plateau conditions were reached after 20/40 minutes. Thus, the whole experiment was carried out in approximately two hours because the return to basal conditions takes longer than the increase to plateau. Water samples were filtered with Whatman GF/F membranes and stored in an ice chest for approximate-

ly two hours until they were transported to the laboratory. Conductivity was determined immediately and soluble reactive phosphorus (SRP) was determined within one day from collection using the ascorbic acid method (APHA, 1998).

### Calculations

Added phosphate concentration (estimated as the difference between plateau and background phosphate concentrations) was standardized, dividing it by conductivity (represented as the difference between plateau and background conductivities) and applying natural logarithm to these quotients. Nutrient uptake length ( $S_w$ ) was calculated as the inverse of the slope ( $k$ ) of the regression between the standardized concentration of injected phosphorus and the distance downstream (Newbold *et al.*, 1981; Stream Solute Workshop, 1990; Martí & Sabater, 1996).

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

Two other retention metrics were calculated (Martí & Sabater, 1996; Webster & Valett, 2007), the mass transfer coefficient or velocity of assimilation,  $V_f$  (mm/min) and the nutrient uptake rate,  $U$  ( $\mu\text{g}/\text{m}^2\text{min}$ ).

$$V_f = h.v / S_w \quad \text{and} \quad U = C.Q / S_w.w$$

**Table 2.** Percent of representation. Organisms of different algae Divisions at Subtropical and temperate streams. *Porcentaje de representación. Organismos de diferentes Divisiones de algas en los ríos subtropicales y templados.*

Algae groups %	Potrerrillos	San Javier	Noque	Las Flores	Choza	Haras
Bacillariophyta	55	30	50	80	60	90
Chlorophyta	0	70	40	10	30	8
Cianophyta	45	0	10	10	10	2

Where:  $h$  is the depth,  $v$  the water velocity, and  $S_w$  the nutrient uptake length as previously was mentioned and;  $C$  is the phosphorus concentration,  $Q$  is the discharge and  $w$  is the width of the stream.

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

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

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 tracer injection rate. The conductivity curve was also used to estimate the size of the transient storage zone ( $A_s$ ) using OTIS, a one-dimensional solute transport model (Runkel, 1998). The advection–dispersion equation under-

lies the model with additional terms to account for transient storage and lateral inflow. Dispersion coefficient and transient storage zone size ( $A_s$ ) was estimated adjusting conductivity curves generated by OTIS to empirical data obtained at the farthest downstream station. The transient storage zones represent zones of the stream channel where water moves slower than in the main channel and retains solutes in a transitory way (Harvey *et al.*, 1996).

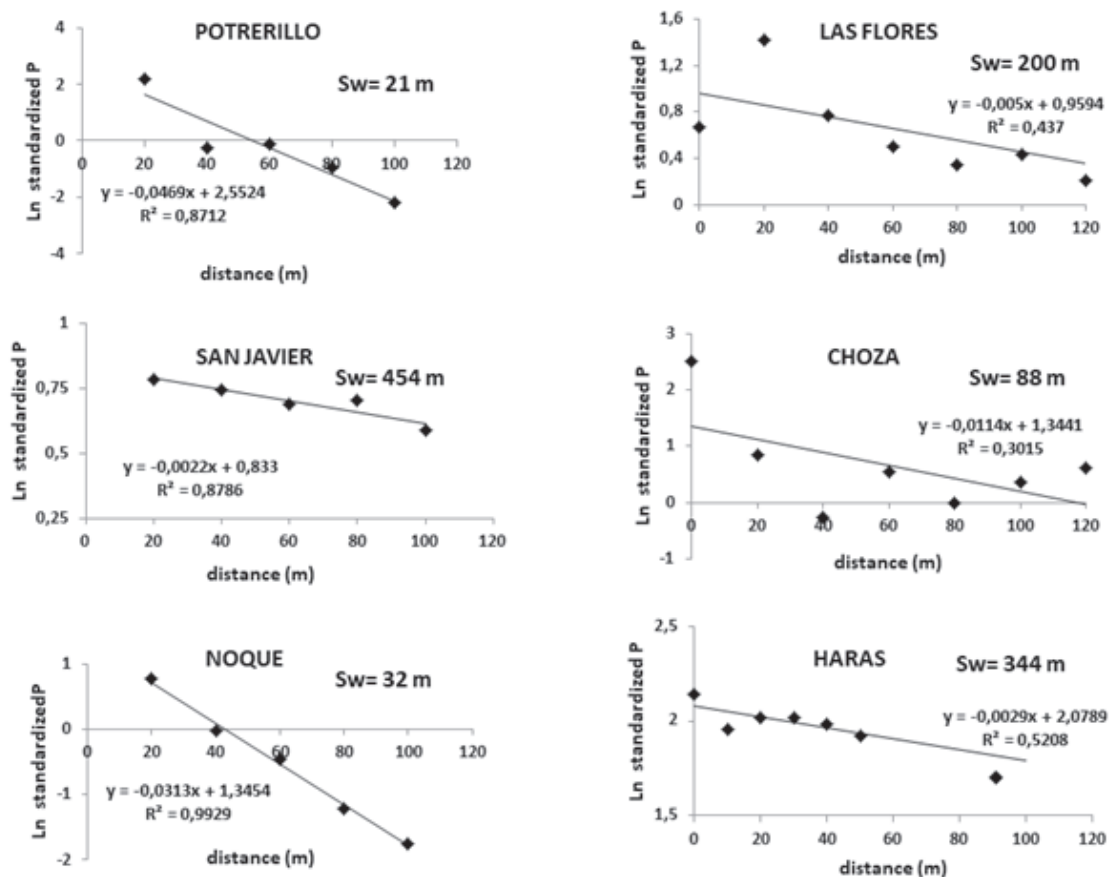
### Analysis

We compared background phosphate concentration of the two regions using a Mann-Whitney U analysis. Non parametric Spearman correlation analysis of all the variables registered in each stream and a PCA analysis was performed to explore the associations between variables and spiralling metrics using the software Statistica 6.0® (StatSoft, Inc., Tulsa, Oklahoma, USA) (Sokal & Rohlf, 1995).

**Table 3.** Spiralling metrics gained from field experiments in streams of subtropical and temperate regions. Results of constant rate additions of phosphate in streams of different regions: Potrerillo, San Javier and Noque streams belong to the subtropical region while Las Flores, Chozas and Haras streams are in the temperate region.  $S_w$ : nutrient uptake length;  $V_f$ : mass transfer coefficient;  $U$ : nutrient uptake rate. *Métricas de retención obtenidas de los experimentos de campo en los ríos de las regiones subtropicales y templadas. Resultados de las adiciones en continuo de fosfato en los ríos de las diferentes regiones: Potrerillo, San Javier y Noque pertenecientes a la región subtropical, Las Flores, Chozas y Haras de la región templada.  $S_w$ : distancia de asimilación;  $V_f$ : velocidad de asimilación;  $U$ : tasa de asimilación de nutriente.*

Stream	Background P-PO <sub>4</sub> <sup>3-</sup> concentration (mg/L)	Injected P-PO <sub>4</sub> <sup>3-</sup> concentration (mg/L)	Spiralling metrics		
			$S_w$ (m)	$V_f$ (m/s)	$U$ (mg/m <sup>2</sup> s)
Potrerillo	0.012	0.018	21	6.9 10 <sup>-4</sup>	0.011
San Javier	0.06	0.09	454	3.6 10 <sup>-5</sup>	0.0034
Noque	0.09	0.18	32	7.6 10 <sup>-5</sup>	0.014
Las Flores	0.878	2.3	200	9.5 10 <sup>-5</sup>	0.090
Chozas	0.160	0.4	88	1.32 10 <sup>-4</sup>	0.065
Haras	0.25	0.8	344	9.2 10 <sup>-5</sup>	0.023





**Figure 2.** Semi-log plot of dilution-corrected concentration of phosphate (in (mgSRP/L)/( $\mu$ S/cm)) and distance. Regression equations and Sw estimated are indicated. *Diagrama semi-logarítmico de la concentración de fosfato corregida por dilución (en (mg FRS/L)/( $\mu$  S/cm)) y la distancia. Se muestra la ecuación de regresión y el Sw estimado.*

## RESULTS

### Patterns of physicochemical variables

Hydraulic and biological variables of the studied streams are shown in Table 1. The conductivity values were 1180, 325 and 320  $\mu$ S/cm in Potrerillo, San Javier, and Noque streams respectively and 873, 1408 and 942  $\mu$ S/cm in Las Flores, Choza, and Haras streams respectively. There were no statistical differences between pH values of the regions being the Noque stream the one with the highest value, of 8.9. Subtropical streams showed higher temperatures (18, 25 and 23.5 °C in Potrerillo, San Javier, and Noque rivers respectively) than temperate streams (16,

17 and 13 °C in Las Flores, Choza, and Haras rivers respectively). The highest flow value was 60 L/s in San Javier River and the lowest value was 14 L/s in Noque river. Regarding  $A_s$  the values were 0.001, 0.001 and 0.004 m<sup>2</sup> in Potrerillo, San Javier, and Noque streams respectively and 0.006, 0.003 and 0.004 m<sup>2</sup> in Las Flores, Choza, and Haras streams respectively. Finally, the background PRS concentration differed between regions (Mann-Whitney U test,  $p < 0.05$ ); with temperate streams showing the highest values (mean value 0.43 mg/L). The values for depth and width are not related to the regions. The depth values ranged from 0.08 to 0.38 meters and width values ranged from 1 to 3 meters.

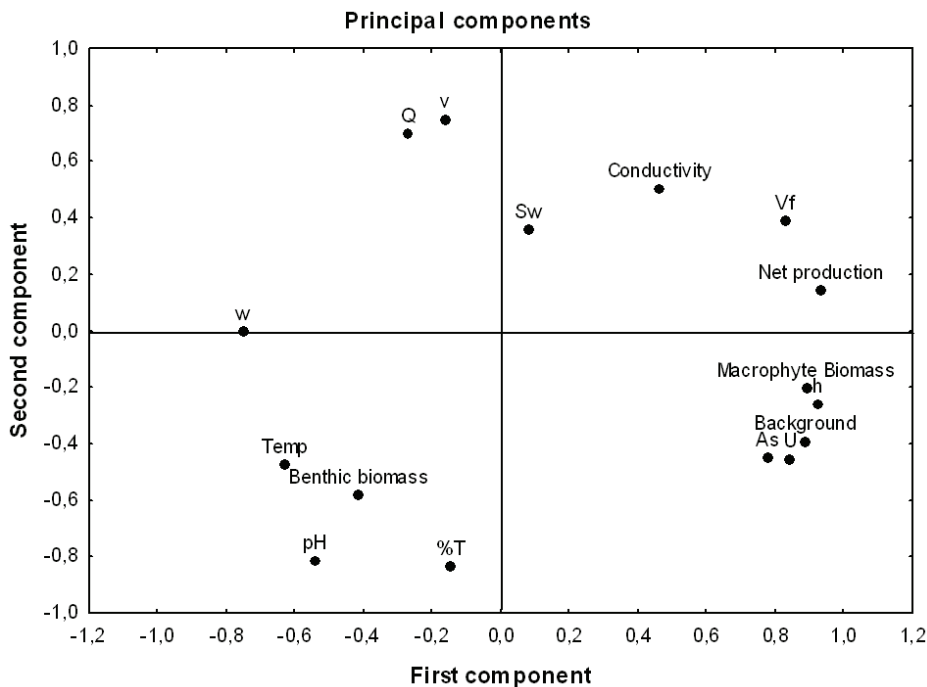
### Photoautotrophic community

Subtropical streams showed higher benthic algae biomass (28.85, 2.14 and 35.5 mg/m<sup>2</sup> in Potrerillo, San Javier, and Noque rivers respectively) than temperate streams (12.27, 8.80 and 0.77 mg/m<sup>2</sup> in Las Flores, Choza, and Haras rivers respectively) (Table 1). On the other hand, the highest macrophyte biomass was 130.6 g/m<sup>2</sup> from Las Flores river. All the temperate rivers had more macrophyte biomass than the subtropical ones while subtropical watercourses had the highest values of benthic algae biomass. The principal group of algae found in all the temperate streams was Bacillariophyta although Cyanophyta, Chlorophyta, and Euglenophyta were also present at a smaller extent (Table 2). These are algae that grow principally over macrophytes but also *Cladophora* and *Spirogyra* were found at the streams and the first genus was colonized by microalgae. Differences were found among subtropical streams. Potrerillo was characterized

by Bacillariophyta and Cyanophyta with similar abundance and codominance. San Javier stream had Chlorophyta as the dominant group, particularly *Cladophora* and *Ulothrix* genera, and Bacillariophyta (*Achnantes*, *Fragillaria*, *Meridion*, and *Cymbella*). Finally, in Noque stream Chlorophyta and Bacillariophyta were codominant while *Spirogyra* and *Cladophora* were the most important genera of Chlorophyta. The diatoms of the genus *Cymbella*, *Surirella*, *Diatoma*, and *Hippodonta* were the most abundant. In subtropical streams, there were scarce macrophytes as *Hydrocotyle ranunculoides* but in the temperate streams *Egeria densa*, *Elodea canadense*, and *Ludwigia peploides* were found in the reaches, mostly colonized by algae.

### Uptake rates

The spiralling metrics determined for the different streams are shown in Table 3. The uptake length ( $S_w$ ) estimated, ranged from 20 to 450 meters in



**Figure 3.** PCA analysis to explore the associations between physics, chemical and biological variables and retention metrics. Temp: indicates temperature and % T is the percent of transparenca in the stream. *Análisis PCA en la búsqueda de asociaciones entre variables físicas, químicas y biológicas y las métricas de retención* Temp: indica temperatura and % T es el porcentaje de transparenca en el arroyo.



both environments and the nutrient uptake rate ( $U$ ) ranged from 0.003 to 0.09 mg/m<sup>2</sup>min. Hence, there was no clear relation between metrics and regions. Figure 2 shows a semi-log plot of standardized concentrations of phosphate vs. distance.  $S_w$ , estimated as the inverse of the slope of the regression, was also indicated.

The results of a non-parametric Spearman correlation analysis are shown in Table 4. Among these results is important to highlight a positive correlation between background concentration of phosphorus and the size of the transient storage zone. We also found a negative correlation between benthic algal biomass and uptake length. On the other hand, we found a positive correlation between  $U$  and background phosphate concentration, and between transient storage zone and  $U$ . The other significant correlations found were between macrophyte biomass with background phosphate concentration.

The PCA analysis explained a high percent-

age of variability (70 % by summing the first and second component) and the first component highlighted the association of background phosphate,  $V_f$ ,  $U$ , and  $A_s$  with the macrophyte biomass while the relation with benthic algae and temperature was negative. Also, the relation between the benthic algal biomass and  $S_w$  was negative. The retention uptake lengths responded inversely to benthic algae biomass but also to macrophyte biomass (Fig. 3). The plot of scores of the PCA analyses differentiated temperate and subtropical streams clearly although algae composition was not considered as a variable (Fig. 4, Table 5).

**DISCUSSION**

This study is a comparison of the retention capability in streams from two regions with climate differences basically in temperature and without limiting resources. We expected differences in retention between both regions and we found that

**Table 4.** Spearman Rank Order Correlations of the variables registered for each stream. Significant correlation values between retention metrics ( $p < 0.05$ ) are marked with an asterisk. *Coefficientes de correlación de Spearman de las variables registradas para cada río. Los valores significativos de correlación entre las métricas ( $p < 0.05$ ) están marcados con un asterisco.*

	As	Macrophyte biomass	Benthic biomass	T	%T	U	$V_f$	$S_w$	Background P- $PO_4^{3-}$	v	h	w	Q	pH	Conductivity
Conductivity	-0.26	0.09	-0.26	-0.49	-0.62	0.2	0.77	-0.26	0.03	0	0.43	-0.2	0.2	-0.78	1
pH	-0.13	-0.52	0.64	0.84*	0.72	-0.55	-0.49	-0.17	-0.46	-0.35	-0.61	0.49	-0.32	1	
Q	-0.62	-0.003	-0.77	0.09	-0.18	-0.43	-0.09	0.60	-0.31	0.84*	0.03	-0.26	1		
w	-0.35	-0.60	0.37	0.66	0	-0.54	0.09	-0.31	-0.49	-0.58	-0.6	1			
h	0.44	0.89*	-0.49	-0.66	0.09	0.71	-0.14	0.42	0.77	0.03	1				
v	-0.22	0.14	-0.70	-0.2	-0.22	-0.12	-0.14	0.58	-0.06	1					
Background P- $PO_4^{3-}$	0.88*	0.94*	-0.31	-0.77	0	0.94*	-0.26	0.37	1						
$S_w$	0.09	0.60	-0.83*	-0.09	0.26	0.09	-0.71	1							
$V_f$	-0.26	-0.37	0.20	-0.31	-0.79	0.03	1								
U	0.88*	0.83	-0.14	-0.89	-0.18	1									
%T	0.09	0.09	0.26	0.53	1										
T (°C)	-0.62	-0.71	0.37	1											
Benthic biomass	0.09	-0.54	1												
Macrophyte biomass	0.71	1													
As	1														

**Table 5.** Results of PCA analysis. Weight of variables in the first two components. *Resultados de los análisis de PCA. Peso de las variables en los dos componentes principales.*

Variables	First Component	Second Component
Background phosphate	<b>0.89</b>	-0.33
S <sub>w</sub>	0.08	0.37
Vf	<b>0.83</b>	0.43
U	<b>0.84</b>	-0.40
As	<b>0.78</b>	-0.40
Q	-0.27	<b>0.69</b>
W	<b>-0.75</b>	-0.066
V	-0.15	<b>0.75</b>
H	<b>0.93</b>	-0.20
% transparency	-0.14	<b>-0.84</b>
Temperature	-0.63	-0.50
Benthic Biomass	-0.41	-0.60
Macrophyte biomass	<b>0.89</b>	-0.14
Conductivity	0.46	0.53
pH	-0.54	<b>-0.84</b>
Net production	0.93	<b>0.14</b>
Eigenvalue	7.08	4.20
% of Variance	44.22	26.28
% cumulative variance	44.22	70.50

temperate and subtropical streams differed mainly in background phosphorus concentration, temperature, macrophyte biomass, size of transient storage zone, and nutrient uptake rate. The macrophytes may have more development at temperate zones because of the background P-PO<sub>4</sub><sup>3-</sup> concentration and they may have a direct or an indirect influence on the retention process, because they can contribute to the transient storage zone or because they are substrate to periphyton development. Haggard *et al.* (2001) attributed the retention capability to the transient storage due to macrophyte density, and this may also be the case in this study, especially in temperate streams. The high correlation found between nutrient uptake rate and the size of the transient storage zone particularly affects temperate streams which showed the biggest transient storage zones. These results agree with those obtained by Feijoó *et al.* (2011), who observed that macrophytes may not enhance nutrient retention by direct assimilation, but by

enlarging the transient storage zone and giving a substrate for the attachment of microscopic and filamentous algae that control nutrient retention in a temperate stream. In the same way, Argerich *et al.* (2008) showed the importance of A<sub>s</sub> by seasonal litter input. Furthermore, these results agree with those obtained by Gucker & Boechat (2004) where channel morphology controlled transient storage and ammonium retention in tropical headwater streams. Also, the importance of the size of the transient storage zone in P-capture during the experiments could be explained by an increase of autotrophic metabolism, at least in the season of the addition experiments (Fellows *et al.*, 2006).

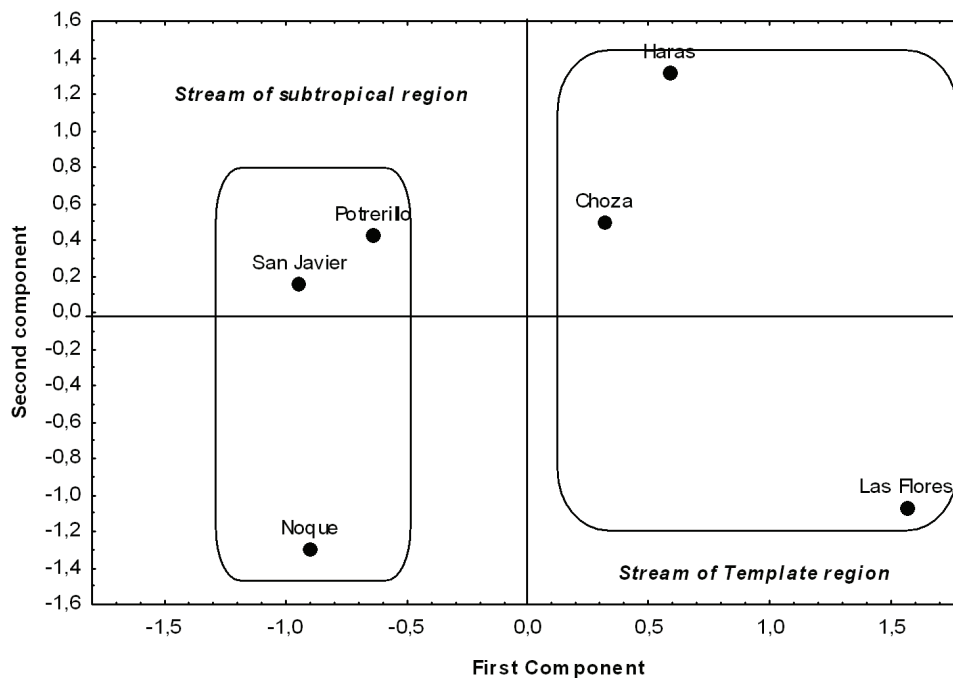
Additionally, we found a negative relation between nutrient uptake length of the different reaches and the active biomass of benthic algae (e.g.-Chlorophyll-*a* estimations). This means that when algal biomass is higher, assimilation distance is shorter and the system will be more efficient in nutrient retention. These results also

agree with those obtained by Feijoó *et al.* (2011), who performed a previous estimation of spiralling length carried out specifically in a temperate stream in South America. At this study, the authors hypothesized that the retention capability could be related by macrophyte biomass but found that the retention increase more by the presence of periphytic algae. So values of 1 mg of periphytic Chlorophyll-*a*. per g. of macrophyte dry weight or higher had more retention than lower values. In our study the highest chlorophyll-*a* values retain more phosphorus at both regions. Other study in temperate streams showed negative relation between nutrient uptake length and the active biomass of benthic algae. The chlorophyll-*a* values were in the same order as the present work but the heterotrophic biofilms become more important than the autotrophic ones (García *et al.*, 2017). This also concurs with nutrient additions performed in European ecosystems (Elosegui *et al.*, 1995; Martí & Sabater, 1996). The negative correlation between nutrient uptake length and algal active biomass was more relevant at subtropical streams when more algal biomass was present. Clearly, our hypothesis that phosphorus retention depended on algal biomass in both study areas has been demonstrated. In temperate streams the high basal concentration of phosphorus contributes to the development of macrophytes that increase the transient storage zones enhancing algae nutrient uptake from the water column. In the case of subtropical streams, high temperatures and radiation could enhance algal growth and, hence, nutrient uptake. This is supported by the negative correlation between  $S_w$  and algal biomass but also is possible that also the heterotrophic component of periphyton contributed to the dissolved phosphorus absorption as García *et al.* (2017) postulate. Because both, autotrophic and heterotrophic periphyton have organisms with a high rate of growth and reproduction that need to absorb nutrients to their development. These results also agree with those found by Sabater *et al.* (2000) where unshaded streams had more algal biomass and thus lower uptake lengths. An alternative explanation is that larger transient storage zones could favor the action of abiotic processes as precipitation. The high values of pH

and the presence of calcium ion in the water at temperate streams could reduce P- solubility and favors precipitation (Rolandi *et al.*, 2011). This possibility offers an alternative explanation on the retention capability that could be attributed at least partially, to abiotic processes. Aldridge *et al.* (2009) showed the importance to abiotic processes for phosphorus retention. They also affirmed that both abiotic and biotic processes are important to phosphorus uptake.

It is relevant to remark that retention metrics obtained show high retention capability. The longest  $S_w$  in this study was around 450 meters, but other studies with similar phosphorus concentration registered 800 to 1250 meters. (Sabater *et al.*, 2000; Haggard *et al.*, 2001; Sabater *et al.*, 2005). Moreover  $S_w$  and  $V_f$  in all studied streams were within the range reported for pristine streams (Marcé & Armengol, 2009). However  $U$  was one order of magnitude higher than the values presented by other authors (Ensign & Doyle, 2006).

Other authors postulate that coarse particulate organic matter (CPOM) regulates the  $S_w$  length in fall and winter periods (Mulholland *et al.*, 1985). Acuña (2002) sustains that the physical characteristics of the streams jointly with biological communities regulate the spiralling metrics in Mediterranean streams. Also, invertebrates can play an important role in P or N ecosystem retention by biofilm ingestion and by incorporation of nutrients to their own biomass, as Solimini *et al.* (2005) asserted; but this possibility could not explain the quick P retention in addition experiments as the ones carried out in our study. It is also possible that flooding and the limitation of light penetration reduce algae development in Mediterranean ecosystems and consequently their capability to retain phosphorus (Acuña *et al.*, 2007). Other factors could regulate nutrient uptake in streams with light, floods or drought-induced limitation. In this study, we can assert that the streams had a high P-retention capability and this situation is related to the presence of algae and their growth. This development is possible in conditions of hydrologic and thermic stability. Background phosphate,  $V_f$ ,  $A_s$  and  $U$  were associated with macrophyte biomass, but P absorption seemed to be



**Figure 4.** Plot of the scores of the first two axes of principal content building with the physical, chemical and biological variables and retention metrics. *Ordenamiento de los ríos sobre los dos primeros ejes de los componentes principales basado en los valores de variables físicas, químicas, biológicas y métricas de retención.*

carried out by benthic algae (related with the second component of the PCA) due to its capability to absorb phosphorous despite its high background concentration in water.

Clearly, more studies in other streams of both regions are necessary to determine if other patterns of P retention associated with regional characteristics are present. This study highlights the importance of benthic algae in relation to nutrient retention in two different regions and its association with the size of transient storage zones.

## CONCLUSIONS

This study shows the importance of macrophytes and chlorophyll-*a* of benthic algae, in retention when background phosphate is high. More efficient retention processes would be related to the further development of macrophytes because they increase the transient storage zones and provide the surface for algae colonization and growth. It is possible that in spring, with proper

conditions of irradiance, algal growth in both regions increases retention. Both, subtropical and temperate streams with favorable light conditions and without other environment restrictions have a high P-retention ability during spring. However, it is necessary to explore the stream responses in other seasons of the year to determine the potential to retain P and other nutrients.

The results obtained in this study showed that gross P uptake rates are high in subtropical and temperate streams of Argentina. Moreover, the underlying mechanisms driving P uptake rates in both systems are similar. They, in temperate streams, depended to algal growth and macrophytes because they increase the transient storage zones. However the mechanism, in subtropical stream, depended to algal growth and favorable light condition. This is a novel study because there was no comparative information regarding P uptake in these ecosystems. In spite of difference between regions there are similar absorption mechanisms in both regions.

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