

High nutrient retention in chronically nutrient-rich lowland streams

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Abstract: Nutrient retention has been studied intensively in streams, but some mechanisms and processes are not yet entirely understood, especially in open-canopy streams. We evaluated PO_4^{3-} , as soluble reactive P (SRP), and NH_4^+ uptake in 2 chronically enriched Pampean streams with different macrophytic abundance. We performed short-term nutrient additions to quantify SRP and NH_4^+ uptake metrics throughout the year, and we investigated which hydrological and biological factors influenced nutrient uptake. The results showed that SRP and NH_4^+ uptakes were high in relation to pristine and impaired streams elsewhere, and they did not saturate despite elevated background nutrient levels. NH_4^+ areal uptake rate fit in a 1st-order uptake model, but an exponential model described the relationship between SRP areal uptake rate and SRP concentration. Consistent with this finding, SRP uptake velocity tended to increase linearly, and SRP uptake length decreased linearly with SRP concentration. The analysis of factors influencing uptake metrics suggested that SRP uptake mainly depended on heterotrophic demand, whereas NH_4^+ uptake was enhanced under more autotrophic conditions. Our results showed that nutrient uptake metrics of enriched Pampean streams were similar to those observed in streams with low nutrient levels.

Key words: phosphate uptake, ammonium uptake, non-saturation, biogeochemistry, metabolism, macrophytes, aquatic biofilms

Nutrient retention is a paramount function of streams and rivers that has been intensively studied across different geographic regions in the last 25 y. However, some mechanisms and processes of this functional attribute are not yet fully understood, especially in open-canopy streams. Nutrient retention in lotic ecosystems results from the interaction of hydrological, chemical, and biological properties (Valett et al. 1996). Hydrological retention is determined by the higher residence times of solutes in pools, backwaters, and the hyporheic zone compared with the main channel, which favors the exposure of dissolved nutrients to biochemically reactive substrata. The abiotic processes of sorption, flocculation, and precipitation result in chemical retention by retarding solute transport, which can be relevant for certain solutes, such as NH_4^+ and PO_4^{3-} . Biological retention is caused by

bacteria, fungi, algae, and macrophytes that generate, immobilize, transform, or remove biologically active nutrients from water (Valett et al. 1996, Ensign and Doyle 2006).

Three interrelated parameters are useful for describing nutrient retention: uptake length (S_w), which is the average distance a nutrient travels downstream before being removed from the water column; uptake velocity (V_f), which indicates the velocity at which a nutrient moves through the water column toward the stream benthos; and uptake rate (U), which is the nutrient mass retained per unit time and area of stream bottom (Stream Solute Workshop 1990). Studies performed in streams of the Northern Hemisphere showed that the efficiency of retention (expressed as V_f) decreased as nutrient concentrations increased, giving lower efficiencies in impaired streams with higher nutrient levels

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DOI: 10.1086/690598. Received 8 January 2016; Accepted 25 October 2016; Published online 22 December 2016.
Freshwater Science. 2017. 36(1):000–000. © 2017 by The Society for Freshwater Science.

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than in pristine streams (Hoellein et al. 2007, Mulholland et al. 2008, Marcé and Armengol 2009).

O'Brien et al. (2007) proposed 3 models to describe the relationship between biological processing and nutrient concentration in streams. In the 1st-order response model, U is directly proportional to nutrient concentration, whereas S_w and V_f remain constant as nutrient concentration increases. The 2nd model assumes Michaelis–Menten uptake kinetics, where processing rates are saturated when the nutrient supply exceeds the biological demand. Hence, S_w will increase and V_f will decrease along the nutrient gradient. In the 3rd model, the rate of nutrient processing by the biota increases with nutrient availability, but the efficiency of the process relative to the concentration declines. In this efficiency-loss model, U and S_w will increase nonlinearly, whereas the V_f will decrease nonlinearly along the nutrient gradient.

Nutrient retention generally has been studied in pristine streams with low nutrient levels, but some investigators focused on nutrient retention and efficiency in streams receiving point-source inputs (Martí et al. 2004, Haggard et al. 2005). Uptake experiments have been conducted mainly in streams with short-term inputs of nutrients, but less is known about uptake in streams with chronic nutrient loading (O'Brien et al. 2007). O'Brien and Dodds (2010) compared the response of NO_3^- uptake to short-term (acute) and long-term (chronic) enrichment, defining chronic loading as increases in ambient N concentration from months to years. They observed that $\text{NO}_3^- U$ was higher in streams with acute enrichments than in chronically enriched streams, suggesting a lower capacity of the latter streams to retain N. However, how the uptake process will operate in streams that have been eutrophic for centuries or even millennia and where biological communities have a long history of coexistence in an environment with high nutrient availability is unknown.

Pampean streams in central Argentina are an example of fluvial systems with elevated nutrient concentrations that cannot be attributed solely to the extent of agricultural activities in the region. The eutrophic status of Pampean waterbodies seems to be a common, persistent feature from long ago, 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). Evidence of the existence of waterbodies in advanced stages of eutrophication in the Pampean region has been found for the intervals 10,000 to 8000 y before present (ybp) and 3900 to 3000 ybp (Prieto 1996, Zárate et al. 2000). Consequently, biological communities living in Pampean aquatic environments have coexisted for a very long period with high nutrient levels and should be well-adapted to enriched conditions.

Some evidence suggests that nutrient retention could be high in Pampean streams, despite the elevated nutrient concentrations registered in these systems (Feijoó et al. 2011,

Rodríguez Castro 2015). Pampean streams support high biomass and a diversity of macrophytes. However, increased macrophyte abundance is not always reflected in a higher SRP uptake (Feijoó et al. 2011). Macrophytes can reduce flow velocity and delay solute transport, enlarging the size of the transient storage zone in streams (Gücker and Boëchat 2004, Wilcock et al. 2004). The presence of abundant macrophyte communities could enlarge the transient storage (Feijoó et al. 2011), thereby explaining why nutrient uptake may be higher than expected in eutrophic Pampean streams. Thus, in Pampean streams, macrophytes would not enhance nutrient retention mainly by direct assimilation, but by expanding the transient storage zone of slow waters and providing a substrate for attachment of microalgae and macroalgae (Feijoó et al. 2011).

We evaluated PO_4^{3-} (as SRP) and NH_4^+ uptake in 2 Pampean streams with different macrophytic abundance. We used short-term nutrient additions to quantify SRP and NH_4^+ uptake metrics throughout the year. In addition, we investigated which hydrological and biological variables influenced nutrient uptake in these streams. We considered flow (Q) and the relative size of the transient storage zone (A_S/A) as the hydrological variables. The biological variables included the biomass and metabolism of macrophytes, macroalgae, epiphyton, epipelon (biofilm developing on streambed fine sediment), and whole-stream metabolism. We also analyzed the relationship between SRP and NH_4^+ processing and nutrient concentrations to assess whether the biological uptake function was saturated in these streams. We hypothesized that nutrient uptake was inversely associated with Q and directly with A_S/A , which in turn, depends on the abundance of macrophytes. Thus, we expected that low Q and high macrophytic biomass would increase SRP and NH_4^+ uptake. We also hypothesized that NH_4^+ and SRP uptake was driven mainly by algal biofilm uptake, with macrophytes playing a minor role. Therefore, we predicted that increases in algal biomass and productivity would enhance SRP and NH_4^+ uptake.

METHODS

Study sites

We conducted our study in a 30-m reach of Las Flores (LF) stream (lat 34°27'35"S, long 59°04'27"W) and a 50-m reach of De la Cruz (DLC) stream (lat 34°24'51"S, long 59°18'08"W). Pampean streams have laminar flow, and finding places where good mixing can occur is difficult. This difficulty, and the entrance of tributaries downstream, limited the length of our study reaches.

Pampean streams run along plains in the center–east of Argentina called Pampa Húmeda. This region has fertile soils formed by loess deposition during the Quaternary and a temperate climate, with rainfall distributed throughout the year (600–1200 mm) and an average annual temperature

between 13 and 17°C. In natural conditions, the area is covered by grasslands, with annual grasses adapted to the occurrence of fires in summer and frosts in winter. Both 2nd-order streams are tributaries of the Paraná–La Plata River system and are considered representative of many Pampean streams (Feijoó and Lombardo 2007, Acuña et al. 2011, Feijoó et al. 2011). They originate in small depressions, and their beds are characterized by fine sediments (primarily silt and clay) underlain by a hard and homogeneous substrata with high CaCO₃ content and without stones and pebbles (Feijoó and Lombardo 2007). They show slow laminar flow because of their gentle slopes ($\leq 0.02\%$) and constricted channels, and they possess high abundance of algae and macrophytes (Giorgi et al. 2005).

Nutrient uptake

We conducted 5 combined PO₄³⁻ and NH₄⁺ enrichment experiments in September (early spring), December (late spring), February (summer), April (autumn), and July (winter) in both streams between 2011 and 2013. We dissolved NaCl, HK₂PO₄ and NH₄ Cl in stream water in a carboy and released the solution to the stream as an instantaneous slug (Wilcock et al. 2002, Ruggiero et al. 2006, Covino et al. 2010). We adjusted PO₄³⁻ and NH₄⁺ concentrations in the added solution to increase stream nutrient concentrations within the natural concentration range of the streams (0.1–1.5 mg PO₄³⁻-P/L and 0.01–0.6 mg NH₄⁺-N/L) (Feijoó et al. 2011). To obtain reliable measurements, we added the solution to the streams at a narrow location to ensure adequate mixing (Stream Solute Workshop 1990, Martí and Sabater 2009). Additions lasted 15 to 80 min. During releases, the increase in conductivity (as surrogate for the NaCl concentration) in stream water was monitored with a WTW 3310 portable conductivity meter with data logger connected to a TetraCon 325 conductivity probe (WTW, Weilheim, Germany) at 10-s intervals. The change in tracer concentration over time at the downstream location is the tracer breakthrough curve (BTC).

We collected grab samples across the full range of the NH₄⁺-N, PO₄³⁻-P, and Cl BTCs at the end of the reach ($n = 25$ grab samples per release) at 20-s to 15-min intervals. We intensified sampling when conductivity was changing rapidly. Upstream of the addition point, we monitored pH, conductivity, temperature, and dissolved O₂ (DO) with a multiparameter HQ40d (HACH, Loveland, Colorado). We collected 5 additional water samples (before and during the addition) to measure background nutrient concentrations. We used grab samples collected upstream to correct the slug-addition grab samples for background concentrations.

A few hours before the nutrient-addition experiment, we released NaCl as an instantaneous addition and measured streamwater conductivity in real time with a WTW 3310 portable conductivity meter with data logger connected to a TetraCon 325 conductivity probe (WTW) at 10-s intervals.

We used conductivity BTCs monitored in real time to guide the collection of grab samples at the base of the reach during nutrient-addition experiments to ensure characterization of the entire NH₄⁺-N, PO₄³⁻-P, and Cl BTCs. We used NaCl dilution gaging to measure Q at the end of the reach based on the linear relationship ($r^2 > 0.99$, $p < 0.0001$) between conductivity and the NaCl concentration.

We filtered grab samples through fiberglass filters (Whatman GF/F) and analyzed them within 6 h for soluble reactive P (SRP) with the ascorbic acid–molybdenum blue method and for NH₄⁺ with the phenol–hypochlorite method (APHA 2005). We ran NO₃⁻ and NO₂⁻ analyses on a FUTURA Auto-analyzer (Alliance Instruments, Frepillon, France) through a reaction with sulfanilamide with a previous Cu–Cd reduction for NO₃⁻ (APHA 2005).

We analyzed the slug BTC data with the BTC-integrated approach (e.g., Ruggiero et al. 2006), where the amount of tracer recovered is estimated via integration of the tracer BTCs to calculate one suite of spiralling metrics (e.g., single S_w , V_f , and U values) from each slug addition. We calculated the mass recovery of the added nutrients as the product of the time-integrated tracer concentration and Q at the downstream sampling location (Covino et al. 2010). We estimated the temporal uptake rate coefficient (k_t) using a mass balance between the nutrient mass leaving the bottom of the reach (Md) and the mass of the initial nutrient released into the stream (Mi):

$$k_t = \frac{\ln\left(\frac{Mi}{Md}\right)}{tp}, \quad (\text{Eq. 1})$$

where tp is the mean water travel time in the experimental stream reaches (i.e., the time taken for the concentration centroid to arrive at the downstream end of the reach). Md was estimated by integrating the nutrient temporal BTC at the downstream sampling point multiplied by Q . From the estimates of k_t , we calculated S_w (m) as v/k_t , where v (m/s) is the average water velocity. We calculated v by dividing the reach length by the time elapsed between solution release and arrival of the conductivity centroid at the downstream sampling point. From S_w we calculated the V_f (m/min) as:

$$V_f = \frac{v z}{S_w} \times 60, \quad (\text{Eq. 2})$$

where z is the average depth (see below). We also calculated the areal nutrient uptake rate ($\text{mg m}^{-2} \text{min}^{-1}$) as:

$$U = \frac{QC}{S_w w} \times 60, \quad (\text{Eq. 3})$$

where Q is the flow (L/s), C is the background nutrient concentration (mg/L), and w is the average width (m) (Stream Solute Workshop 1990, Wilcock et al. 2002). S_w and V_f were

also expressed on a stoichiometric basis by dividing them by the molecular weight of the corresponding element (P for SRP uptake metrics and N for NH_4^+ metrics).

We used the 1-dimensional transport with inflow and storage (OTIS) model to quantify the cross-sectional area of the storage zone (A_S) and the relative transient storage zone size (A_S/A), where A is the main-channel cross-sectional area (Runkel 1998). To estimate whether any of the added NH_4^+ was nitrified, we compared the increase of NO_3^- or NO_2^- concentration during the additions with the expected NO_3^- or NO_2^- concentration if all of the NH_4^+ were transformed into any of these N forms (Ribot et al. 2012).

Reach characterization

The day after each addition experiment, we measured the wetted width (w) and 3 equidistant depths along transects spaced 0.5 m apart in the LF stream (for a total of 60 transects) and 1 m apart in the DLC stream (50 transects) to estimate the reach surface area. We mapped the distribution of each basal compartment (submerged macrophytes, floating macroalgae, epipelton, and epiphyton) from these transects and estimated their covers as described by Feijoó and Menéndez (2009). We collected 6 samples of each community for estimation of biomass. We transported the biological samples to the laboratory in the dark and cold for processing within 6 h. We estimated macrophyte and epiphyton biomass as described by Vilches and Giorgi (2010) and epipelton biomass as described by Leggieri et al. (2013). Floating macroalgae were dried at 60°C to constant dry mass and combusted at 480°C for ~4 h to estimate the ash-free dry mass (AFDM). Reach characterization and biomass collection were done on the same days as chamber metabolism measures were made in each stream (see below).

To evaluate the contribution of algal biomass to biofilms (epiphyton and epipelton), we calculated the autotrophic index (AI) as $\text{AI} = \text{AFDM}/\text{chlorophyll } a$. Values >200 suggest a heterotrophic community (APHA 2005). We combined the community biomass data with the data from map plotting to calculate the mean biomass of every basal compartment standardized by the reach surface ($\text{g AFDM}/\text{m}^2$).

Whole-stream metabolism

Whole-stream measures at each stream began on the same day and before each addition experiment was carried out and ended the following day, once chamber measurements were concluded. We estimated whole-stream rates of gross primary production (GPP) and ecosystem respiration (ER) with the open-channel 1-station technique (Odum 1956, Uehlinger and Naegeli 1998). We calculated rates by measuring changes in DO concentrations in the streams in 5-min increments for 24 to 32 h during and immediately after nutrient-addition experiments with a field-calibrated oximeter (HQ40D; HACH, Loveland, Colorado). We esti-

mated reaeration (k) as the decline of the DO concentration after dusk (linear regressions to obtain k showed $r^2 \geq 0.60$; Hornberger and Kelly 1975). We calculated net ecosystem production (NEP) ($\text{g O}_2 \text{ m}^{-2} \text{ min}^{-1}$) as described by Leggieri et al. (2013). Daily NEP was the sum of net production (1/min) over 24 h. Daily ER was the sum of the NEP during the night and the respiration rate during the day (obtained by the linear interpolation between the NEPs ~2 h before sunrise in the subsequent day and the NEPs an hour after sunset of the addition day). Daily GPP was the sum of daily NEP and daily ER (Odum 1956), and the photosynthesis/respiration (P/R) index was estimated as $\text{GPP}:\text{ER}$.

Chamber metabolism

We evaluated the metabolism of 4 basal compartments (macrophytes, floating macroalgae, macrophyte–epiphyton complex, and epipelton) with the chamber method. We conducted chamber measurements at each stream on the same sampling days that whole-stream measurements were made.

We estimated net community production (NCP) and respiration (CR) of each compartment from incubations in acrylic rectangular chambers (volume = 6.6 L) without circulation (giving low current velocity; Velasco et al. 2003). We measured the variation of the DO concentration with a field-calibrated oximeter (HQ40D; HACH). During each measurement, the probe was immersed in the chamber and was kept in constant motion to break up any possible diffusion gradient.

We collected samples of each basal compartment from random locations throughout the study reach and incubated them in triplicate in the chambers. We used macrophyte–epiphyton complex and floating macroalgae samples as they were collected from the streams. We washed macrophyte samples with a brush to remove the attached epiphyton. We put 3 epipelton core samples (diameter = 4 cm) in each chamber. We ran 3 additional chambers filled with stream water to correct the possible influence of seston on the metabolism of the other compartments. We used this compartment only as a correction because the metabolic activity of seston is very low in these streams (Vilches and Giorgi 2010).

We covered the chambers with a plastic black sheet and incubated them in the streams for 2 h in the dark to estimate CR. Afterward, we removed the plastic sheet and incubated the chambers for 1 more hour (to avoid supersaturation) to estimate NCP of each compartment. We calculated gross community production (GCP) as the sum of NCP and CR. We subtracted metabolic rates obtained in the chambers filled with stream water from the rates obtained in the chambers with samples of the other 4 compartments. We measured the volume occupied by the samples of each basal compartment used in the incubations and deducted it from the total volume of each chamber.

Metabolic rates of epiphyton were calculated as the difference between the rates of macrophytes alone and the macrophytes+epiphyton complex (Vilches and Giorgi 2010). We used the methods described above to estimate the biomass of each compartment in the chambers. We calculated CR, NCP, and GCP for each chamber in units of O₂ production or consumption per unit AFDM and time and transformed values to g O₂ m⁻² h⁻¹ by considering the proportion of the stream reach covered by the different compartments obtained from the mapping.

Statistical analyses

We used simple linear regression to test relationships between Q and A_S/A and between A_S/A and the macrophytic biomass. We also analyzed the relationships between uptake metrics and background nutrient concentrations. We evaluated their fit to the models proposed by O'Brien et al. (2007) based on least squares regression solved with the Levenberg–Marquardt estimation algorithm. First, we analyzed the fit of the relationship to a linear model. Both SRP and NH₄⁺ showed a good fit to the linear relationship. We did not observe deceleration of nutrient uptake with increasing concentration (indicating no saturation), so we did not test models that included saturation of retention (loss of efficiency or Michaelis–Menten). We also tested the relationship between U_{SRP} and SRP concentration for an exponential response. We used Spearman rank correlations to evaluate the possible relationships between uptake metrics and hydrological, biological, and metabolic variables ($n = 10$). When ≥ 2 explanatory variables were redundant (e.g., NEP, ER, and P/R), we considered only 1 of them. Following this criterion, the total number of correlation tests performed for each uptake metric was 14 (42 tests for each nutrient). The explanatory variables included in the analysis were: temperature; specific discharge (Q/w); AFDM of macrophytes, epiphyton, and epipelon; AI of epipelon and epiphyton; NCP of macrophytes, epiphyton, and epipelon; RC of macrophytes, epiphyton, and epipelon; and ecosystem P/R. Floating macroalgae were absent on 1/2 of the sampling dates, so we excluded them from this analysis. We also analyzed the covariation of SRP and NH₄⁺ uptake metrics (S_w and V_f) with Spearman rank correlations. We checked the normality of all the response variables with the Kolmogorov–Smirnov test.

RESULTS

Environmental characteristics of the stream reaches

The streams had high values of specific conductivity and dissolved nutrient concentration (Table 1). NO₃⁻ concentration was higher but N:P was lower in LF than in DLC, suggesting a higher P deficiency in DLC. In addition, LF had lower Q and higher A_S/A than DLC (Table 1). SRP concentration was maximum in summer for DLC and in late spring

for LF, and minimum in early spring in both streams, in winter for DLC, and in autumn for LF (data not shown). In the case of NH₄⁺, concentrations were maximum in autumn for DLC and in late spring for LF, and minimum in winter for DLC and in early spring for LF (data not shown). When considering the data from both streams, we observed a negative relationship between A_S/A and Q (Fig. 1). However, A_S/A was not significantly related to the macrophytic biomass.

Biomass of basal compartments

The sum of biomasses of all basal compartments (as AFDM) ranged from 322 to 612 g/m² in DLC and from 556 to 975 g/m² in LF. AFDM differed significantly among functional compartments in both streams. The AFDM was higher in the epipelic compartment than in the other ones (Table 2). Macrophytic biomass was higher in DLC than in LF, with a maximum in summer and in late spring, respectively. The highest epiphyton biomass was registered in winter in DLC and in early spring in LF, and the lowest value was registered in late spring for both streams. Floating macroalgae were present on only 1/2 of the sampling occasions. The highest biomass was registered in winter in DLC and in early spring in LF.

The AFDM method did not allow us to separate living from dead organic matter, so we might have underestimated epipelic biomass with this method. On the other hand, when comparing biofilms based on chlorophyll a content (Table 2), we observed that epiphyton and epipelon biomasses were lower and even similar in some sampling periods (especially in DLC stream).

Whole-reach and chamber metabolism

Metabolic ecosystem rates were elevated relative to other streams and rivers of the world (Acuña et al. 2011). P/R was >1 in both streams, suggesting the dominance of autotrophic processes over heterotrophic processes (Table 3).

Table 1. Mean (\pm SD) values of physicochemical and hydrological characteristics of Las Flores and De la Cruz streams. SRP = soluble reactive P.

Stream	De la Cruz	Las Flores
pH	8.99 (0.63)	8.72 (0.37)
Conductivity (μ S/cm)	892 (36)	750 (216)
Flow (Q L/s)	26.8 (13.0)	7.6 (6.1)
Stream velocity (m/s)	0.21 (0.06)	0.07 (0.05)
A_S/A	0.37 (0.13)	0.54 (0.12)
SRP (mg/L)	0.11 (0.14)	0.15 (0.15)
NO ₃ ⁻ -N (mg/L)	6.26 (3.05)	11.71 (9.93)
NO ₂ ⁻ -N (mg/L)	0.32 (0.48)	0.28 (0.27)
NH ₄ ⁺ -N (mg/L)	0.02 (0.02)	0.06 (0.09)
Molar N:P	426 (677)	295 (253)

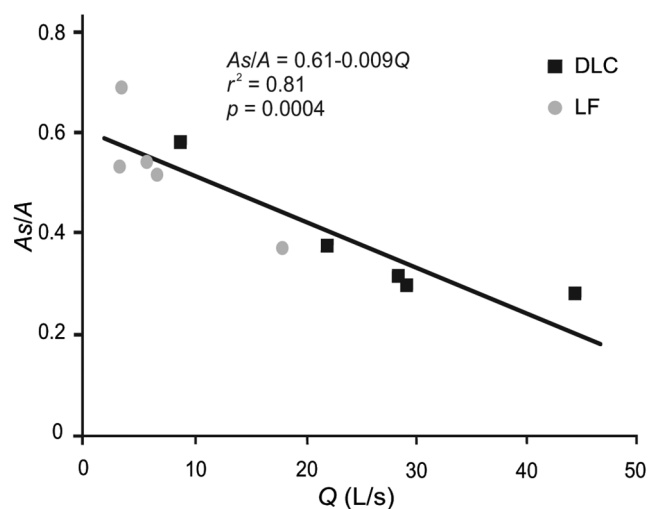


Figure 1. The relative size of the transient storage zone (A_S/A) decreased with increasing flow (Q) Las Flores (LF) and De la Cruz (DLC) streams.

CR and GCP differed among different compartments in both streams, and epipelon had the highest rates (Table 4). Overall, epipelon was the compartment that contributed most to metabolism at the whole-reach scale on most sampling dates (21–89% of CR for DLC and 0–96% for LF; 34–69% of GCP for DLC and 33–95% for LF) in both streams and considering the basal compartments analyzed.

Nutrient uptake metrics

In comparison to previous studies, nutrient uptake was high in both streams (Table 5). The mean percentage of SRP retained from the $P-PO_4^{3-}$ added in the different addition experiments was 65% (range: 44–95%) for DLC and 49% (range: 26–67%) for LF. The percentage of NH_4^+ retained was even higher, at 87% (range: 73–96%) in DLC and 84% (range: 69–97) in LF. U_{SRP} was maximum in summer for DLC ($4724 \mu g m^{-2} min^{-1}$) and in late spring for LF ($11,899 \mu g m^{-2} min^{-1}$), and minimum in winter for DLC ($187 \mu g m^{-2} min^{-1}$) and autumn for LF ($44 \mu g m^{-2} min^{-1}$). The higher SRP uptake in DLC was in accordance with the higher P demand expected for this stream. $U_{NH_4^+}$ was maximum in autumn ($1166 \mu g m^{-2} min^{-1}$) for DLC and in late spring for LF ($7435 \mu g m^{-2} min^{-1}$), and minimum in winter for DLC ($54 \mu g m^{-2} min^{-1}$) and in au-

tumn for LF ($76 \mu g m^{-2} min^{-1}$). The uptake metrics estimated in both streams reflected the elevated nutrient retention, with low S_w and high V_f and areal uptake rates for both SRP and NH_4^+ (Table 5). When the uptake metrics were estimated on a molar basis, no significant relationships were detected between S_{w-SRP} and $S_{w-NH_4^+}$ and between V_{f-SRP} and $V_{f-NH_4^+}$.

Uptake kinetics

The relationships between the uptake metrics and background nutrient concentrations in the stream water indicated that the uptake function was not saturated along the wide nutrient gradient that we observed (Fig. 2A–F). The relationship between U_{SRP} and SRP concentration fit an exponential model ($r^2 = 0.86$, $p = 0.0001$; Fig. 2A) and this relationship remained significant when the highest SRP concentration point was removed from the analysis ($r^2 = 0.77$, $p < 0.0001$). This relationship also fit in a linear model, but the coefficient of determination was lower than for the exponential model one ($r^2 = 0.74$). S_{w-SRP} decreased linearly with SRP concentration ($r^2 = 0.61$, $p = 0.008$; Fig. 2C) supporting the exponential model. V_{f-SRP} tended to increase with SRP concentration, but this relationship was not significant (Fig. 2B). $U_{NH_4^+}$ also increased with NH_4^+ concentration but followed a linear model ($r^2 = 0.98$, $p = 0.00001$; Fig. 2D). This relationship remained significant ($r^2 = 0.64$, $p = 0.006$) when the point with the highest NH_4^+ concentration was eliminated. $V_{f-NH_4^+}$ and $S_{w-NH_4^+}$ showed no significant relationships with NH_4^+ concentration (Fig. 2E, F). Our data showed a good fit to the linear relationship for both SRP and NH_4^+ , which is characteristic of the 1st-order response model, so we did not test their fit to the saturation models.

Environmental and functional variables controlling nutrient uptake

U_{SRP} was positively associated ($r = 0.80$, $p = 0.005$), whereas S_{w-SRP} was negatively associated with temperature ($r = -0.71$, $p = 0.021$) (Fig. 3A, B). Epiphyton AI was positively correlated with U_{SRP} ($r = 0.75$, $p = 0.013$) and negatively correlated with S_{w-SRP} ($r = -0.70$, $p = 0.025$) (Fig. 3C, D). Among the metabolic variables, the epipelon NCP was negatively related to U_{SRP} ($r = -0.77$, $p = 0.009$) and V_{f-SRP} ($r = -0.81$, $p = 0.005$) (Fig. 3E, F). U_{SRP} decreased with

Table 2. Mean (range) ash-free dry mass (AFDM) and chlorophyll a concentration of each basal compartment in Las Flores (LF) and De la Cruz (DLC) streams ($n = 5$ for all compartments, except for floating macroalgae where $n = 2$ for DLC and $n = 3$ for LF).

Stream	AFDM of basal compartments (g/m^2)				Chlorophyll a (mg/m^2)	
	Macrophytes	Floating macroalgae	Epiphyton	Epipelon	Epiphyton	Epipelon
DLC	25.76 (11.8–55.2)	0.63 (0–2.41)	0.69 (0.03–1.77)	422.6 (265–576)	17.0 (0.64–37.8)	32.6 (3.18–57.3)
LF	5.53 (1.53–14)	1.25 (0–4.47)	0.13 (0.02–0.39)	717.8 (545–972)	1.66 (0.02–7.18)	37.5 (11.5–55.6)

Table 3. Mean (\pm SD) gross primary production (GPP), net ecosystem production (NEP), ecosystem respiration (ER), and GPP:ER (P/R) of Las Flores (LF) and De la Cruz (DLC) streams.

Ecosystem metabolic rate	Stream	
	DLC	LF
GPP ($\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$)	4.40 ± 2.16	3.80 ± 2.27
ER ($\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$)	3.27 ± 2.70	3.03 ± 0.71
NEP ($\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$)	1.13 ± 3.40	0.77 ± 2.80
P/R	1.97 ± 1.59	1.41 ± 1.11

increasing P/R ($r = -0.68$, $p = 0.029$), was highly variable when $P/R < 1$, and was low when $P/R > 1$ (Fig. 3G).

The factors correlated with NH_4^+ uptake included specific discharge (Q/w), A_S/A , and ecosystem P/R. Q/w was positively correlated with $V_{f\text{-NH}_4}$ ($r = 0.82$, $p = 0.004$; Fig. 4A), whereas A_S/A was negatively associated with $V_{f\text{-NH}_4}$ ($r = -0.85$, $p = 0.002$; plot not shown). This last correlation possibly reflected the negative relationship between flow and A_S/A . $V_{f\text{-NH}_4}$ increased with ecosystem P/R ($r = 0.64$, $p = 0.048$; Fig. 4B).

NO_2^- concentration increased with NH_4^+ loss, but NO_3^- concentration did not change during additions. Therefore, we considered nitrification only in terms of NO_2^- gain (data not shown). Nitrification in DLC was very low in summer ($<1\%$), but varied between 22 and 37% in the other seasons. In LF, the estimated nitrification represented between 8 and 43% of total NH_4^+ uptake during the year (Fig. 5).

DISCUSSION

Despite high background nutrient levels, SRP and NH_4^+ uptakes were high compared to previous reports and were not saturated. U_{NH_4} fit in a 1st-order model (O'Brien et al. 2007), but the relationship between U_{SRP} and SRP concentration was better described by an exponential model (Fig. 2A).

Supporting this model, $V_{f\text{-SRP}}$ tended to increase linearly and $S_{w\text{-SRP}}$ decreased linearly with SRP concentration. The models of the relationship between stream nutrient processing and concentration (O'Brien et al. 2007) assumed saturation or no change in the nutrient uptake efficiency (expressed as V_f) as the nutrient concentration increased. However, our results suggested a higher efficiency of SRP uptake at high SRP levels, and the relationship between U_{SRP} and SRP concentration fit an exponential model.

Nutrient uptake metrics in the Pampean streams

The uptake metrics estimated in our study were high when compared with data from other fluvial systems of the world (Table 5). $S_{w\text{-SRP}}$ in both Pampean streams were within the range reported for pristine streams (Marcé and Armengol 2009) or for streams of the same order (Ensign and Doyle 2006). However, in our study, $V_{f\text{-SRP}}$ was 1 order of magnitude higher and U_{SRP} was 2 orders of magnitude higher than the values presented by other authors. The same situation was observed for NH_4^+ uptake. $S_{w\text{-NH}_4}$ was lower and $V_{f\text{-NH}_4}$ and U_{NH_4} were higher than the values reported by Ensign and Doyle (2006) and Hall et al. (2013). The differences were even larger when compared to values from impaired streams with high nutrient levels, where S_w generally was on the order of kilometers (Martí et al. 2004, Haggard et al. 2005, Marcé and Armengol 2009). The uptake metrics in our study were similar to those estimated in the LF stream downstream of our sampling point and in other Pampean streams (Feijoó et al. 2011, Rodríguez Castro 2015) (Table 5). Hence, our results confirmed the previous evidence of high efficiency of nutrient uptake in streams of the region.

Lack of saturation of nutrient uptake

U_{SRP} and U_{NH_4} increased with nutrient concentrations, but followed different models. U_{NH_4} fit in a linear model,

Table 4. Mean (\pm SD) net community production (NCP), community respiration (CR), and gross community production (GCP) for macrophytes, floating macroalgae, epiphyton, and epipelon in Las Flores (LF) and De la Cruz (DLC) streams ($n = 5$ for all compartments, except for macroalgae where $n = 2$ for DLC and $n = 3$ for LF).

Stream	Compartment	Metabolic rate ($\text{g O}_2 \text{ m}^{-2} \text{ h}^{-1}$)		
		NCP	CR	GCP
DLC	Macrophytes	0.173 ± 0.224	0.038 ± 0.030	0.210 ± 0.247
	Floating macroalgae	0.004 ± 0.006	0.001 ± 0.001	0.005 ± 0.006
	Epiphyton	0.021 ± 0.015	0.011 ± 0.011	0.032 ± 0.025
	Epipelon	0.118 ± 0.123	0.091 ± 0.053	0.209 ± 0.130
LF	Macrophytes	0.056 ± 0.101	0.007 ± 0.009	0.063 ± 0.110
	Floating macroalgae	0.023 ± 0.042	0.001 ± 0.001	0.024 ± 0.043
	Epiphyton	0.033 ± 0.034	0.003 ± 0.004	0.100 ± 0.110
	Epipelon	0.199 ± 0.155	0.036 ± 0.038	0.299 ± 0.129

Table 5. Uptake metrics (range) estimated in the studied Pampean streams and in other fluvial systems of the world. Means are reported for data collected by Marti et al. 2004, Haggard et al. 2005, Hall et al. 2013, Rodríguez Castro 2015, and in our study. Medians are presented for the other studies. Ranges in Ensign and Doyle (2006) are interquartile ranges. S_w = uptake length, V_f = uptake velocity, U = uptake rate.

Stream	S_{w-SRP} (m)	V_f-SRP (mm/min)	U_{SRP} ($\mu\text{g m}^{-2} \text{min}^{-1}$)	S_{w-NH4+} (m)	V_f-NH4+ (mm/min)	U_{NH4+} ($\mu\text{g m}^{-2} \text{min}^{-1}$)	Source
De la Cruz	56 (18–95)	38 (22–52)	1483 (187–4724)	26 (17–42)	92 (23–165)	400 (54–1166)	This study
Las Flores	47 (29–71)	20 (1–59)	2776 (44–11899)	15 (9–27)	45 (6–63)	1820 (76–7435)	This study
Las Flores (in spring)	250	3.7	1491				Feijóo et al. 2011
Las Flores	75	30	4680				Rodríguez Castro 2015
La Choza	1208 (35–4463)	21 (2–72)	870 (120–2640)				Rodríguez Castro 2015
Indio Rico	37	360	7380				Rodríguez Castro 2015
Arroyo Grande	38	240	4920				Rodríguez Castro 2015
27 streams of 2 nd order	62 (26–223)	2.6 (1.2–6.9)	17 (9–35)	120 (60–266)	5.1 (2.6–10)	32 (11–59)	Ensign and Doyle 2006
2 impaired streams	8700 (6800–13400)	0.3 (0.1–0.5)	1320 (720–1740)	863 (400–1400)	2.9 (0.8–5.9)	11400 (4500–19500)	Haggard et al. 2005
2 impaired streams	3700			3200			Marti et al. 2004
46 pristine streams	88 (6–2955)	4.5 (0.3–41)					Marcé and Armengol 2009
20 impaired streams	3495 (50–188115)	0.3 (0.1–79)					Marcé and Armengol 2009
271 streams	1267 (2–87879)	4.17 (0.03–38.6)					Hall et al. 2013
360 streams				419 (5–5609)	6.15 (0.3–119)		Hall et al. 2013

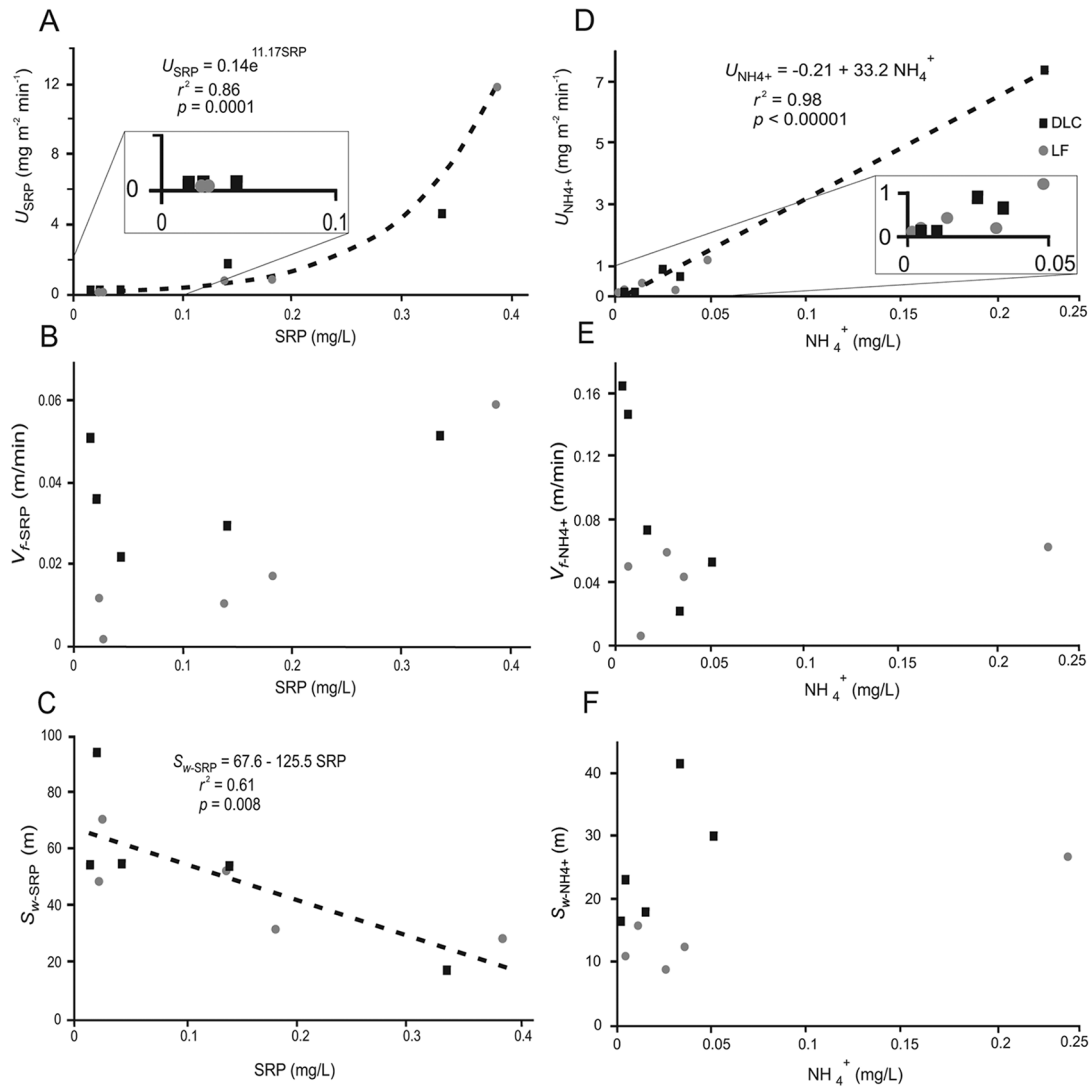


Figure 2. Relationships between uptake rate (U) for soluble reactive P (SRP) (A) and NH_4^+ (D), uptake velocity (V_f = relative efficiency) for SRP (B) and NH_4^+ (E), and uptake length (S_w) for SRP (C) and NH_4^+ (F) and background nutrient concentration (in mg/L of SRP and mg/L of NH_4^+ -N) indicate nonsaturation of SRP and NH_4^+ uptake in both streams. NH_4^+ uptake fits a 1st-order model, whereas an exponential model best explains SRP uptake.

whereas $V_{f\text{-NH}_4^+}$ did not change consistently with the NH_4^+ concentration. Dodds et al. (2002) found no relationship between V_f and NO_3^- concentration, as expected with a linear model, in some of the additions that they performed in a prairie stream. Levi et al. (2015) also observed a linear relationship between $U_{\text{NH}_4^+}$ and NH_4^+ concentration when they pooled data from different macrophyte-rich streams reported in the literature. However, we injected NH_4^+ but not NO_3^- because NO_3^- is the predominant form of N in aquatic ecosystems (Allan and Castillo 2007), including our streams. In theory, NH_4^+ is an energetically less costly N source, and thus should be preferred over NO_3^- to cover the autotrophic demand (Feijóo et al. 2002, Oviedo-Vargas et al. 2013). In the case of SRP, U_{SRP} increased exponentially with SRP concentration, suggesting that the stream

was well below saturation (Earl et al. 2006). We did not detect lower V_f with increasing nutrient availability, but we expect that saturation might be reached both in the linear and in the exponential model at higher nutrient concentrations. In the case of the linear model, we can assume Michaelis–Menten kinetics, whereas the exponential model suggests a sigmoidal function that can be described by the Hill function (Scheffer 1990). The Michaelis–Menten function is a special case of the Hill function (Scheffer 1990).

Previous researchers generally have shown that stream nutrient V_f decreases under high nutrient loading because of saturation of the uptake function in biological communities (e.g., Martí et al. 2004, Hoellein et al. 2007, Mulholland et al. 2008, Marcé and Armengol 2009, O'Brien and Dodds 2010). However, Diemer et al. (2015) observed an increase

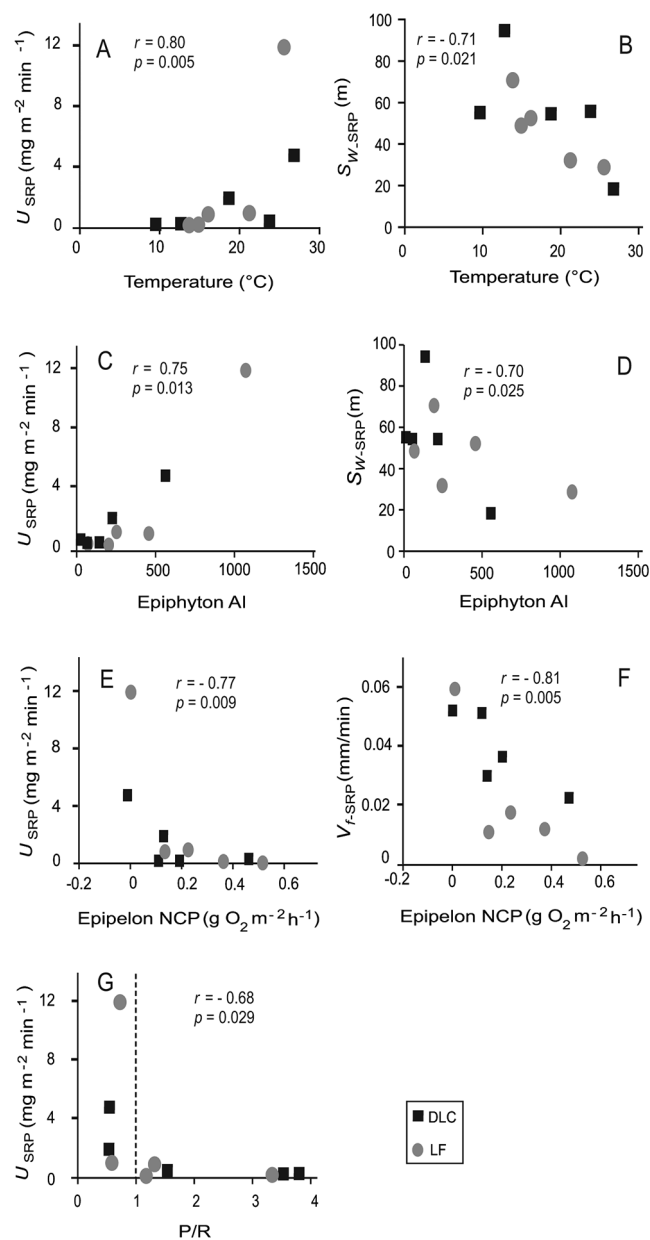


Figure 3. Significant Spearman correlations for soluble reactive P (SRP) were found between temperature (A, B), epiphyton autotrophic index (AI) (C, D), epilpelon net production (NCP) (E, F), and ecosystem gross primary production:respiration ratio (P/R) (G) and uptake rate (U) (A, C, E, G), uptake length (S_w) (B, D), and uptake velocity (V_f = relative efficiency) (F) in Las Flores (LF) and De la Cruz (DLC) streams.

in V_f with increasing concentration in some of their studied streams in burned watersheds of Siberia. They proposed the term “biostimulation model” to describe this pattern and argued that the demand for a particular nutrient is stimulated by greater nutrient concentration. Even though this condition has not been described often in the literature, Rodriguez Cardona et al. (2016) observed an increase in V_f at higher nutrient concentrations, and Covino et al. (2012) found a de-

crease in S_w with higher added N concentrations in a stream with chronic N inputs. Ribot et al. (2013) compared the uptake kinetics of biofilms from 2 streams differing in background N concentrations. They observed that the saturation of NH_4^+ uptake occurred at lower U in the low-N than in the high-N stream and concluded that the response capacity of biofilms for short-term increases in nutrient concentration was determined by the ambient nutrient concentration under which they developed. A similar mechanism might explain the lack of saturation of SRP and NH_4^+ uptake that we observed.

Another possible explanation for the lack of saturation of nutrient uptake could be related to the high biological diversity and the presence of various basal compartments that were characteristic of Pampean streams (Giorgi et al. 2005, Feijoó et al. 2014). Experimental evidence indicates that aquatic communities with higher species richness take better advantage of niche opportunities than those with fewer species because the coexistence of different species that are best adapted for different habitats allows diverse systems to capture greater proportions of nutrients (Cardinale 2011). In addition, Levi et al. (2015) proposed that macrophyte-rich

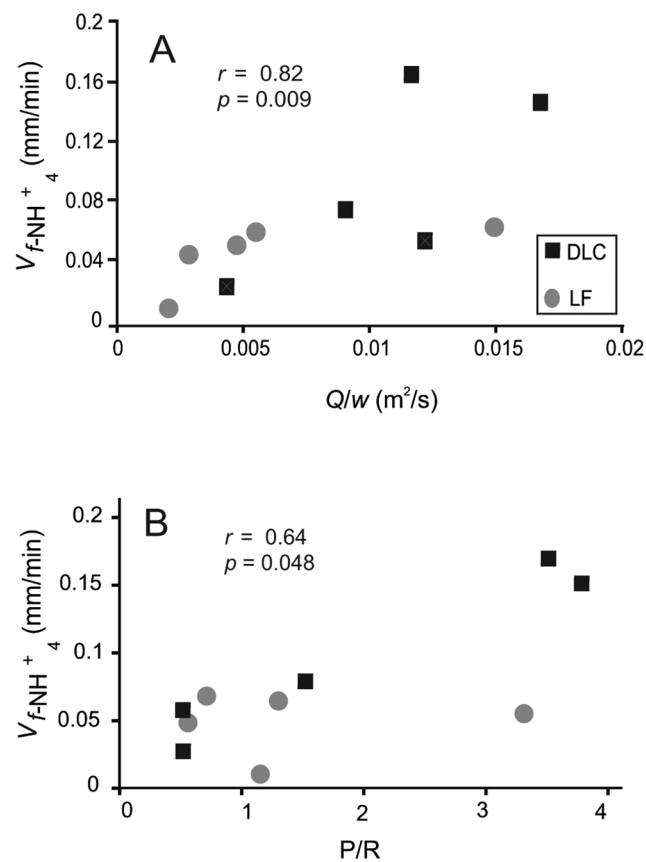


Figure 4. Significant Spearman correlations for NH_4^+ were found between specific discharge (Q/w) (A) and ecosystem gross primary production:respiration ratio (P/R) (B) and uptake velocity (V_f = relative efficiency) in Las Flores (LF) and De la Cruz (DLC) streams.

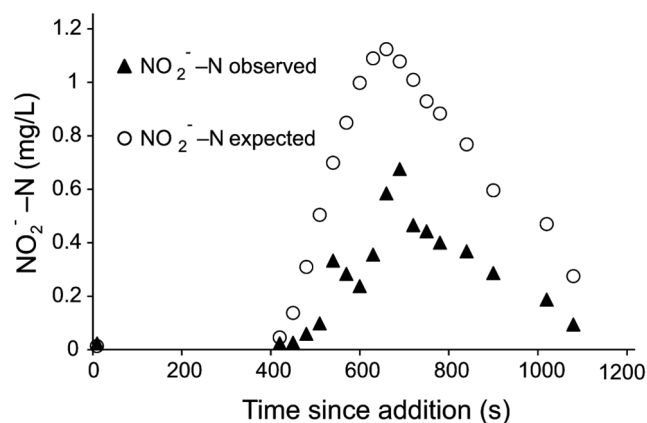


Figure 5. Example of the estimation of nitrification (Las Flores [LF] in summer). Observed NO_2^- concentrations and expected NO_2^- concentrations if all of the added NH_4^+ were transformed to NO_2^- . In this case, the estimated nitrification was 43% of total NH_4^+ uptake.

streams were able to process higher concentrations of NH_4^+ before uptake was saturated relative to streams without macrophytes. Mechanisms like these could be operating in Pampean streams where biotic diversity and macrophyte cover are high.

Factors influencing nutrient uptake

Transient storage has been proposed as one of the determinants of nutrient uptake because in these localized regions, water moves slower, favoring exposure of solutes to biogeochemically reactive substrates (Webster et al. 2003). However, in a review of nutrient spiralling, Ensign and Doyle (2006) found little evidence of a causal relationship between transient storage and nutrient uptake. In addition, others have proposed that in macrophyte-dominated streams, the size of the transient storage zone will be associated with plant growth that induces changes in both stream flow and hyporheic exchange patterns (Salehin et al. 2003, Gücker and Boëchat 2004). In accordance with the results of Ensign and Doyle (2006), we did not find a significant relationship between A_S/A and uptake metrics or macrophytic biomass. Our data showed that A_S/A was associated with flow, a result suggesting that in these streams, transient storage is mainly driven by hydrology and may not depend on vegetation abundance or the development of a hyporheic zone. The uptake metrics also were not related to macrophytic biomass. Thus, the results do not support our hypothesis that greater plant abundance enhanced nutrient uptake by enlargement of the transient storage zone.

With the exception of $V_{f\text{-SRP}}$, all SRP uptake metrics were significantly related to temperature, indicating higher SRP uptake with increasing temperature. U_{SRP} was positively associated with epiphyton AI (but $S_{w\text{-SRP}}$ was negatively associated), and SRP uptake and efficiency decreased when the

epipelon community production increased. These results suggest that SRP uptake would be favored by the presence of more heterotrophic epiphytic biofilms and less productive epipelic biofilms. In addition, U_{SRP} was higher under more heterotrophic conditions ($P/R < 1$). The positive associations of SRP uptake metrics with temperature, epiphyton, and ecosystem heterotrophy provided evidence that SRP uptake may be dominated by the heterotrophic demand. This result was unexpected. We had predicted a significant effect of photoautotrophic activity, considering the abundance of macrophytes and the expected autotrophic nature of Pampean streams. Few investigators have reported a heterotrophic demand on nutrient retention, especially in autotrophic streams. However, Hoellein et al. (2007) found evidence for heterotrophic control on $V_{f\text{-SRP}}$ and U_{SRP} across biomes when they combined their data with other studies from the literature. Webster et al. (1991) also found that the primary mechanism of PO_4^{3-} uptake in southern Appalachian streams was heterotrophic uptake. Several other investigators have found strong relationships with low explanatory power between metabolism and U (Hoellein et al. 2007), results suggesting that additional factors may influence nutrient uptake dynamics in headwater streams (von Schiller et al. 2008a).

S_w generally increases with Q (Valett et al. 1996, Peterson et al. 2001, Hall et al. 2002, Niyogi et al. 2004, Marcé and Armengol 2009) and with Q/w for both N and P (Hall et al. 2009). However, no significant relationships between Q/w and V_f have been reported (Hanafi et al. 2006, Marcé and Armengol 2009). We found that $V_{f\text{-NH}_4^+}$ increased with Q/w , suggesting a higher efficiency in NH_4^+ uptake at elevated flows. However, the current velocities are very low in Pampean streams, and an increase in flow might facilitate the exchange of nutrients between the water column and the benthic community through the disruption of the boundary layer. Mass transfer can control nutrient uptake by benthic and epiphytic algae (Larned 2010). As velocity increases, the depth of the boundary layer decreases. Consequently, diffusion distance is reduced, algae encounter higher nutrient concentrations, and nutrient U increases (Horner et al. 1990, Larned et al. 2004).

$\text{NH}_4^+ V_f$ increased with the degree of autotrophy of the ecosystem in our study. Previous investigators typically reported a coupling between NH_4^+ processing and metabolism (Hall and Tank 2003, Gücker and Pusch 2006, Rasmussen et al. 2011, Cohen et al. 2013), but some authors found no relationship between GPP, ER, or P/R with NH_4^+ uptake (von Schiller et al. 2008b, O'Brien et al. 2014). Our results suggested that NH_4^+ uptake was enhanced under more autotrophic conditions.

The addition experiments did not allow us to separate direct autotrophic uptake from other N removal processes mediated by bacteria, such as nitrification and denitrification. Many previous investigators have found that nitrification is common in streams and varies substantially across

biomes (Peterson et al. 2001, Bernhardt et al. 2002). In particular, nitrification could be high in eutrophic streams (Merseburger et al. 2005, Gammons et al. 2011). According to our data (not shown), nitrification could potentially explain up to ~40% of total NH_4^+ uptake in these streams in some periods of the year (Fig. 5). Thus, the heterotrophic biofilms could have influenced NH_4^+ spiralling through direct uptake or nitrification, but to a proportionally lower degree than the autotrophic demand.

Macrophytes can increase nutrient uptake in streams directly by assimilating P and N from the water column and storing them as standing stock biomass, with longer retention times than fluvial biofilms. They also can enhance nutrient processing indirectly by providing surface areas for epiphytic communities or promoting denitrification (Riis et al. 2012, O'Brien et al. 2014). Macrophytes can have higher U than other autotrophs as a result of their large nutrient biomass and relatively high V_f (Peipoch et al. 2014, Levi et al. 2015). However, a clear link between macrophytic abundance and nutrient U has not always been detected. Rasmussen et al. (2011) found that nutrient uptake and GPP increased with macrophyte cover in 4 Icelandic streams, and Hensley et al. (2014) observed greater removal rates in reaches with high macrophyte cover relative to in less vegetated reaches of 6 spring-fed rivers. However, O'Brien et al. (2014) did not see an increase in whole-stream nutrient uptake resulting from macrophyte growth, and Riis et al. (2012) could not verify their hypothesis that whole-stream U was higher in vegetated than in other streams. In addition, the ability of macrophytes to gain nutrients can be influenced by attributes such as plant life form (Wilcock et al. 2004), taxonomic group (macroalgae or vascular plants), species type, and tissue stoichiometry (Hensley et al. 2014).

Previous results in LF stream suggested that the influence of SRP uptake by algal biofilms depended on their biomass and metabolic activity, whereas macrophytes influenced nutrient uptake by giving structure to the physical environment and generating habitat heterogeneity for other autotrophs (Feijoó et al. 2011). Based on this information, we expected that nutrient uptake would be driven mainly by algal biofilms, with macrophytes playing a minor role. In the case of NH_4^+ , we found a significant relationship between V_f and ecosystem P/R, but we did not detect positive associations for algal biomass. In the case of SRP, we did not detect positive associations between algal biomass and productivity and SRP uptake. Consequently, our data gave partial support to our 2nd hypothesis for NH_4^+ but not for SRP.

Given the abundance of autotrophic communities in Pampean streams, we sampled all autotrophic basal communities and analyzed their influence on nutrient uptake. However, other abiotic and biotic mechanisms can influence the nutrient uptake in lotic systems. For instance, P adsorption to the sediment can be important (Davis and Minshall 1999),

especially in chalk streams (Boar et al. 1995, Jarvie et al. 2006). P coprecipitation could be important in Pampean streams, given the high CaCO_3 content in their substrata. The percentage of P retained in the LF stream did not vary during the day (Martí et al. 2011, unpublished data), a result suggesting that an abiotic mechanism not linked to diel variation of light and temperature also influenced P. Therefore, we cannot discard P adsorption to sediments as another mechanism that contributes to total P uptake in these streams. On the other hand, adsorption should not be relevant in the case of NH_4^+ given that NH_4^+ uptake strongly increased during the day compared to the night (Martí et al. 2011, unpublished data).

Conclusions

Studies of in-stream biogeochemical transformations across climates, landscapes, and biomes are critical for enlarging our view of how nutrient retention works and its geomorphological and biological determinants. Our results challenged some of the assumptions made by previous researchers on stream nutrient processing. First, nutrient uptake and efficiency in enriched Pampean streams can be very high and similar to those in streams with low nutrient levels. Second, the role of macrophytes in nutrient retention seemed to be minor in these streams despite the high macrophytic abundance. Last, we assumed that nutrient uptake is governed by autotrophs in open-canopy streams (Fellows et al. 2006), but nutrient processing in Pampean streams is likely to rely on both autotrophic and heterotrophic demand, especially in the case of P. Thus, further research is needed to clarify the role of the heterotrophic demand in highly productive and autotrophic streams.

ACKNOWLEDGEMENTS

Author contributions: VJG performed the research, analyzed the data, and wrote the paper; PG, LG, CH and NF performed the research; FS conceived the study and contributed new methods; CF conceived and designed the study, analyzed the data, and wrote the paper. All authors discussed the results and commented on the manuscript.

We thank Adonis Giorgi for theoretical and methodological advice, Susana Filippini for statistical advice, Carolina Vilches for some interesting comments on the results, and Daniel von Schiller for his suggestions on an early draft. Special thanks are extended to Associate Editor Robert Hall and 2 anonymous referees for helpful comments on the manuscript. We are grateful to Andrés Solá and Juan Rojas for providing access to the study sites. VJG was funded through a CONICET doctoral fellowship. Financial support for this research was provided by projects PICT-2011-0163 (Agencia Nacional de Promoción Científica y Tecnológica), and A1/037685/11 (Agencia Española de Cooperación Internacional para el Desarrollo).

LITERATURE CITED

- Acuña, V., C. Vilches, and A. Giorgi. 2011. As productive and slow as a stream can be: the metabolism of a Pampean stream. *Journal of the North American Benthological Society* 30:71–83.
- Allan, J. D., and M. M. Castillo. 2007. *Stream ecology: structure and function of running waters*. 2nd edition. Springer Science and Business Media, Dordrecht, The Netherlands.
- APHA (American Public Health Association). 2005. *Standard methods for the examination of water and wastewater*. 20th edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.
- Bernhardt, E. S., R. O. Hall, and G. E. Likens. 2002. Whole-system estimates of nitrification and nitrate uptake in streams of the Hubbard Brook Experimental Forest. *Ecosystems* 5:419–430.
- Boar, R. R., D. H. Lister, and W. T. Clough. 1995. Phosphorus loads in a small groundwater-fed river during the 1989–1992 East Anglian drought. *Water Research* 29:2167–2173.
- Cardinale, B. J. 2011. Biodiversity improves water quality through niche partitioning. *Nature* 472:86–89.
- Cohen, M. J., M. J. Kurz, J. B. Heffernan, J. B. Martin, R. L. Douglass, C. R. Foster, and R. G. Thomas. 2013. Diel phosphorus variation and the stoichiometry of ecosystem metabolism in a large spring-fed river. *Ecological Monographs* 83:155–176.
- Covino, T., B. McGlynn, and M. Baker. 2010. Separating physical and biological nutrient retention and quantifying uptake kinetics from ambient to saturation in successive mountain stream reaches. *Journal of Geophysical Research* 115:G04010.
- Covino, T., B. McGlynn, and R. McNamara. 2012. Land use/land cover and scale influences on in-stream nitrogen uptake kinetics. *Journal of Geophysical Research: Biogeosciences* 117(G2). doi:10.1029/2011JG001874
- Davis, J. C., and G. W. Minshall. 1999. Nitrogen and phosphorus uptake in two Idaho (USA) headwater wilderness streams. *Oecologia (Berlin)* 119:247–255.
- Diemer, L. A., W. H. McDowell, A. S. Wymore, and A. S. Prokushkin. 2015. Nutrient uptake along a fire gradient in boreal streams of Central Siberia. *Freshwater Science* 34:1443–1456.
- Dodds, W. K., A. J. López, W. B. Bowden, S. Gregory, N. B. Grimm, S. K. Hamilton, A. E. Hershey, E. Martí, W. H. McDowell, J. L. Meyer, D. Morrall, P. J. Mulholland, B. J. Peterson, J. L. Tank, H. M. Valett, J. R. Webster, and W. Wollheim. 2002. N uptake as a function of concentration in streams. *Journal of the North American Benthological Society* 21:206–220.
- Earl, S. R., H. M. Valett, and J. R. Webster. 2006. Nitrogen saturation in stream ecosystems. *Ecology* 87:3140–3151.
- Ensign, S. H., and M. W. Doyle. 2006. Nutrient spiraling in streams and river networks. *Journal of Geophysical Research* 111:1–13.
- Feijoó, C., M. E. García, F. Momo, and J. Toja. 2002. Nutrient absorption by the submerged macrophyte *Egeria densa* Planch: effect of ammonium and phosphorus availability in the water column on growth and nutrient uptake. *Limnética* 21:93–104.
- Feijoó, C., A. Giorgi, and N. Ferreiro. 2011. Phosphate uptake in a macrophyte-rich Pampean stream. *Limnologia* 41:285–289.
- Feijoó, C., L. Leggieri, C. Ocón, I. Muñoz, A. Rodríguez Capítulo, A. Giorgi, D. Colautti, N. Ferreiro, M. Licursi, N. Gómez, and S. Sabater. 2014. Stoichiometric homeostasis in the food web of a chronically nutrient-rich stream. *Freshwater Science* 33:820–831.
- Feijoó, C., and R. J. Lombardo. 2007. Baseline water quality and macrophyte assemblages in Pampean streams: a regional approach. *Water Research* 41:1399–1410.
- Feijoó, C., and M. Menéndez. 2009. La biota de los ríos: Macrófitos. Pages 242–251 in A. Elósegui and S. Sabater (editors). *Conceptos y técnicas para el estudio de la ecología de ríos*. Fundación BBVA, Bilbao, Spain.
- Fellows, C. S., H. M. Valett, C. N. Dahm, P. J. Mulholland, and S. A. Thomas. 2006. Coupling nutrient uptake and energy flow in headwater streams. *Ecosystems* 9:788–804.
- Gammons, C. H., J. N. Babcock, S. R. Parker, and S. R. Poulson. 2011. Diel cycling and stable isotopes of dissolved oxygen, dissolved inorganic carbon, and nitrogenous species in a stream receiving treated municipal sewage. *Chemical Geology* 283:44–55.
- Giorgi, A., C. Feijoó, and G. Tell. 2005. Primary producers in a Pampean stream: temporal variation and structuring role. *Biodiversity and Conservation* 14:1699–1718.
- Gücker, B., and I. G. Boëchat. 2004. Stream morphology controls ammonium retention in tropical headwaters. *Ecology* 85:2818–2827.
- Gücker, B., and M. T. Pusch. 2006. Regulation of nutrient uptake in eutrophic lowland streams. *Limnology and Oceanography* 51:1443–1453.
- Haggard, B. E., E. H. Stanley, and D. E. Storm. 2005. Nutrient retention in a point-source-enriched stream. *Journal of the North American Benthological Society* 24:29–47.
- Hall, R. O., M. A. Baker, E. J. Rosi-Marshall, J. L. Tank, and J. D. Newbold. 2013. Solute-specific scaling of inorganic nitrogen and phosphorus uptake in streams. *Biogeosciences* 10:7323–7331.
- Hall, R. O., E. S. Bernhardt, and G. E. Likens. 2002. Relating nutrient uptake with transient storage in forested mountain streams. *Limnology and Oceanography* 47:255–265.
- Hall, R. O., and J. L. Tank. 2003. Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. *Limnology and Oceanography* 48:1120–1128.
- Hall, R. O., J. L. Tank, D. J. Sobota, P. J. Mulholland, J. M. O'Brien, W. K. Dodds, J. R. Webster, H. M. Valett, G. C. Poole, B. J. Peterson, J. L. Meyer, W. H. McDowell, S. L. Johnson, S. K. Hamilton, N. B. Grimm, S. V. Gregory, C. N. Dahm, L. W. Cooper, L. R. Ashkenas, S. M. Thomas, R. W. Sheibley, J. D. Potter, B. R. Niederlehner, L. T. Johnson, A. M. Helton, C. M. Crenshaw, A. J. Burgin, M. J. Bernot, J. J. Beaulieu, and C. P. Arango. 2009. Nitrate removal in stream ecosystems measured by ¹⁵N addition experiments: total uptake. *Limnology and Oceanography* 54:653–665.
- Hanafi, S., M. R. Grace, and B. T. Hart. 2006. Can nutrient spiraling be used to detect seasonal nutrient uptake in a forested stream? *Water, Air, and Soil Pollution* 6:403–411.
- Hensley, R. T., M. J. Cohen, and L. V. Korhnak. 2014. Inferring nitrogen removal on large rivers from high-resolution longitudinal profiling. *Limnology and Oceanography* 59:1152–1170.
- Hoellein, T. J., J. L. Tank, E. J. Rosi-Marshall, S. A. Entekin, and G. A. Lamberti. 2007. Controls on spatial and temporal variation of nutrient uptake in three Michigan headwater streams. *Limnology and Oceanography* 52:1964–1977.

- Hornberger, G. M., and M. G. Kelly. 1975. Atmospheric reaeration in a river using productivity analysis. *Journal of Environmental Engineering* 101:729–739.
- Horner, R. R., E. B. Welch, M. R. Seeley, and J. M. Jacoby. 1990. Responses of periphyton to changes in current velocity, suspended sediment and phosphorus concentration. *Freshwater Biology* 24:215–232.
- Jarvie, H. P., C. Neal, M. D. Jürgens, E. J. Sutton, M. Neal, H. D. Wickham, L. K. Hill, S. A. Harman, J. J. L. Davies, A. Warwick, C. Barrett, J. Griffiths, A. Binley, N. Swannack, and N. McIntyre. 2006. Within-river nutrient processing in chalk streams: the Pang and Lambourn, UK. *Journal of Hydrology* 330:101–125.
- Larned, S. T. 2010. A prospectus for periphyton: recent and future ecological research. *Journal of the North American Benthological Society* 29:182–206.
- Larned, S. T., V. I. Nikora, and B. J. Biggs. 2004. Mass-transfer-limited nitrogen and phosphorus uptake by stream periphyton: a conceptual model and experimental evidence. *Limnology and Oceanography* 49:1992–2000.
- Leggieri, L., C. Feijóo, A. Giorgi, N. Ferreira, and V. Acuña. 2013. Seasonal weather effects on hydrology drive the metabolism of non-forest lowland streams. *Hydrobiologia* 716:47–58.
- Levi, P. S., T. Riis, A. B. Alnøe, M. Peipoch, K. Maetzke, C. Bruus, and A. Baattrup-Pedersen. 2015. Macrophyte complexity controls nutrient uptake in lowland streams. *Ecosystems* 18:914–931.
- Marcé, R., and J. Armengol. 2009. Modelling nutrient in-stream processes at the watershed scale using nutrient spiralling metrics. *Hydrology and Earth System Sciences* 13:953–967.
- Martí, E., J. Aumatell, L. Godé, M. Poch, and F. Sabater. 2004. Nutrient retention efficiency in streams receiving inputs from wastewater treatment plants. *Journal of Environmental Quality* 33:285–293.
- Martí, E., and F. Sabater. 2009. Retención de nutrientes en ecosistemas fluviales. Pages 117–132 in A. Elósegui and S. Sabater (editors). *Conceptos y técnicas para el estudio de la ecología de ríos*. Fundación BBVA, Bilbao, Spain.
- Martí, E., F. Sabater, C. Feijóo, P. Gantes, A. Giorgi, C. Vilches, N. Ferreira, A. Torremorell, C. Rodríguez, C. Ranieri, M. L. Gultemiriam, E. Zunino and V. García. 2011. Daily variation of N and P uptake in a highly productive Pampean stream. Annual Meeting of the North American Benthological Society, Providence, Rhode Island (unpublished abstract).
- Merseburger, G. C., E. Martí, and F. Sabater. 2005. Net changes in nutrient concentrations below a point source input in two streams draining catchments with contrasting land uses. *Science of the Total Environment* 347:217–229.
- Mulholland, P. J., A. M. Helton, G. C. Poole, R. O. Hall, S. K. Hamilton, B. J. Peterson, J. L. Tank, R. L. Ashkenas, L. W. Cooper, C. N. Dahm, W. K. Dodds, S. E. G. Findlay, S. V. Gregory, N. V. Grimm, S. L. Johnson, W. H. McDowell, J. L. Meyer, M. Valett, J. R. Webster, C. P. Arango, J. J. Beaulieu, M. J. Bernot, A. J. Burgin, C. L. Crenshaw, L. T. Johnson, B. R. Niederlehner, J. M. O'Brien, J. D. Potter, R. W. Sheibley, D. J. Sobota, and S. M. Thomas. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature* 452:202–205.
- Niyogi, D. K., K. S. Simon, and C. R. Townsend. 2004. Land use and stream ecosystem functioning: nutrient uptake in streams that contrast in agricultural development. *Archiv für Hydrobiologie* 160:471–486.
- O'Brien, J. M., and W. K. Dodds. 2010. Saturation of NO_3^- uptake in prairie streams as a function of acute and chronic N exposure. *Journal of the North American Benthological Society* 29:627–635.
- O'Brien, J. M., W. K. Dodds, K. C. Wilson, J. N. Murdock, and J. Eichmiller. 2007. The saturation of N cycling in Central Plains streams: ^{15}N experiments across a broad gradient of nitrate concentration. *Biogeochemistry* 84:31–49.
- O'Brien, J. M., J. L. Lessard, D. Plew, S. E. Graham, and A. R. McIntosh. 2014. Aquatic macrophytes alter metabolism and nutrient cycling in lowland streams. *Ecosystems* 17:405–417.
- Odum, H. T. 1956. Primary production in flowing waters. *Limnology and Oceanography* 1:102–117.
- Oviedo-Vargas, D., T. V. Royer, and L. T. Johnson. 2013. Dissolved organic carbon manipulation reveals coupled cycling of carbon, nitrogen, and phosphorus in a nitrogen-rich stream. *Limnology and Oceanography* 58:1196–1206.
- Peipoch, M., E. Garcia, A. Pastor, M. Ribot, J. L. Riera, F. Sabater, and E. Martí. 2014. Intrinsic and extrinsic drivers of autotrophic nitrogen cycling in stream ecosystems: results from a translocation experiment. *Limnology and Oceanography* 59:1973–1986.
- Peterson, B. J., W. M. Wollheim, P. J. Mulholland, J. R. Webster, J. L. Meyer, J. L. Tank, E. Martí, W. D. Bowden, H. M. Valett, A. E. Hershey, W. H. McDowell, W. K. Dodds, S. K. Hamilton, S. Gregory, and D. D. Morrall. 2001. Control of nitrogen export from watershed by headwater streams. *Science* 292:86–90.
- Prieto, A. R. 1996. Late Quaternary vegetational and climatic changes in the Pampa grassland of Argentina. *Quaternary Research* 45:73–88.
- Rasmussen, J. J., A. Baattrup-Pedersen, T. Riis, and N. Friberg. 2011. Stream ecosystem properties and processes along a temperature gradient. *Aquatic Ecology* 45:231–242.
- Ribot, M., E. Martí, D. von Schiller, F. Sabater, H. Daims, and T. J. Battin. 2012. Nitrogen processing and the role of epilithic biofilms downstream of a wastewater treatment plant. *Freshwater Biology* 51:1057–1069.
- Ribot, M., D. von Schiller, M. Peipoch, F. Sabater, N. B. Grimm, and E. Martí. 2013. Influence of nitrate and ammonium availability on uptake kinetics of stream biofilms. *Freshwater Science* 32:1155–1167.
- Riis, T., W. K. Dodds, P. B. Kristensen, and A. J. Baisner. 2012. Nitrogen cycling and dynamics in a macrophyte-rich stream as determined by a ^{15}N - NH_4 release. *Freshwater Biology* 57:1579–1591.
- Rodríguez-Cardona, B., A. S. Wymore, and W. H. McDowell. 2016. DOC: NO_3^- ratios and NO_3^- uptake in forested headwater streams. *Journal of Geophysical Research: Biogeosciences* 121:205–217.
- Rodríguez Castro, C. 2015. Capacidad de depuración de sustancias bioaprovechables en arroyos de llanura y su relación con el arsénico. PhD Dissertation, Universidad de Buenos Aires, Buenos Aires, Argentina.
- Ruggiero, A., A. G. Solimini, M. Anello, A. Romano, M. De Cicco, and G. Carchini. 2006. Nitrogen and phosphorus retention in a human altered stream. *Chemistry and Ecology* 22(Supplement 1). (Available from: <http://dx.doi.org/10.1080/02757540600556753>)

- Runkel, R. L. 1998. One-dimensional transport with inflow and storage (OTIS): a solute transport model for streams and rivers. US Geological Survey, Water-Resources Investigations Report 98-4018. US Geological Survey, Reston, Virginia.
- Salehin, M., A. I. Packman, and A. Wörman. 2003. Comparison of transient storage in vegetated and unvegetated reaches of a small agricultural stream in Sweden: seasonal variation and anthropogenic manipulation. *Advances in Water Resources* 26: 951–964.
- Scheffer, M. 1990. Multiplicity of stable states in freshwater systems. *Hydrobiologia* 200:475–486.
- Stream Solute Workshop. 1990. Concepts and methods for assessing solute dynamics in stream ecosystems. *Journal of the North American Benthological Society* 9:95–119.
- Uehlinger, U., and W. M. Naegeli. 1998. Ecosystem metabolism, disturbance, and stability in a prealpine gravel bed river. *Journal of the North American Benthological Society* 17:165–178.
- Valett, H. M., J. A. Morrice, C. N. Dahm, and M. E. Campana. 1996. Parent lithology, surface-groundwater exchange, and nitrate retention in headwaters streams. *Limnology and Oceanography* 41:333–345.
- Velasco, J., A. Millard, M. R. Vidal-Abarca, M. L. Suárez, C. Guerrero, and M. Ortega. 2003. Macrophytic, epipelic and epilithic primary production in a semiarid Mediterranean stream. *Freshwater Biology* 48:1408–1420.
- Vilches, C., and A. Giorgi. 2010. Metabolism in a macrophyte-rich stream exposed to flooding. *Hydrobiologia* 654:57–65.
- von Schiller, D., E. Martí, J. L. Riera, M. Ribot, A. Argerich, P. Fonolla, and F. Sabater. 2008a. Inter-annual, annual, and seasonal variation of P and N retention in a perennial and an intermittent stream. *Ecosystems* 11:670–687.
- von Schiller, D., E. Martí, J. L. Riera, M. Ribot, J. C. Marks, and F. Sabater. 2008b. Influence of land use on stream ecosystem function in a Mediterranean catchment. *Freshwater Biology* 53:2600–2612.
- Webster, J. R., D. J. D'Angelo, and G. T. Peters. 1991. Nitrate and phosphate uptake in streams at Coweeta Hydrologic Laboratory. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* 24:1681–1686.
- Webster, J. R., P. J. Mulholland, J. L. Tank, H. M. Valett, W. K. Dodds, B. J. Peterson, W. B. Bowden, C. N. Dahm, S. Findlay, S. V. Gregory, N. B. Grimm, S. K. Hamilton, S. L. Johnson, E. Martí, W. H. McDowell, J. L. Meyer, D. D. Morrall, S. A. Thomas, and W. M. Wollheim. 2003. Factors affecting ammonium uptake in streams—an inter-biome perspective. *Freshwater Biology* 48:1329–1352.
- Wilcock, R. J., M. R. Scarsbrook, J. G. Cooke, K. J. Costley, and J. W. Nagels. 2002. Controlled release experiments to determine the effects of shade and plants on nutrient retention in lowland stream. *Hydrobiologia* 485:153–162.
- Wilcock, R. J., M. R. Scarsbrook, J. G. Cooke, K. J. Costley, and J. W. Nagels. 2004. Shade and flow effects on ammonia retention in macrophyte-rich streams: implications on water quality. *Environmental Pollution* 132:95–100.
- Zárate, M., R. A. Kemp, M. Espinosa, and L. Ferrero. 2000. Pedosedimentary and palaeo-environmental significance of a Holocene alluvial sequence in the southern Pampas, Argentina. *Holocene* 10:481–488.