

# Diel variation of nutrient retention is associated with metabolism for ammonium but not phosphorus in a lowland stream

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**Abstract:** In-stream nutrient retention is an important ecosystem function because it can regulate nutrient fate and export to downstream ecosystems. Temporal variation in nutrient retention in streams has been studied extensively at the annual and seasonal scale but less thoroughly at the diel scale. However, understanding temporal variability in nutrient uptake at the diel scale can increase understanding of the role of photoautotrophic primary production on nutrient uptake in streams, especially open-canopy streams. We hypothesized that nutrient retention mostly depends on autotrophic demand in open-canopy streams and that it varies following the diel pattern of gross primary production (GPP). We therefore evaluated the temporal variation in phosphate ( $\text{PO}_4^{3-}$ ) and ammonium ( $\text{NH}_4^+$ ) uptake at a daily scale in a highly-productive Pampean stream that is dominated by a dense assemblage of macrophytes and filamentous algae. We conducted 6 slug additions of  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  over a 24-h period and quantified reach-scale nutrient uptake concurrently with measurements of whole-stream metabolism and chemical variables during additions (including nitrates and nitrites). The study stream had extremely high uptake of  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  (>90 and >75% retention of the P and N mass added, respectively). Uptake of  $\text{PO}_4^{3-}$  did not vary throughout the day. Estimated  $\text{PO}_4^{3-}$  uptake from GPP accounted for only a small fraction of observed  $\text{PO}_4^{3-}$  uptake. Thus, another mechanism, such as heterotrophic demand by microbial assemblages or adsorption onto sediments, could also have contributed to  $\text{PO}_4^{3-}$  uptake in the study stream. In contrast,  $\text{NH}_4^+$  uptake clearly varied throughout the day. Up to 48% of the observed  $\text{NH}_4^+$  uptake rate could be explained by  $\text{NH}_4^+$  estimated from GPP, and  $\text{NH}_4^+$  demand was positively associated with GPP, indicating a high dependence on photoautotrophic demand. An increase of nitrite ( $\text{NO}_2^-$ ) concentration during additions (representing up to 70% of the added mass of  $\text{NH}_4^+$ ) suggests that nitrification contributed to the diel pattern of  $\text{NH}_4^+$  uptake. Our results indicate that nutrient uptake does not always rely on autotrophic demand in open-canopy streams and that other abiotic and dissimilatory mechanisms may explain the diel patterns of nutrient retention. In addition, our study highlights the need to measure uptake metrics throughout the day to obtain an accurate estimate of nutrient retention on a daily scale.

**Key words:** nutrient uptake, nitrogen, phosphorus, macrophytes, biofilms, metabolism, nitrification, Pampean streams

Nutrient uptake is a paramount ecosystem function in lotic ecosystems and has traditionally been evaluated with the nutrient spiraling concept (Newbold et al. 1981, Stream

Solute Workshop 1990). Nutrient uptake results from the interaction between hydro-geomorphological and biogeochemical processes, both of which operate as water and

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nutrients are transported downstream (Valett et al. 1996). Hydro-geomorphological factors determine the residence time of water and the exposure of dissolved nutrients to biochemically-reactive substrata (Valett et al. 1996, Argerich et al. 2011). Abiotic geochemical processes (i.e., sorption, flocculation, and precipitation) chemically retain certain solutes, such as phosphate ( $\text{PO}_4^{3-}$ ) and ammonium ( $\text{NH}_4^+$ ). In particular,  $\text{PO}_4^{3-}$  can be removed from the water column through co-precipitation with calcium carbonate ( $\text{CaCO}_3$ ) or adsorption onto calcium-rich substrata in streams draining catchments with calcareous lithology (Reddy et al. 1999, Jalali and Peikam 2013, Corman et al. 2016). Nutrient uptake is also regulated by biotic processes such as assimilatory uptake by autotrophic and heterotrophic organisms as well as dissimilatory uptake processes such as nitrification (Mulholland et al. 2008, Ribot et al. 2017, Tank et al. 2018). Nutrient uptake has been studied extensively at annual and seasonal scales in streams from different biomes (see, for instance, Mulholland et al. 1985, von Schiller et al. 2008, García et al. 2017). However, nutrient uptake has been less thoroughly investigated at shorter time scales, such as hourly or daily scales (but see Martí et al. 1994, Johnson and Tank 2009, Heffernan and Cohen 2010).

Biotic activity relies on nutrient uptake because nitrogen (N) is needed to produce proteins and phosphorus (P) is required to synthesize adenosine triphosphate, which is a crucial energy source in enzymatic reactions. In particular, assimilatory nutrient demand by photoautotrophic activity can greatly influence whole-reach nutrient uptake in open-canopy streams (Cohen et al. 2013, Hanrahan et al. 2018, Tank et al. 2018). Moreover, light and thermal regimes show remarkable diel oscillations in these types of streams (Johnson and Tank 2009, Heffernan and Cohen 2010, Bernhardt et al. 2017). Both factors influence the metabolic activity of photoautotrophic assemblages, so nutrient retention may vary greatly on a diel basis in open-canopy streams.

The relationship between stream metabolism (i.e., gross primary production [GPP], ecosystem respiration [ER], and net primary production) and nutrient uptake has been investigated in several types of fluvial systems. Some authors report no clear relationship, or the lack of any relationship, between metabolism and nutrient uptake metrics (Webster et al. 2003, Hoellein et al. 2007, von Schiller et al. 2008, O'Brien et al. 2014). These results suggest that biotic or abiotic processes other than stream metabolism control nutrient retention. In contrast, many studies have shown positive relationships between metabolic measures, especially GPP, and the uptake of P, N, or both (Sabater et al. 2000, Hall and Tank 2003, Fellows et al. 2006, Gücker and Pusch 2006, Newbold et al. 2006, Rasmussen et al. 2011, Gibson and O'Reilly 2012, Cohen et al. 2013). These results suggest that stream metabolism influences nutrient uptake. In open-canopy streams, where GPP rates are high, we would expect that stream metabolism influences nutrient uptake. Accordingly, we would also expect clear diel variation in nutrient

uptake that is associated with the photoautotrophic activity that controls GPP.

In this study, we investigated the diel variation of soluble reactive P (SRP) and  $\text{NH}_4^+$  uptake relative to primary production in a highly-productive Pampean stream (central Argentina). Pampean streams lack riparian forests and have low gradients, high irradiances, dense assemblages of algae and macrophytes and the highest GPP in warm months (Acuña et al. 2011, García et al. 2017). In addition, Pampean streams are eutrophic under USEPA (2000) guidelines because the mean concentration of  $\text{PO}_4^{3-}$  is  $>0.15$  mg/L and the mean concentration of  $\text{NO}_3^-$  is  $>1.5$  mg/L (Amuchástegui et al. 2016). The  $\text{PO}_4^{3-}$  present in these streams is probably derived mainly from sedimentary parent material, whereas the  $\text{NO}_3^-$  is derived mostly from the agricultural activities in the region (Amuchástegui et al. 2016). However, paleolimnological records suggest that eutrophic conditions were common in Pampean water bodies even before the rise of agriculture (Feijoó and Lombardo 2007). Thus, the biological communities present in these streams may be well adapted to enriched conditions (García et al. 2017). Pampean streambeds are commonly composed of a  $\text{CaCO}_3$ -rich tuff layer covered by fine sediments (Zárate et al. 2000), which can influence geochemical mechanisms of  $\text{PO}_4^{3-}$  uptake (Reddy et al. 1999). These characteristics of Pampean streams make them valuable study sites. Further, these streams are comparable to nutrient-rich, low-gradient streams elsewhere in the world.

To understand fine-scale nutrient uptake in this stream, we did 6 nutrient slug additions over the course of a 24-h period and quantified reach-scale nutrient uptake while we also took measurements of whole-stream metabolism. We hypothesized that nutrient uptake in Pampean streams relies mostly on photoautotrophic activity. Therefore, we expected that diel variation in uptake of both P and N would follow diel patterns of light and instantaneous GPP. We expected that diel patterns of P uptake would be coupled more strongly with GPP than would  $\text{NH}_4^+$  uptake because: 1) high N:P ratios of stream water (i.e., range of molar N:P from 295–426; García et al. 2017) suggest that P is the limiting nutrient for photoautotrophic communities, and 2) P can be removed from the water column by adsorption into the sediments (Jalali and Peikam 2013) or by co-precipitation with calcite. Both of these processes can occur at high pH, which varies with GPP (Cohen et al. 2013).

## METHODS

### Study site

We did this study in the Las Flores stream, a 2<sup>nd</sup>-order stream in the Luján River basin (northeast of Buenos Aires province, Argentina; lat 34°27'35''S, long 59°04'27''W). The sub-catchment upstream of the study reach is dominated by agricultural land use (Amuchástegui et al. 2016). This stream is characteristic of Pampean streams, and it has primarily grassy riparian vegetation, low stream water velocity,

a CaCO<sub>3</sub>-rich tuff layer of streambed substrata, and elevated nutrient levels (0.77 mg P-PO<sub>4</sub><sup>3-</sup>/L and 4.14 mg N-NO<sub>3</sub><sup>-</sup>/L; Giorgi et al. 2005). The macrophyte assemblage in the reach was dense and diverse during this study and included submerged (*Ceratophyllum demersum*, *Stuckenia striata*, *Elodea ernstae*), emergent (*Lolium* spp., *Poligonum* spp., *Leersia* spp., *Nasturtium* spp., *Eleocharis* spp., *Hydrocotyle modesta*, *H. ranunculoides*), and floating (*Lemna* spp.) species. Filamentous algae (*Spirogyra* spp. and *Cladophora* spp.) were also present.

We did the nutrient slug additions in a 28-m stream reach with no detectable lateral inflows. Despite the relatively short reach length, the water residence time was 45 min on average, which is a reasonable time frame in which to measure nutrient uptake throughout the reach with slug additions (Martí and Sabater 2009). In addition, measuring nutrient uptake in longer reaches would have prevented sequential and separate slug additions throughout the day since the water residence time was relatively high.

### Field nutrient addition experiments

We did the nutrient slug additions in December (austral spring) under low-flow conditions. We used the slug addition method instead of the constant-rate addition method because we wanted to do several nutrient uptake measurements within 24 h. This would have been challenging with the constant-rate addition method because that method requires longer addition times to reach a plateau, and it is difficult to ensure that all the solution is gone from the study reach before the beginning of the next addition. Some studies have indicated that results from the 2 methods are comparable, especially under low flow conditions (Powers et al. 2009, Álvarez et al. 2010).

We did 6 slug additions of PO<sub>4</sub><sup>3-</sup> and NH<sub>4</sub><sup>+</sup> to cover a range of photosynthetic active radiation (PAR) and temperature conditions throughout a 24-h period from 1000 h December 3 to 1000 h December 4 2010. We took measurements during the day (1000, 1500, and 1900 h of the 1<sup>st</sup> d, and 1000 h of the 2<sup>nd</sup> d) and at night (0300 and 0700 h of the 2<sup>nd</sup> d). For each addition we prepared a 2-L solution with distilled water that contained 4 g K<sub>2</sub>HPO<sub>4</sub><sup>3-</sup> (as the PO<sub>4</sub><sup>3-</sup> source), 10 g NH<sub>4</sub>Cl (as a NH<sub>4</sub><sup>+</sup> source), and 250 g NaCl (as a conservative tracer). These reagent concentrations were high enough to increase nutrient concentrations above the background levels and allow the added nutrients to be chemically traced at the downstream end of the reach throughout the slug passage. We used distilled water to prepare the solutions to ensure that each addition contained the same mass of N and P. We placed 1 conductivity meter with a data logger (HQ40d; HACH, Loveland, Colorado) at each end (upstream and downstream) of the reach. Before the solute addition, we recorded the initial conductivity and collected a 100-mL water sample at the downstream end of the reach. We then added the solute as a single pulse at a narrow location (to ensure adequate mixing) 2 m upstream of the up-

stream end of the reach. We then started collecting water samples at the downstream end of the reach. Time intervals for water sampling were set to capture the full breakthrough curve of the solutes at the downstream end of the reach. Time intervals were identified a priori based on results from a preliminary NaCl slug addition (Martí and Sabater 2009). On average, each slug addition lasted 45 min, and we collected 33 water samples during each addition. We also monitored electrical conductivity (EC) and pH with a multiparameter HQ40d probe (HACH) and collected water samples upstream of the addition point every 5 min. The upstream samples were used to check that stream nutrient concentration did not change during the additions. We also used the upstream samples to determine background nutrient concentrations during the additions and the background variability of nutrient levels over the 24-h period. We confirmed that enough time had elapsed between each slug addition when conductivity measurements at the upstream and downstream ends of the reach were equivalent.

We estimated stream metabolism during the whole period of slug additions with the open-system, single-station approach (Odum 1956, Uehlinger and Naegeli 1998). We placed a dissolved oxygen (DO) probe (HQ40d, HACH) 5 m upstream of the study reach to avoid potential effects of the slug on metabolism measurements. Stream characteristics around the probe including riparian vegetation, hydrology, chemistry, and macrophyte abundance were similar to the characteristics in the reach. Therefore, we assumed that measurements of stream metabolism near the probe were representative of the study reach. The probe automatically recorded oxygen and temperature data at 5-min intervals and was left in place from 0900 h on 3 December to 1300 on 4 December, spanning the whole period of slug additions. We also collected water samples every 30 min from 1300 to 1730 h at the location of the DO probe and measured DO concentrations in these samples with the Winkler method. These additional estimations were done because extremely high rates of GPP increased DO concentration beyond the DO range detectable by the probe (i.e., >20 mg/L). We used data from the GERSolar Station at the National University of Luján, located close to the study site (<20 km), to estimate instantaneous PAR during the study period (measured every 10 min).

We collected data to describe the physical characteristics of the reach and the abundance of the different basal biological assemblages. We measured channel width (m) at 14 equidistant transects along the reach to determine average channel width and surface area of the reach. We mapped macrophyte abundance (including filamentous algae) by measuring the length of the transect occupied by each plant species. These data were used to calculate stream surface covered by the different macrophyte life forms (emergent, submerged, and floating) following Feijoó and Menéndez (2009). Five 650-cm<sup>2</sup> quadrat macrophyte samples were taken along the reach to estimate the biomass of each plant

species. We also took 12 samples of fine benthic organic matter (FBOM) with a 6-cm diameter core along the reach to analyze benthic biomass and chlorophyll-*a* (Chl-*a*) content. Water, macrophyte, and sediment samples were kept cold and in the dark until we transported them to the laboratory within 6 h of collection.

### Laboratory analyses

We manually analyzed samples for concentrations of  $\text{PO}_4^{3-}$  (as SRP) and  $\text{NH}_4^+$  following standard colorimetric methods (ascorbic acid-molybdenum blue and phenol hypochlorite methods, respectively; APHA 2005). Concentrations of nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ) were determined with a TRAACS 2000 Autoanalyzer (Bran+Luebbe®, Nordestedt, Germany) following standard colorimetric methods (sulphanilamide method with a previous Cu-Cd reduction for  $\text{NO}_3^-$ ; APHA 2005). Winkler samples were analyzed for DO (APHA 2005).

We sorted macrophyte and filamentous algae samples by species and separated fragments of each species to measure their Chl-*a* content. The remaining samples were dried at 60°C until they reached a constant weight, combusted at 500°C for 4 h, and weighed again. We used the pre- and post-combustion weight difference divided by the total area from which a macrophyte species was collected to determine the biomass of its standing stocks, expressed as ash-free dry mass ( $\text{g/m}^2$ ). We measured Chl-*a* content by extracting the photosynthetic pigments from macrophyte subsamples in 90% acetone at 4°C for 24 h. We used a spectrophotometer to measure the Chl-*a* content of the extract following APHA (2005). We estimated the biomass of each species at a reach scale with the biomass of the standing stocks and % areal cover of each species. This allowed us to estimate the relative contribution of different categories of primary producers to total standing stocks.

We took 12 FBOM samples and used 6 to estimate biomass content and the other 6 samples to determine Chl-*a* content. Methods used to characterize FBOM were the same as those described above for macrophytes. We used the biological characteristics of FBOM to characterize the reach in terms of standing stocks of primary producers.

### Parameter and metric estimates

**Hydraulic parameters** We measured discharge ( $Q$ , L/s) and average water velocity ( $v$ , m/s) with the breakthrough curve of EC recorded at the downstream end of the reach. Calculation of  $Q$  was based on a tracer mass balance approach (Gordon et al. 1992). Mean water velocity was calculated by dividing the reach length by the time needed to reach the peak of the EC breakthrough curve (i.e.,  $t_n$  or nominal travel time, in s). We estimated the water transient storage zone ( $A_s$ ,  $\text{m}^2$ ) with a 1-dimensional transient storage model, which accounts for advection, dispersion, dilution from lateral water inflow, and exchange with transient stor-

age zones (Runkel 1998). We calculated the cross-sectional area of the stream channel ( $A$ ,  $\text{m}^2$ ) by dividing  $Q$  ( $\text{m}^3/\text{s}$ ) by  $v$  and used it to obtain the ratio between the cross section of the water transient storage zone and that of the stream channel ( $A_s:A$ ).

**Nutrient uptake metrics** We calculated nutrient uptake metrics by comparing the breakthrough curves of measured and predicted concentrations of  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  from the additions. The predicted concentrations represent the null hypothesis that nutrients behave conservatively and their variation over time is based solely on hydrologic factors (i.e., advection, dispersion, and dilution). We calculated predicted nutrient concentrations at different times of the breakthrough curves ( $N_{(\text{pred})t}$ ) with the following equation:

$$N_{(\text{pred})t} = \left( \frac{\text{Cond}_t - \text{Cond}_b}{\text{Cond}_s} \times N_s \right) + N_b \quad (\text{Eq. 1}),$$

where  $\text{Cond}_t$  is the observed EC at time of the breakthrough curve,  $\text{Cond}_b$  and  $N_b$  are the background EC and nutrient concentration, respectively, and  $\text{Cond}_s$  and  $N_s$  are the EC and the nutrient concentration in the added solution, respectively. We estimated the nutrient uptake rate coefficient ( $k_t$ , s) following the exponential decay model (Wilcock et al. 2002):

$$M_{\text{obs}} = M_{\text{pred}} \times e^{-k_t \times t_n} \quad (\text{Eq. 2}),$$

where  $M_{\text{obs}}$  and  $M_{\text{pred}}$  are the mass of nutrients (mg) observed and predicted at the bottom of the reach, respectively, and  $t_n$  is the nominal water travel time (s) along the reach. Calculation of  $M_{\text{obs}}$  and  $M_{\text{pred}}$  was based on the integrated area of the background-corrected nutrient concentrations of the breakthrough curves ( $\text{mg L}^{-1} \text{s}^{-1}$ ) multiplied by stream  $Q$  (L/s) for observed and predicted nutrient concentrations, respectively. We used  $k_t$  to estimate uptake demand ( $V_f$ , m/min) and areal uptake rate ( $U$ ,  $\text{mg m}^{-2} \text{min}^{-1}$ ) with the following equations:

$$V_f = \frac{Q \times k_t}{w \times v} \quad (\text{Eq. 3})$$

and

$$U = V_f \times N_b \quad (\text{Eq. 4}),$$

where  $Q$  is in  $\text{m}^3/\text{s}$ ,  $w$  is average stream channel width (m), and  $v$  is mean water velocity (m/s).

To estimate the percentage of added  $\text{NH}_4^+$  that was nitrified, we compared the increase in  $\text{NO}_3^-$  or  $\text{NO}_2^-$  mass during the slug passage at the downstream end of the reach with the mass of  $\text{NO}_3^-$  or  $\text{NO}_2^-$  expected if all  $\text{NH}_4^+$  added was transformed into either of these N forms.

**Metabolism parameters** We estimated daily rates of GPP and ER by integrating the DO measurements at the single

station upstream of the reach during a 24-h period (Uehlinger and Naegeli 1998). We estimated the nighttime reaeration coefficient and respiration based on DO change rates and DO deficits with the nighttime regression method (Young and Huryn 1996). Respiration at night was extrapolated to a period of 24 h to estimate the daily rate of ER. We calculated the daily rate of GPP by integrating the difference between the measured net DO change (corrected by the reaeration flux) and the extrapolated daytime respiration. Daily rates of GPP and ER were then multiplied by the mean reach depth to obtain areal estimates ( $\text{mg O}_2 \text{ m}^{-2} \text{ d}^{-1}$ ). We also expressed GPP in  $\text{mg C m}^{-2} \text{ d}^{-1}$  with a 1:1 molar conversion between produced oxygen and consumed carbon dioxide. We used these values to calculate the net ecosystem production ( $\text{NEP} = \text{GPP} - \text{ER}$ ) and the GPP:ER ratio.

We estimated the average instantaneous GPP for the duration of each nutrient addition (~45 min). We estimated the autotrophic P and N uptake ( $U_{\text{GPP}}$ ) from instantaneous GPP following Webster et al. (2003). We used a C:N ratio of 15.7, which is characteristic of macrophytes from the study stream (Feijóo et al. 2014), to convert C fixation (i.e., instantaneous GPP) into autotrophic N uptake ( $U_{\text{NH}_4 - \text{GPP}}$ ). We then used an N:P ratio of 17.1 (Feijóo et al. 1996) to convert  $U_{\text{NH}_4 - \text{GPP}}$  into autotrophic P uptake ( $U_{\text{P} - \text{GPP}}$ ). We compared the observed values of P and N uptake with  $U_{\text{P} - \text{GPP}}$  and  $U_{\text{NH}_4 - \text{GPP}}$ , respectively, to evaluate the relative contribution of photoautotrophic activity to whole-reach nutrient uptake.

## Data analyses

We explored the association between nutrient uptake efficiency ( $V_f$ ) and several variables that may explain nutrient retention in the stream. We described the response of instantaneous GPP to variation in light availability (PAR) in the study stream with a hyperbolic single rectangular model ( $n = 273$ ). We examined the relationship between  $Q$  and  $A_s/A$  and between nutrient uptake efficiency ( $V_f - \text{P}$  and  $V_f - \text{NH}_4$ ) and potentially-related independent variables (water temperature, concentrations of  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$ , pH, and GPP) with simple linear regression analysis. These independent variables were selected because they have previously been associated with nutrient uptake in the Las Flores stream (García et al. 2017). We used linear regressions to detect likely associations between variables rather than as confirmatory analyses because of the low number of replicates ( $n = 6$ ; Wasserstein et al. 2019) and the possibility of temporal pseudo-replication (Hurlbert 1984). Associations that emerged from linear regressions were then contrasted with additional evidence to understand the diel pattern of nutrient retention (Wasserstein et al. 2019). All statistical analyses were done with SPSS® for Microsoft Windows (version 12.0; IBM®, Chicago, Illinois).

## RESULTS

### Diel variation of physical and chemical variables

Most physical and hydrological variables varied markedly during the experiment (Table 1). Water temperature had

Table 1. Diel variation of physical and chemical variables. Data are from measurements done during 6 slug additions conducted over the 24-h study. PAR = photosynthetically active radiation,  $A_s$  = cross section of the transient storage zone,  $A$  = cross section of the stream channel, SRP = soluble reactive phosphorus,  $\text{NH}_4^+$  = ammonium,  $\text{NO}_2^-$  = nitrites,  $\text{NO}_3^-$  = nitrates, DIN = dissolved inorganic nitrogen, P = phosphorus, DO = dissolved oxygen.

Variable	Time of day when slug additions were conducted					
	1000	1500	1900	0300	0700	1000
Temperature (°C)	22.0	27.5	25.9	21.1	19.8	19.9
PAR ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )	1572.7	1781.5	224.2	0.0	73.3	593.3
Discharge (L/s)	6.1	6.2	5.4	4.7	4.2	4.3
Water depth (m)	0.096	0.091	0.085	0.088	0.087	0.086
Velocity (m/s)	0.061	0.065	0.061	0.051	0.046	0.048
Conductivity ( $\mu\text{S/cm}$ )	843	830	827	851	852	852
$A_s/A$	0.57	0.58	0.64	0.67	0.69	0.70
SRP (mg/L)	0.064	0.066	0.066	0.065	0.064	0.066
$\text{NH}_4^+$ (mg/L)	0.018	0.014	0.042	0.021	0.014	0.012
$\text{NO}_2^-$ (mg/L)	0.299	0.258	0.263	0.294	0.472	0.339
$\text{NO}_3^-$ (mg/L)	3.01	3.00	3.23	3.14	3.10	2.71
DIN:SRP	107.3	104.4	111.8	109.9	111.9	95.1
pH	8.1	8.8	8.7	7.8	7.6	7.8
DO (mg/L)	15.6	22.2	17.0	2.2	1.9	6.7
DO saturation (%)	184.3	285.4	211.6	25.1	20.9	74.0

~8°C daily amplitude, and the highest water temperature was recorded at 1500 h. PAR, integrated over the 24-h study period, was 51.98  $\mu\text{mol m}^{-2} \text{d}^{-1}$  with a lower value during the 2<sup>nd</sup> day because of cloudier weather conditions (Fig. 1A). Stream Q was low overall and decreased slightly over the study period, as did water depth and velocity (Table 1). The estimated values of  $A_s/A$  were relatively high and negatively associated with Q ( $r^2 = 0.97$ ). EC was relatively constant during the study period, whereas DO concentration varied in a diel pattern with values ranging from supersaturation conditions at 1500 h to hypoxic conditions at night (Table 1, Fig. 1A). Values of pH also had a diel pattern of variation similar to DO and pH (Table 1).

Dissolved P and N concentrations showed different patterns during the sampling period. The SRP concentration was constant throughout the study, but  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations varied and were highest near sunset (1900 h; Table 1). Concentration of  $\text{NO}_2^-$  was highest in the morning (0700 and 1000 h; Table 1). The molar ratio between dissolved inorganic N ( $\text{DIN} = \text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$ ) and SRP concentrations did not show a diel pattern. DIN:SRP ratios were high, indicating potential P limitation.

### Ecosystem metabolism

As expected for this open-canopy stream, metabolic rates showed a wide range of variation during the study. The daily variation of DO concentration and the relatively-low reaera-

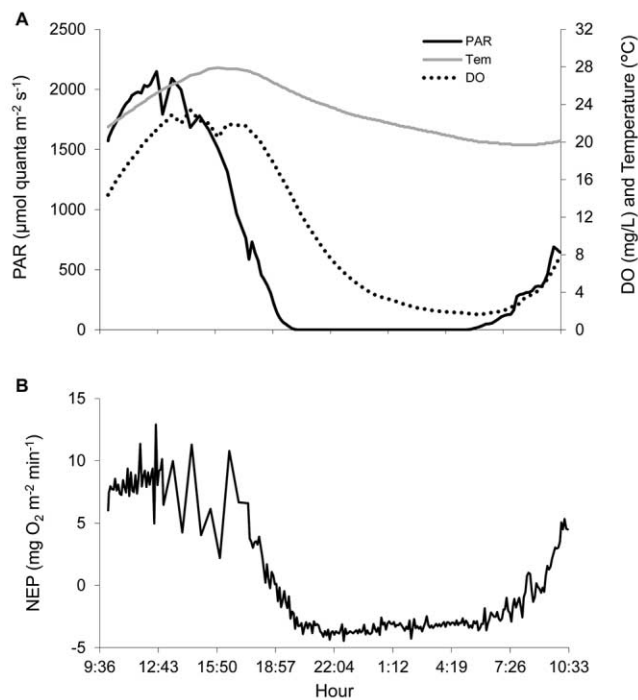


Figure 1. Diel variation of photosynthetically active radiation (PAR), water temperature (Tem), and dissolved oxygen concentration (DO; A) and net ecosystem production (NEP; B) during the 24-h study period.

tion flux (i.e., 0.005/min) resulted in a diel oscillation of instantaneous rates of NEP, which followed the temporal variation in PAR (Fig. 1A, B). NEP at 1200 h was different on each sampling day because of differences in irradiance caused by variation in cloud cover. NEP values were positive (i.e.,  $\text{GPP} > \text{ER}$ ) from 0900 to 1900 h, with a maximum of 12.9  $\text{mg O}_2 \text{m}^{-2} \text{min}^{-1}$  at 1235 h. At night, NEP rates were relatively constant and averaged  $-3.37 \text{mg O}_2 \text{m}^{-2} \text{min}^{-1}$ . Instantaneous rates of GPP ranged from 1.37 to 10.94  $\text{mg O}_2 \text{m}^{-2} \text{min}^{-1}$  during the day and were positively related to PAR following a hyperbolic single rectangular equation ( $r^2 = 0.95$ ), where maximum GPP is 19.13  $\text{g O}_2 \text{m}^{-2} \text{d}^{-1}$  and the PAR at which GPP is  $\frac{1}{2}$  the maximum is 1238  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The daily rate of GPP (6.03  $\text{g O}_2 \text{m}^{-2} \text{d}^{-1}$ ) was higher than the daily rate of ER (4.86  $\text{g O}_2 \text{m}^{-2} \text{d}^{-1}$ ), resulting in a positive daily rate of NEP (1.18  $\text{g O}_2 \text{m}^{-2} \text{d}^{-1}$ ; Fig. 1B) and a GPP:ER ratio of 1.24.

### Diel variation in nutrient uptake

As we observed for nutrient concentrations, diel dynamics of nutrient retention differed between SRP and  $\text{NH}_4^+$ . The percentage of added  $\text{PO}_4^{3-}$  that was retained along the 28-m reach varied between 92.6 and 94.6% (Table 2).  $\text{PO}_4^{3-}$  retention did not show a clear diel pattern and instead decreased gradually over the 24-h study period. Values of  $V_{f-p}$  were consistently high (mean  $\pm$  standard error:  $0.030 \pm 0.002 \text{m/min}$ ; Table 2) and followed a similar pattern to the percentage of retained  $\text{PO}_4^{3-}$ . Diel variation in  $V_{f-p}$  was not associated with either instantaneous GPP or SRP concentration but did increase with temperature ( $r^2 = 0.62$ ) and pH ( $r^2 = 0.68$ ) (Fig. 2A–C). Temperature and pH were correlated with each other ( $r = 0.98$ ) but neither of them was correlated with GPP. The measured  $U_p$  was also consistently high ( $1.94 \pm 0.17 \text{mg P m}^{-2} \text{min}^{-1}$ ) throughout the day but decreased at night (Table 2). Uptake rates of  $\text{PO}_4^{3-}$  estimated from GPP ( $U_{p-GPP}$ ), a proxy of photoautotrophic  $\text{PO}_4^{3-}$  uptake, were almost 2 orders of magnitude lower than  $U_p$  and accounted for <1% of measured  $U_p$  ( $0.022 \pm 0.007 \text{mg P m}^{-2} \text{min}^{-1}$ ; Table 2).

The percentage of added  $\text{NH}_4^+$  retained in the reach was highest during the day (Table 2).  $V_{f-NH4}$  was relatively high and varied almost 1 order of magnitude over the 24-h period (Table 2), following a pattern similar to that of the percentage of  $\text{NH}_4^+$  retained. The  $V_{f-NH4}:V_{f-p}$  ratio averaged  $0.65 \pm 0.17$  and was highest during the day (i.e., daytime range: 0.61–1.33). The daily variation of  $V_{f-NH4}$  was positively associated with instantaneous GPP ( $r^2 = 0.78$ ; Fig. 2F) but not with water temperature,  $\text{NH}_4^+$  concentration, or pH (Fig. 2D–E). Values of  $U_{NH4}$  also varied by an order of magnitude over the 24-h period and followed a daily pattern similar to that of  $V_{f-NH4}$  (Table 2). The uptake rate of  $\text{NH}_4^+$  estimated from GPP ( $U_{NH4-GPP}$ ), as a proxy of photoautotrophic  $\text{NH}_4^+$  uptake, was highly variable throughout the day and followed the same pattern as  $U_{NH4}$  (Table 2). On average,

Table 2. Diel variation of metabolic and uptake metrics. Data are from measurements done during 6 slug additions conducted over a 24-h study period. GPP = gross primary production,  $\text{PO}_4^{3-}$  = phosphate,  $\text{NH}_4^+$  = ammonium,  $\text{NO}_2^-$  = nitrites,  $\text{NO}_3^-$  = nitrates.

Metric	Time of the day when slug additions were conducted					
	1000	1500	1900	0300	0700	1000
GPP ( $\text{mg C m}^{-2} \text{ min}^{-1}$ )	4.10	3.17	0.94	0.00	0.52	2.90
$\text{PO}_4^{3-}$ retained from added (%)	94.2	94.3	94.6	93.8	92.9	92.6
$\text{PO}_4^{3-}$ demand ( $V_{f-P}$ , $\text{m/min}$ )	0.036	0.036	0.033	0.026	0.023	0.023
$\text{PO}_4^{3-}$ uptake from slug addition ( $U_P$ , $\text{mg P m}^{-2} \text{ min}^{-1}$ )	2.325	2.409	2.167	1.725	1.464	1.527
$\text{PO}_4^{3-}$ uptake from GPP ( $U_P - \text{GPP}$ , $\text{mg P m}^{-2} \text{ min}^{-1}$ )	0.039	0.031	0.009	0.000	0.005	0.028
$U_P - \text{GPP} : U_P$	0.017	0.013	0.004	0.000	0.003	0.018
$\text{NH}_4^+$ retained from added (%)	97.8	93.9	69.2	61.9	47.5	79.6
$\text{NH}_4^+$ demand ( $V_{f-NH_4}$ , $\text{m/min}$ )	0.048	0.036	0.013	0.009	0.006	0.014
$\text{NH}_4^+$ uptake from slug addition ( $U_{NH_4}$ , $\text{mg N m}^{-2} \text{ min}^{-1}$ )	0.859	0.518	0.552	0.194	0.080	0.169
$\text{NH}_4^+$ uptake from GPP ( $U_{NH_4} - \text{GPP}$ , $\text{mg N m}^{-2} \text{ min}^{-1}$ )	0.305	0.236	0.070	0.000	0.038	0.215
$U_{NH_4} - \text{GPP} : U_{NH_4}$	0.355	0.456	0.127	0.000	0.478	1.276
$\text{NO}_2^-$ obtained from $\text{NH}_4^+$ -N added (%)	58.3	10.3	7.7	11.5	13.6	70.0
$\text{NO}_3^-$ -N mass lost (%)	6.4	0.9	1.0	1.7	6.8	5.5

the contribution of  $U_{NH_4 - GPP}$  to measured  $U_{NH_4}$  accounted for 45% of measured  $U_{NH_4}$  ( $0.0173 \pm 0.051 \text{ mg N m}^{-2} \text{ min}^{-1}$ ; Table 2).

We added N into the stream as  $\text{NH}_4^+$ , but we found that background concentration of  $\text{NO}_2^-$  tended to increase (Fig. 3A, B) and concentration of  $\text{NO}_3^-$  tended to decrease (Fig. 4A) as the solution passed through the downstream end of the reach. These patterns were not observed for samples collected upstream of the reach during the additions, where concentrations of these compounds remained constant. The relative increase in  $\text{NO}_2^-$  concentration varied among the different slug additions (Table 2, Fig. 3C). The resulting increase in  $\text{NO}_2^-$  mass accounted up to 70.0% of the

added mass of  $\text{NH}_4^+$ , with the highest values measured at 1000 h each day (Table 2, Fig. 3C). The relative decreases in  $\text{NO}_3^-$  concentration also varied among the different slug additions but was always  $\leq 6.8\%$  (Table 2, Fig. 4C). Higher percentages of  $\text{NO}_3^-$  mass reduction occurred at 0700 and 1000 h, similar to the diel pattern of the increases in  $\text{NO}_2^-$ . Decreases in  $\text{NO}_3^-$  concentration during the additions resulted in declines in the DIN:SRP ratio (Fig. 4B).

### Characterization of primary producers

The reach hosted 13 species of emergent and submerged macrophytes and filamentous algae. The stream channel

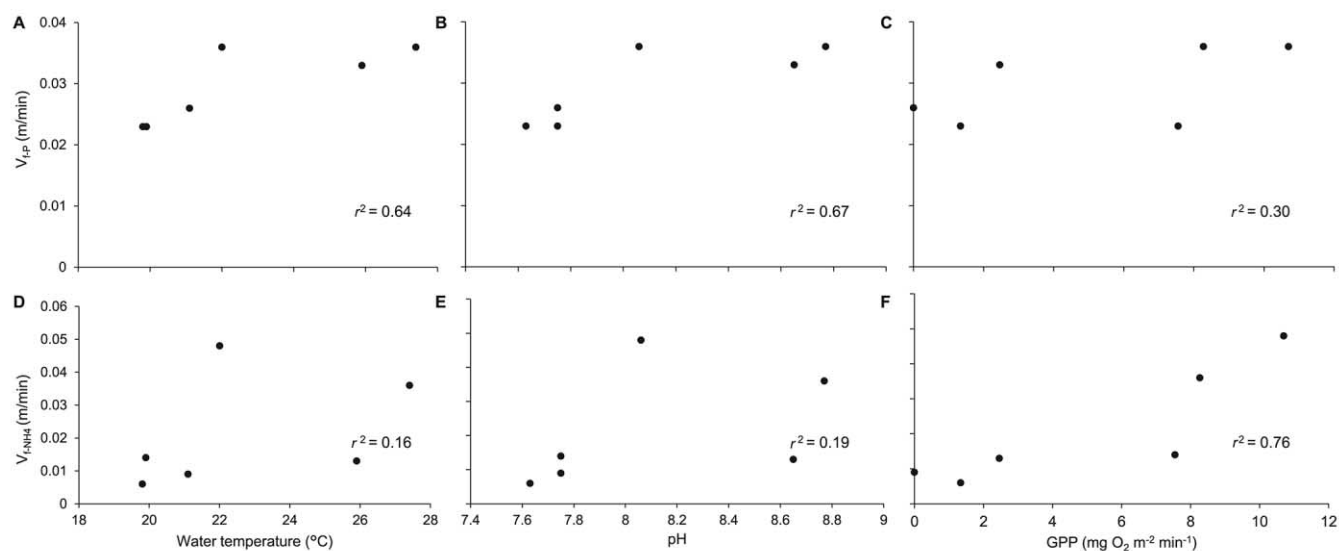


Figure 2. Linear regressions of nutrient uptake efficiency ( $V_{f-P}$  and  $V_{f-NH_4}$ ) with water temperature (A and D), pH (B and E), and gross primary production (GPP, C and F). Linear regression coefficients are indicated for each relationship.

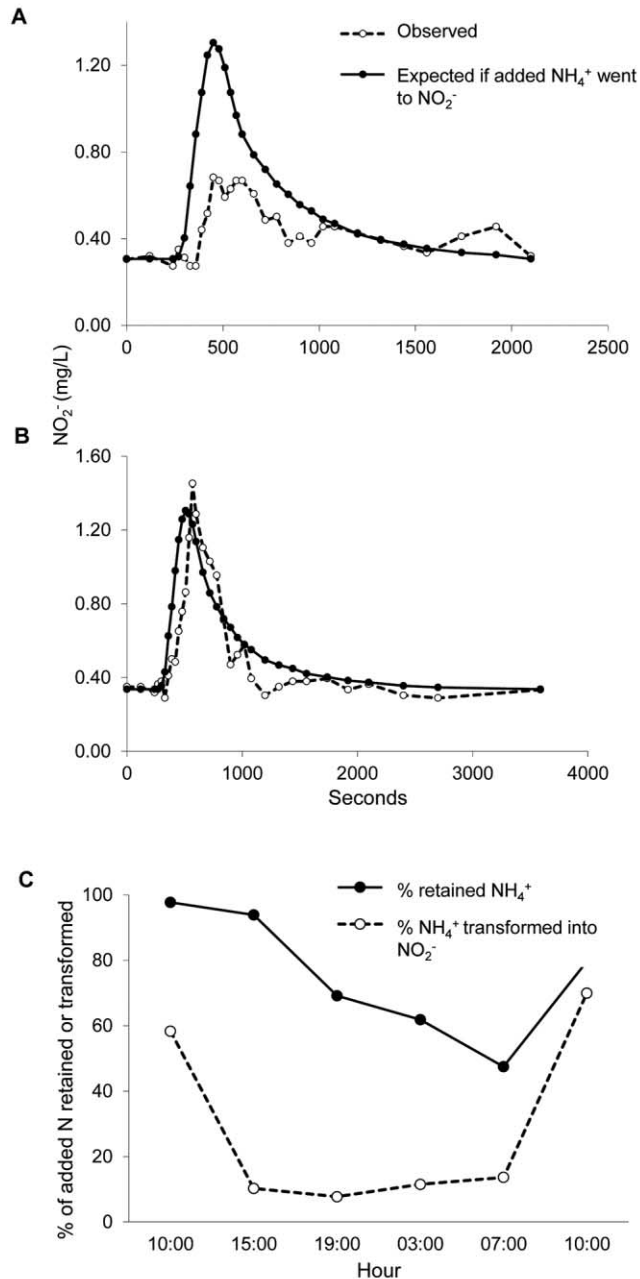


Figure 3. Observed and expected nitrite ( $\text{NO}_2^-$ ) concentration if all added ammonium ( $\text{NH}_4^+$ ) went to  $\text{NO}_2^-$  through nitrification in the additions at 1000 h of the 1<sup>st</sup> day (A) and 2<sup>nd</sup> day (B). Percentage of total  $\text{NH}_4^+$  retained and percentage of  $\text{NH}_4^+$  transformed into  $\text{NO}_2^-$  during the additions (C).

area covered by macrophyte stands was 177 m<sup>2</sup>, which was 45% of the total reach surface. Macrophytes were dominated by the emergent species *Rorippa nasturtium-aquaticum* (72% of the total biomass), followed by another emergent plant, *Eleocharis* spp. (9% of the total biomass). Submerged vegetation (*Elodea ernstae*, *Ceratophyllum demersum*, and the filamentous algae *Cladophora* spp.) accounted for only 7% of the total biomass. The rest of the biomass was repre-

sented by *Stuckenia striata*, *Lolium* spp., *Polygonum hydro-piperoides*, *Leersia* spp., *Lemna* spp., *Hydrocotyle modesta*, *Hydrocotyle ranunculoides*, and the filamentous algae *Spirogyra* spp.

The standing stock of FBOM biomass was lower than that of macrophytes and filamentous algae. FBOM contained less Chl-*a* than macrophytes but an amount similar to filamentous algae (Table 3).

## DISCUSSION

We expected that nutrient uptake would vary on a diel scale that followed the variation of autotrophic metabolism in the highly-productive Las Flores stream. Our results support this expectation for  $\text{NH}_4^+$  but not for  $\text{PO}_4^{3-}$ . Diel variation in  $\text{NH}_4^+$  uptake was strongly associated with GPP, explaining 48% of the total uptake rate. In contrast, the  $\text{PO}_4^{3-}$  uptake rate showed no clear differences between day and night. Moreover,  $\text{PO}_4^{3-}$  uptake estimated from GPP only accounted for a small fraction of the total  $\text{PO}_4^{3-}$  uptake (<2%).

The study stream had high daily rates of both GPP and ER, which were within the range of previously-published values for the Las Flores and other Pampean streams under similar seasonal conditions (Acuña et al. 2011, Rodríguez Castro 2015, García et al. 2017). These values of stream metabolism are much higher than those of headwater streams with well-developed riparian forests in temperate regions (Fellows et al. 2006, von Schiller et al. 2008, Bernhardt et al. 2017). High metabolism is common in open-channel streams, where light does not limit primary production (Bernot et al. 2010, Alnoe et al. 2016).

Metabolism was dominated by autotrophic activity because  $\text{GPP} > \text{ER}$ , probably because of the high abundance of macrophytes, filamentous algae, and benthic biofilm that all had high Chl-*a* content. High PAR irradiance levels caused by the lack of riparian forest, laminar flow, and low discharge are factors that favor the development of dense primary producer assemblages. The high nutrient availability in Pampean streams further contributes to the development and metabolism of the primary producers. In fact, several of the macrophyte species in this stream indicate eutrophic conditions (for instance, *R. nasturtium-aquaticum*, *C. demersum*, *S. striata*, and *Lemna* spp. [Palmer and Roy 2001]). A previous study of the Las Flores stream (García et al. 2017) reported that the benthic assemblage accounted for the largest fraction of GPP (~75% on average), followed by epiphytes and then macrophytes. Thus, it is possible that microbial organisms associated with benthic organic matter may explain a high proportion of the metabolic rates that we observed in the stream.

NEP varied strongly throughout the day, following the diel pattern of PAR variation. The laminar flow caused the reaeration flux to be low (range: 11.3–66.2/d) and, thus, high NEP variation caused large diel oscillations of DO concentrations and pH. Therefore, the stream changes from a



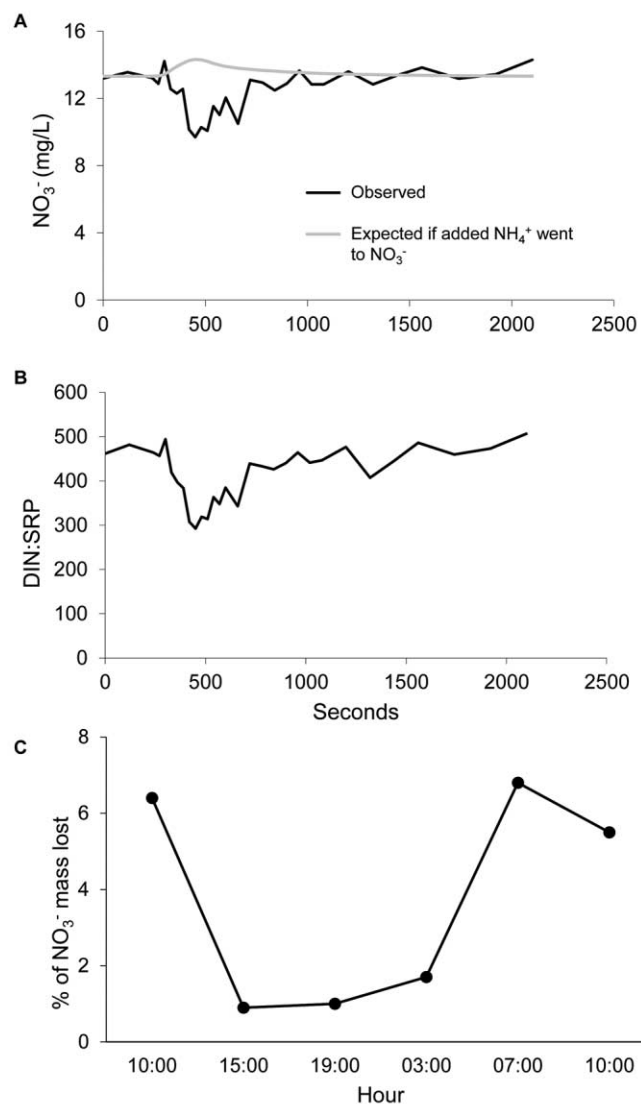


Figure 4. A.—Observed and expected nitrite ( $\text{NO}_3^-$ ) concentration if all added ammonium ( $\text{NH}_4^+$ ) was transformed to  $\text{NO}_3^-$  by nitrification throughout the addition at 1000 h of the 1<sup>st</sup> day. B.—Change in dissolved inorganic N to soluble reactive P (DIN:SRP) throughout the same addition. C.—Percentage of  $\text{NO}_3^-$  mass lost during the additions.

supersaturation state to a nearly-anoxic state over short-term periods. DO saturation (21–285%) was even higher than that reported for intensively-agricultural streams in the US (maximum saturation range: 61–229%; Griffiths et al. 2013). These marked changes in oxygen concentrations may influence the dynamics of in-stream biogeochemical processes associated with nutrient cycling.

The study stream had extremely high P and  $\text{NH}_4^+$  uptake, which makes sense given the high whole-reach metabolic rates. On average, >90 and >75% of the P and  $\text{NH}_4^+$  mass, respectively, added by the slug additions were removed from the water column along the 28-m reach. This removal was

related to high areal uptake rates of both  $\text{NH}_4^+$  and P, which were 2 orders of magnitude higher than those reported by Ensign and Doyle (2006) in their meta-analysis of 87 streams and rivers from different biomes but are consistent with previous measurements done in the Las Flores stream (Feijóo et al. 2011, Rodríguez Castro 2015, García et al. 2017). In addition, values of nutrient demand (expressed as  $V_f$ ) were 1 order of magnitude higher than values estimated by Hall et al. (2013) and Hanrahan et al. (2018) for open-canopy streams (see García et al. 2017 for a more detailed comparison of uptake metrics of Pampean streams with streams elsewhere). These differences indicate high nutrient retention efficiency, which we did not expect because nutrient concentrations are elevated in the Las Flores stream. Previous studies reported saturation of the nutrient demand at high nutrient concentrations. For instance, Newbold et al. (2006) found that nutrient demand of  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  in urbanized streams saturated at concentrations of 0.012 mg total dissolved P/L and 1.02 mg total dissolved N/L, respectively. These nutrient concentrations are below the concentrations measured in the study stream (0.07 mg P- $\text{PO}_4^{3-}$ /L and 12.36 mg DIN/L).

One possible explanation of high nutrient uptake in the Las Flores stream is the presence of relatively-large transient storage zones. Low current velocity in transient storage zones may allow increased interaction between the nutrients in the water column and the microbial assemblages on the benthos and on macrophytes, favoring nutrient uptake (Salehin et al. 2003, Webster et al. 2003, Argerich et al. 2011, Cunha et al. 2018). In a previous study in the Las Flores stream, transient storage was related to flow but not macrophyte biomass, suggesting that hydrology drives the total transient storage (García et al. 2017). Accordingly, our results suggest a negative relationship between  $A_s/A$  and flow, which indicates that transient storage may increase at low velocities. Hence, the size of the transient storage zone in the Las Flores stream may be related to hydraulic conditions and may not depend on the abundance of vegetation or development of hyporheic zones.

Unlike Martí et al. (1994), who reported clear differences in P-uptake rates between day and night, P uptake showed low temporal variation in the Las Flores stream. P demand (expressed as  $V_{f-p}$ ) also showed little variation, but it did

Table 3. Chlorophyll-*a* (Chl-*a*) and organic matter (OM) content in fine benthic organic matter (FBOM), macrophytes, and filamentous algae in the study reach.

	Chl- <i>a</i> (mg/m <sup>2</sup> )	OM (g/m <sup>2</sup> )
FBOM	72 ± 43	288 ± 126
Submerged macrophytes	260 ± 121	29 ± 16
Emergent macrophytes	254 ± 199	28 ± 14
Filamentous algae	79 ± 44	7 ± 4

increase with temperature and pH and was not related to GPP. P-uptake rates were 2 orders of magnitude higher than those estimated from GPP rates during the day and increased to 3 orders higher at night. This finding is consistent with a previous study that reported higher observed uptake rates than those estimated from GPP (O'Brien et al. 2014). Consequently, these results suggest that photoautotrophic activity has only a minor influence on P uptake, even though P is the limiting nutrient in the Las Flores stream.

Divergences between total P-uptake rates and those estimated from GPP indicate that other biogeochemical processes may influence P uptake in the Las Flores stream, overwhelming the diel patterns associated with the influence of GPP. In fact, the dominant contribution of the benthic community to ER rates (García et al. 2017) suggests that heterotrophic P demand by microbial assemblages could have contributed to P uptake in the Las Flores stream. We observed a positive association between P demand (expressed as  $V_f$ ) and temperature, which suggests a metabolic control on the variation of P demand. Few investigators have reported the influence of heterotrophic demand on nutrient retention, especially in autotrophic streams. However, Hoel-lein et al. (2007) found evidence for heterotrophic control on P uptake across biomes when they combined their data with other studies from the literature. Webster et al. (1991) also found that heterotrophic uptake was the primary mechanism of phosphate uptake in southern Appalachian streams.

Other processes that may explain the P uptake are adsorption onto sediments and co-precipitation of  $\text{PO}_4^{3-}$  with  $\text{CO}_3\text{Ca}$ , which can be especially important in calcareous streams like Las Flores (Jarvie et al. 2006, Jalali and Peikam 2013, Corman et al. 2016). Co-precipitation of Ca and  $\text{PO}_4^{3-}$  is associated with an increase of pH in a carbon-carbonate system. Increases in water pH can be caused by the high photosynthetic activity of primary producers (Reddy et al. 1999). However, we did not detect the formation of  $\text{CO}_3\text{Ca}$  precipitates on the leaves and stems of macrophyte species present in our study stream (personal observation, CF). Moreover, we did not observe an association between the daily variation of pH and GPP. Co-precipitation of  $\text{PO}_4^{3-}$  with  $\text{CO}_3\text{Ca}$  is the most cited process (Reddy et al. 1999, Corman et al. 2016) by which P is eliminated from the water column, but other chemical mechanisms can also eliminate P from water, such as the adsorption of  $\text{PO}_4^{3-}$  ions on  $\text{CO}_3\text{Ca}$  that leads to apatite formation (Millero et al. 2001). The typical calcareous layers of the Pampean region can be formed by different mechanisms, such as precipitation from phreatic waters, capillary rise, and pedogenic carbonate leaching (Zárate and Folguera 2009), but not by  $\text{CaCO}_3$  deposition from stream water. Therefore, P adsorption to streambed sediments is the most likely chemical mechanism behind the high P uptake in the Las Flores stream. The almost constant P retention throughout the day supports the occurrence of an abiotic mechanism like P adsorption to sediments.

Unlike P,  $\text{NH}_4^+$  uptake in the Las Flores stream clearly varied throughout the day and was lower at night. This finding is similar to previous studies in which N-uptake rates were greater in daylight than in dark conditions (Martí et al. 1994, Fellows et al. 2006, Johnson and Tank 2009), which occurred because of autotrophic N demand during the day. Moreover,  $\text{NH}_4^+$ -uptake rates were within the range of expected uptake rates based on GPP, suggesting a greater control of primary producers' activity on the uptake of  $\text{NH}_4^+$  than on P. In other studies where uptake rates were estimated from GPP, some authors reported that autotrophic N demand was similar to  $\text{NH}_4^+$ -uptake rate (O'Brien et al. 2014), whereas others observed that both autotrophic and heterotrophic assimilation contributed to N retention (Hall and Tank 2003). Uptake of  $\text{NH}_4^+$  in the Las Flores stream has been associated with autotrophic activity because  $\text{NH}_4^+$  demand (expressed as  $V_f - \text{NH}_4$ ) increased with GPP:ER ratio on a seasonal basis (García et al. 2017). Unlike forested streams, the majority of N uptake in open-canopy streams may result from algal assimilation (Webster et al. 2003, Fellows et al. 2006). Our results and those from a previous study (García et al. 2017) suggest that  $\text{NH}_4^+$  uptake is dominated by autotrophic demand in the Las Flores stream.

Bacterial-mediated processes other than direct heterotrophic uptake can also influence  $\text{NH}_4^+$  retention in the stream. The coupling of  $\text{NH}_4^+$  loss with  $\text{NO}_2^-$  increase during slug additions suggests that nitrification occurred during these additions. However, we did not observe a concomitant increase of  $\text{NO}_3^-$ , indicating that  $\text{NO}_2^-$  was not subsequently oxidized to  $\text{NO}_3^-$ . During nitrification,  $\text{NH}_4^+$  is oxidized to  $\text{NO}_2^-$  by *Nitrosomonas*, and  $\text{NO}_2^-$  is then oxidized to  $\text{NO}_3^-$  by *Nitrobacter*. These microbial chemoautotrophs can be affected differently by factors such as pH and hydraulic retention time (Suthersan and Ganczarczyk 1986, Gee et al. 1990). The activity of *Nitrobacter* can also be reduced when the abundance of *Nitrosomonas* increases (but not vice versa; Gee et al. 1990). Consequently, the accumulation of  $\text{NO}_2^-$  (but not of  $\text{NO}_3^-$ ) could be related to a lower activity of *Nitrobacter* compared to *Nitrosomonas* during the nitrification process.

Nitrification is common in streams and varies substantially across biomes (Peterson et al. 2001, Bernhardt et al. 2002), but it can be especially high in eutrophic streams (Merseburger et al. 2005, Gammons et al. 2011). We calculated that nitrification could explain between 3 and 29% of  $\text{NH}_4^+$  mass retained during additions. These values are within the range of previous estimations in the same stream at a seasonal scale (8–43%; García et al. 2017). Nitrification can be inhibited by light availability in streams (Merbt et al. 2012). During the morning, light was not very intense and DO concentrations were intermediate, and these conditions may explain the higher loss of  $\text{NH}_4^+$  that we observed at 1000 h.

In contrast to  $\text{NO}_2^-$ , we observed that  $\text{NO}_3^-$  concentration decreased during slug additions. It is possible that

the input of additional P during additions alleviated P limitation, dropping the DIN:SRP ratio and enhancing  $\text{NO}_3^-$  uptake. We also observed higher percentages of  $\text{NO}_3^-$  reduction in the morning. Another study that used in-situ high-frequency measurements of  $\text{NO}_3^-$  and DO found a similar diel pattern of  $\text{NO}_3^-$  reduction in a river (Heffernan and Cohen 2010). The authors of that study inferred that there was higher autotrophic assimilation and lower heterotrophic dissimilation of  $\text{NO}_3^-$  throughout the day and estimated that 35% of the denitrification was fueled by photosynthesis that occurred the previous day. Thus, it is possible that the higher percentage of  $\text{NO}_3^-$  reduction observed early in the morning is the result of denitrification in our study site.

To summarize,  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$  retention followed distinct diel patterns in the Las Flores stream.  $\text{NH}_4^+$  retention uptake may vary in association with autotrophic metabolism, and  $\text{PO}_4^{3-}$  retention remained almost constant throughout the day. Hence, our results indicate that the diel variation of autotrophic demand does not always drive nutrient uptake in open-canopy streams, and that other dissimilatory and abiotic mechanisms must be considered to explain the whole pattern of diel nutrient retention. In addition, our study highlights the need to explore variation in uptake over shorter time periods and beyond the more frequently studied seasonal shifts. Nutrient uptake is usually measured with a single nutrient addition measurement during daytime and then extrapolated to the whole day. This practice may lead to overestimated nutrient uptake rates and, thus, overestimated importance of nutrient processing capacities of fluvial systems.

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Author contributions: EM, FS, CF, and AG conceived the study and did the field sampling together with NF, PG, AT, and CRC. AT and CRC prepared and coordinated the sampling. Processing of samples and analysis of results was done by CV, CR, MLG, NF, and PG. FS and EM developed the method for estimating nitrification. FS, EM, and AG adapted methods of nutrient addition and of estimation of metabolism and biofilm biomass to the stream conditions. EM and FS performed data calculation and statistical analysis. EM, FS, and CF wrote the paper with significant contributions from CV.

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