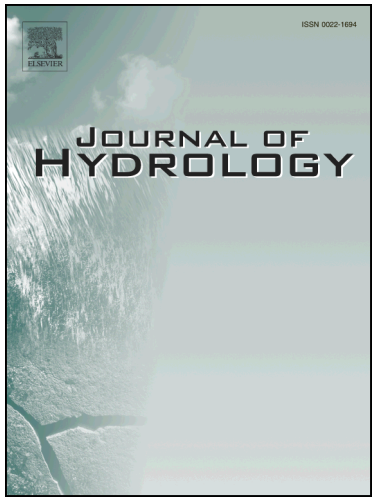

Research papers

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PII: S0022-1694(18)30745-5
DOI: https://doi.org/10.1016/j.jhydrol.2018.09.051
Reference: HYDROL 23148

To appear in: *Journal of Hydrology*

Received Date: 5 May 2018
Revised Date: 11 September 2018
Accepted Date: 15 September 2018

Please cite this article as: Feijoó, C., Messetta, M.L., Hegoburu, C., Vázquez, A.G., Guerra-López, J., Mas-Pla, J., Rigacci, L., García, V., Butturini, A., Retention and release of nutrients and dissolved organic carbon in a nutrient-rich stream: a mass balance approach, *Journal of Hydrology* (2018), doi: https://doi.org/10.1016/j.jhydrol.2018.09.051

Retention and release of nutrients and dissolved organic carbon in a nutrient-rich stream: a mass balance approach

Claudia Feijoó[a,*], María Laura Messetta[a], Cecilia Hegoburu[a], Alicia Gómez Vázquez[a], José Guerra-López[a], Josep Mas-Pla[b], Laura Rigacci[a], Victoria García[a], Andrea Butturini[c]

[a] Programa Biogeoquímica de Ecosistemas Dulceacuícolas (BED), Instituto de Ecología y Desarrollo Sustentable (INEDES, CONICET-UNLu), Rutas 5 y ex 7, (6700) Luján, Argentina. clasife@yahoo.com.ar

[b] Institut Català de Recerca de l'Aigua (ICRA), c/ Emili Grahit 101, 17003 Girona, and Grup de Recerca GAiA-Geocamb, Dept. Ciències Ambientals, Universitat de Girona, Spain.

[c] Departament d'Ecologia, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, 08028 Barcelona, Spain.

[*] Corresponding author

Abstract

The relevance of fluvial systems to process nutrients and carbon is widely accepted, but their role as sinks and sources of nutrients and dissolved organic carbon (DOC) is still under discussion especially in non-forested and highly productive streams. In this study, we used a mass balance approach at a reach scale in a Pampean stream to elucidate the major sources of water, nutrients and DOC as well as to determine net in-stream retention efficiencies of nutrients and DOC under different hydrological conditions.

We measured conductivity, conservative ions (chloride and calcium), soluble reactive phosphorus (SRP), nitrate (NO_3), nitrite (NO_2), ammonium (NH_4) and DOC at the end-point of a reach of Las Flores stream (site A), at two upstream tributaries (B1 and B2), and at each potential hydrological contributors to stream flow (groundwater, overland and subsurface flows, and rainfall). In addition, we monitored one storm event where we collected samples during the rising and the recession limb of the hydrograph.

Stream flow originated from groundwater (≈50%), upstream tributaries (B1 and B2) at baseflow, whereas overland flow contributed >20% during high flows. During baseflow, groundwater provided NO_3 to stream water, while B2, which received a point input of a dairy industry, was the main source of SRP and NH_4. Conversely, SRP and NH_4 were provided by B1, overland flow and subsurface flow during high flows. Overland flow also contributed DOC during high flow periods. Mass balance estimates revealed that the reach acts as a source of DOC, SRP and NO_3 (21.4, 37.4 and 53.5 % mean net in-stream release, respectively) and a sink of NH_4 (-36.8 % mean net in-stream retention). Relevant in-stream processes may be nutrient uptake (as in the case of SRP and NH_4) and biotic production (DOC), as well as decomposition (SRP) and nitrification (NH_4) in this Pampean stream. Our results stress the relevance of nutrient and DOC generation processes within the channel in non-forested and highly productive streams.

Keywords

Phosphorus

Nitrogen

In-stream processing

Groundwater

Storm events

1. Introduction

Stream water chemistry depends on runoff sources that contribute to stream flow and on several hydrological and biogeochemical processes operating within the fluvial channel (Hooper et al., 1990; Stream Solute Workshop, 1990; Valett et al., 1996). Many dissolved compounds are carried by the streams, but only a few are biologically important, including phosphorus, nitrogen and carbon (Cole et al., 2007; Mulholland and Webster, 2010). Phosphorus and nitrogen are key drivers of biological productivity in streams (Mulholland and Webster, 2010). Dissolved organic

carbon (DOC) is the major pool of reduced carbon in transport from the continents to the ocean though fluvial networks, and represents the primary substrata for microbial communities in freshwaters (Findlay, 2010). Phosphorus, nitrogen and carbon greatly influence the metabolic activity of aquatic systems; therefore, determining their origins and transformation processes is essential to understand stream function.

River networks have been identified as active conduits where solutes are produced or removed. Hence, streams may behave as either sinks or sources of nutrients and DOC (Kaushal et al., 2014; Bernal et al., 2015; Casas-Ruiz et al., 2017). However, some studies showed that streams can also act as passive pipes, with little nutrient and DOC transformation (Bernal et al., 2015; Kothawala et al., 2015; Casas-Ruiz et al., 2017). Therefore, the role of fluvial systems on nutrient and DOC biogeochemical cycles is still no clear. Particularly, a better understanding on the actual nutrient and DOC sources and on the behavior of streams (reactors vs. passive pipes) is critical for anticipating downstream alterations of ecosystem function and proposing measures to manage excessive exports. This information is also essential to predict future scenarios based on climate change predictions for each particular region of the world. To explore solute processing in fluvial systems (i.e. retention/release), the mass balance method has been applied using direct field measurements at the stream reach scale (Kaushal et al., 2014; Bernal et al., 2015; Ejarque et al., 2017; Wollheim et al., 2017) or through mathematical catchment modeling (Lyon et al., 2010; Destouni et al., 2010). Most of our knowledge on nutrient and DOC processing is based on studies performed in forested streams, but solute processing in non-forested and highly productive streams is still poorly explored (but see Grimm, 1987). Studies in open fluvial systems are then needed to understand if all streams behave in the same way, or conversely, the capacity to process solutes depends on the structure of microbial communities.

Pampean stream peculiarities provide the opportunity to increase our comprehension on in-stream biogeochemical processes. These streams originate in wetland areas and may be mainly

fed by the shallow unconfined aquifer (Sala et al., 1983). They are characterized by low velocities and high irradiance due to the lack of riparian forest, and show elevated metabolic activity (García et al., 2017). Nutrient levels are high in relation to streams from other regions of the world (see Feijoó and Lombardo, 2007, and Amuchástegui et al., 2016, for a comparison), and such levels cannot solely be attributed to agricultural activities (Feijoó and Lombardo, 2007). Soluble reactive phosphorus (SRP) concentration in Pampean streams shows no relation with any type of land use including the agricultural cover (Amuchástegui et al., 2016). Nevertheless, Quaternary loess deposits contain volcanic glasses rich in phosphorus (Morrás, 1993; Iriodo and Kröling, 1995), and the weathering of volcanic material could supply phosphorus to surface waters through superficial and subsurface flow (Crews et al., 1995). In the case of nitrogen, nitrate (NO_3) concentration in stream water increases with agricultural land cover in basins of the region, but NO_3 levels are lower than might be expected considering the dominant agricultural use (Feijoó and Lombardo, 2007; Amuchástegui et al., 2016). High NO_3 levels were also found in groundwater from farm and urban areas compared to less impacted areas in the region (Momo et al. 1999; Carbó et al. 2009), suggesting that the shallow aquifer itself can be a source of NO_3 to stream water.

DOC levels are also elevated in Pampean streams (6.42 ± 2.79 mg/l; Messetta et al., 2018) in relation to streams and rivers of USA (McKnight et al., 2001; Arreghini et al., 2005; Raymond and Saires, 2010), but they are within the range reported for other streams of the world (Dawson et al., 2008; Knorr, 2013; Lyon et al., 2010; Laudon et al., 2011; Kamjunke et al., 2015). Soils in the region are rich, with a high organic content (3-3.9 % in northeast Pampa) that may derive from the decomposition of herbaceous vegetation typical of the region (Sainz Rozas et al., 2011). So, it is expected that overland flow washes the organic matter accumulated in the top of the soils and releases it to the stream as DOC during storm events (Bernal et al., 2002; Messetta et al., 2018). DOC could also derive from the decomposition of dense macrophyte communities (and associated

epiphyton) that are common in streams of the region (Giorgi et al., 2005; Catalán et al., 2013; Messetta et al., 2018). In addition, epiphytic and benthic biofilms could generate DOC through biological production (Romera-Castillo et al., 2010). Anthropogenic point sources should also provide nutrients and DOC to Pampean fluvial systems; however, they are relatively infrequent in agricultural headwaters (Feijoó and Lombardo, 2007).

Previous research in Pampean streams showed that despite elevated nutrient levels, SRP and ammonium (NH_4) uptake are high and similar to those observed in pristine streams (García et al., 2017). Therefore, these streams may act as sinks for SRP and NH_4. In the case of NO_3, it was observed that NO_3 demand is typically lower than SRP and NH_4 demand (Ensign and Doyle, 2006) and that NO_3 removal efficiency in streams declined with increased NO_3 loading (Mulholland et al., 2008). NO_3 uptake was not measured in streams of the region, but considering that NO_3 levels are elevated, it is expected that Pampean streams behave as passive pipes for NO_3.

The time that DOC spends in the system (i.e. the residence time) may be one of the main factors determining in-stream DOC processing (Battin et al., 2008; Casas-Ruiz et al., 2016). Low flow velocities in Pampean streams allow the development of dense epiphytic and benthic biofilms (Giorgi et al., 2005) and may provide physical opportunities for microorganisms to metabolize organic carbon (Battin et al., 2008). This suggest that Pampean streams may act as active reactors, promoting active retention of DOC.

In this study, a mass balance approach has been used to analyze in-stream transport, retention and release of nutrients and DOC at reach-scale to determine whether the stream acts as a passive pipe, source or sink of phosphorus, nitrogen and DOC. We aimed to: (i) identify and quantify the relevance of different end-members as inputs of soluble reactive phosphorus (SRP), NO_3, nitrite (NO_2), NH_4 and DOC to the stream under different hydrological conditions; and (ii) estimate net in-stream SRP, NO_3, NH_4 and DOC retention/release efficiencies at the reach. Our hypothesis is that

SRP is mainly supplied by overland flow, while DOC is provided by overland flow and in-stream production and NO_3 by groundwater. We also hypothesized that the reach acts as a net sink of SRP, NH_4 and DOC, while it behaves as a passive pipe for NO_3. We predict that NO_3 concentration will increase under baseflow conditions, while SRP and DOC levels will be higher under stormflow conditions.

2. Study area

The study was conducted in Las Flores stream, a third-order tributary of the Luján river (NE Buenos Aires province, Argentina) that is considered representative of many Pampean streams (Giorgi et al., 2005) (Fig. 1). The predominant land use in the basin is cropland (77%; mainly soybean and corn), followed by natural and implanted pastures (11%), natural vegetation (9%), implanted forests (3%) and urban (0.6%) (Amuchástegui et al., 2016).

Fertile soils of the Pampean region were formed by loess deposition during the Quaternary. Climate is temperate, with rainfall evenly distributed throughout the year (between 200-1200 mm) and a mean annual temperature between 13°C and 17°C (Mateucci, 2012). Stream velocity is low (generally < 0.05 m/s) and flow is laminar in Las Flores due to the gentle gradient. The stream bed consists of fine sediments (primarily silt and clay) without sand or pebbles. High nutrient levels favored the development of rich and dense macrophyte communities that act as substrate for biofilms and food and refuge to macroinvertebrate and fishes (Giorgi et al., 2005; Ferreiro et al., 2014).

3. Materials and Methods

3.1. Field survey

We selected a 2.2-km stream reach of Las Flores, which originates at the confluence of two tributaries (B1 and B2) with different subcatchment areas (18.6 km^2 for B1 and 13.3 km^2 for B2) but similar land use (mainly agriculture) (Fig. 1). B2 receives the effluent of wastewater treatment

plant of a dairy industry that produces cheese and milk jam and that is situated 1 km upstream of the beginning of the reach.

Prior to the beginning of the study, a preliminary sampling was conducted to identify the inorganic conservative hydrochemical tracers that better discriminate the different end-members. We collected samples from the stream, their tributaries and all end-members to characterize the major ions (chlorides, sulfates, carbonates, bicarbonates, calcium, magnesium, sodium and potassium) and nutrients (SRP, NO_3, NO_2 and NH_4) according to the methods of APHA (2005). Results of the preliminary sampling indicated that chloride (Cl) and calcium (Ca) were the conservative ions that allowed the best end-member discrimination (Fig. A; Supplementary information).

A periodic sampling was then conducted from 2011 to 2015 at both tributaries (B1 and B2) just before their confluences, and downstream at the end of the reach (sampling site A) (Fig. 1) under different hydrological conditions. Site A was sampled 40 occasions, and sites B1 and B2 could only be sampled 32 occasions due to bad weather conditions. We measured water temperature, pH, dissolved oxygen concentration and electrical conductivity (EC) in B1, B2 and A with a Hach multiprobe (HQ40D). On each sampling occasion, we gathered water samples and measured stream cross-section area and water velocity at the stream (A) and both tributaries (B1 and B2). Velocity data was recorded with a multiprobe anemometer (Schiltknecht MiniAir20), and then used to determine stream discharge by the velocity-area method (Gordon et al 1992).

In July 2013, a levelogger (Solinst 3001 LT F15/M5) was installed at the end of the reach (site A) to register water depth at a 5minute interval. An empirical relationship between stream discharge and water level measured by the levelogger was calculated to obtain a continuous register of discharge from stream water depth:

$$Q = 0.0862 \times h^{2.3662} \quad \text{(eq. 1)}$$

where *Q* is discharge (in l/s) and *h* is stream water level (in m) (R^2 = 0.94, N = 19).

On each sampling occasion, we also sampled water from all potential hydrological sources (also called end-members; Hooper et al., 1990) to the total stream flow. We installed a pluviometer in an open area situated 50 m apart from the end of the reach to collect rainwater (R) during each storm event. Overland flow (OF) was collected using a PVC gutter (2 m long) situated parallel to the channel and connected by a plastic tube to a 20 l tank. The gutter was protected with a grey polycarbonate sheet to avoid the entrance of rainwater. Soil water (thereafter, subsurface flow or SF) was sampled in a tank (0.15 m diameter and 0.30 m long) with slots in the upper 0.15 m, which was located 0.50 m apart from the channel. We buried the tank with the slots located just over the limit between the organic-rich surface soil and the clay subsoil. In this tank, rainwater percolating through the superficial horizons and flowing from the subsoil to the stream was gathered. SF samples were extracted from the tank using a peristaltic pump. Riparian groundwater (GW) was sampled from a piezometer situated 1 m apart from the stream channel. The piezometer consisted of a PVC tube (0.11 m diameter and 4 m depth) with slots in the last 3 m. GW was sampled with a peristaltic pump, and the piezometer was purged with a minimum of 3 piezometer volume prior to sample collection. In July 2013 we installed a levelogger into the well for measuring water table head. The levelogger was barometrically compensated with a barologger Edge (Solinst 3001 LT FE/M15). Using continuous data from leveloggers, we estimated the hydraulic gradient as the difference between the water table level at the piezometer and the stage in the river, divided by the distance between them (Elosegui and Butturini, 2009). GW was sampled at each sampling occasion, except under extreme flow events when stream water flooded the piezometer. R, OF and SF end-members could only be sampled after rain events.

Additionally to the periodic sampling, a storm event was monitored in detail in July 2015 by collecting stream water samples during the rising and the recession limbs of the hydrograph.

3.2. Sample analysis

Water samples were collected in polyethylene bottles, stored in an ice chest and immediately transported to the laboratory, where they were filtered through pre-ashed GF/F glass fiber filters. Nutrient samples were preserved at 4°C until analysis, while DOC samples were collected in amber colored glass bottles (to minimize light exposure) and then acidified with HCl 10% to achieve a final pH=2. SRP and NH_4 concentration were measured within one day of collection, using the molybdate-ascorbic method and the indophenol blue method, respectively (APHA, 2005). NO_3 and NO_2 concentration were determined in the samples with a FUTURA Autoanalyzer (Alliance Instruments, Frepillon, France) through a reaction with sulfanilamide with a previous Cu-Cd-reduction for NO_3 (APHA, 2005). DOC concentration was measured in acidified samples using a Shimadzu TOC Analyzer VCSH. Cl and Ca concentrations were measured using titration methods (argentometric method and the EDTA method, respectively; APHA, 2005).

3.3. Mass balance and data analysis

An input-output solute mass balance at the reach scale was performed when all potential hydrological end-members were sampled. This estimation was possible for a total of 14 surveys (11 under baseflow and 3 under high flow conditions). In the remaining surveys mass balances were not applied because, in some occasions, bad weather conditions or flooding prevented sampling of B1, B2, GW, OF and/or SF.

The input-output water and solute mass balance is a simple mixing model approach similar to that is widely used in small catchment hydrology research (e.g., Hooper et al., 1990; Christophersen and Hooper, 1992, among others). At baseflow, we assumed that the inputs were the sum of water and solute masses from the two tributaries (B1 and B2) and the riparian groundwater (GW). Under high flow conditions, we added the overland flow (OF) as additional end member. The output consisted of the water and solute masses at the downstream sampling point

(A). The inclusion of other potential end-members (SF and R) to the mass balance model did not improve the fit between the observed discharge and the discharge estimated by the model (see below). Therefore, we assumed that SF and R contributions to stream flow was negligible.

In the input-output balance, the discharge at the output (sampling point A; Q_A) is the sum of the input water fluxes (Q_{B1}, Q_{B2}, Q_{GW}, Q_{OF}). Therefore, the water mass balance is:

$$Q_A = Q_{B1} + Q_{B2} + Q_{GW} + Q_{OF} \quad \text{(eq. 2)}$$

Q_A, Q_{B1} and Q_{B2} were measured in the field meanwhile Q_{GW} and Q_{OF} were unknown. Under baseflow condition, the only unknown parameter in eq. 2 was Q_{GW}. Under high flow conditions, contribution of OF could not be ignored, and there were two unknown parameters (Q_{GW} and Q_{OF}). Therefore, it is indispensable that the mass balance equation included the information of one additional solute conservative tracer (C) from each potential hydrological end-member:

$$C_A Q_A = C_{B1} Q_{B1} + C_{B2} Q_{B2} + C_{GW} Q_{GW} + C_{OF} Q_{OF} \quad \text{(eq. 3)}$$

Under high flow conditions, we tested different combinations of hydrological tracers (Cl, Ca and EC). The best fit between measured discharge at point A and estimated discharge with the model (eqs. 2 and 3) were obtained with EC and Ca as conservative tracers. In consequence, water and solute contribution of each end-member were estimated using these tracers.

Solving the system of equations 2 and 3, it is possible to estimate the unknown Q_{GW} and Q_{OF} parameters. Under high flow conditions (three cases), overland flow could not be omitted. Therefore, this additional unknown could be estimated with a system of three equations and using EC and Ca as conservative solutes (see below).

3.4. Net in-stream solutes retention/release efficiencies

Once the hydrological contribution of each hydrological end-member was estimated, the expected concentration (C_{exp}) of the reactive solutes (SRP, NO_3, NO_2, NH_4 and DOC) in stream water was

estimated. C_{exp} is the expected stream solute concentration at the output of the reach (point A) obtained from the conservative mixing of the input sources:

$$C_{exp} = \frac{\sum_{i=1}^{x} C_i Q_i}{Q_A} \text{(eq.4)}$$

where Q_A is the same of eq. 2 and x is the number of potential water sources (x=4 in this case; see equation 2). Once C_{exp} is estimated according to eq. 4, a net in stream retention/release efficiency of solute C ($\eta_{(y)}$; in %) was calculated according to the following formula (Butturini et al., 2016):

$$\eta_{(y)} = \frac{(C_{meas} - C_{exp})}{C_{exp}} \times 100 \qquad \text{(eq. 5)}$$

where C_{meas} is the measured concentration of the solute. $\eta_{(y)}$ is independent of water flow and it can be used to compare the solute C retention/release efficiency across diverse hydrological conditions. $\eta_{(y)} < 0$ indicated a net retention of the solute C. The opposite relationship indicated a net release. To reduce the risk of overestimation/underestimation of net in-stream solutes retention/release, $\eta_{(y)}$ values were corrected using water EC values as conservative tracer (Pellerin et al., 2008). First, the in-stream balance efficiency of EC ($\eta_{(EC)}$) was estimated according to equation 5, and then $\eta_{(y)}$ values were then compared to the $\eta_{(EC)}$ values. Corrected $\eta_{(y)}$ values (named $\eta'_{(y)}$) were estimated according to the methodological criteria detailed in the Supplementary information. In addition, we estimated the net uptake/release ($Nup_{(y)}$; in ug/m · s) of nutrients and DOC according the formula:

$$Nup(y) = \frac{(C_{meas} - C_{exp})Q_A}{x} \text{ (eq. 6)}$$

where x is the distance between the sampling point A and the point of the confluence of the tributaries B_1 and B_2 (x=2210 m).

Differences in chemical characteristics among end-members were evaluated using one-way ANOVA. Relationships among variables and water temperature and discharge were evaluated by

the Pearson's correlation coefficient (r) with simple linear models. Water temperature was considered as a possible driver of nutrient and DOC transformations given its association with biological processes and seasonality. Different models were applied to analyze the relationship between stream flow and the relative hydrological contribution of each end-member. Models that showed the best fit between observed and predicted values were the exponential model for GW and the logarithmic model for B1, while B2 showed not significant relationship with flow. Multiple linear models were used if a variable was significantly related to both water temperature and discharge. Relationships are considered significant at $p<0.05$. Variables that did not meet the assumption of normality were log transformed.

4. Results

4.1. Hydrological characterization: main channel, tributaries and groundwater

Stream flow at site A was highly variable and increased considerably during storm events (Fig. 2). Mean streamflow registered during the recording period (July 15, 2013 – August 5, 2015) was 548±7 L/s (mean±SD), ranging from 6 to 72,209 L/s, and median of 74 L/s. According to the hydrograph shape, most of the baseflow presented a magnitude below 100 L/s. Stream discharge was unrelated to water temperature ($r=0.46$, $p>0.05$). Focusing on tributaries, flow in B1 was higher and slightly more variable (89±2L/s, mean±SE) than in B2 (64±1 L/s).

During both baseflow and stormflow conditions, groundwater level was clearly higher than stream level (Fig. 2). The hydraulic gradient between the aquifer and the stream was always positive indicating the perennial gaining condition of the stream (mean gradient = 0.146±0.000, median = 0.149). Near 78% of the time, hydraulic gradient varied between 0.14 and 0.18 m, while only 6% of the time the level difference was lower than 0.1 m (Fig. 2). During the peak of storm episodes the level difference was reduced to near 0.05 m or less, but the difference was never reversed, indicating that even under high flows, stream water did not recharge the surrounding riparian

groundwater. Nevertheless, the magnitude of the flow from the water table aquifer to the stream may vary about three times between baseflow condition and peak flow events, considering that during recession periods the gradient will be above 0.15 and during floods around 0.05 (and that the recharge will vary accordingly).

4.2 Chemical characterization

Hydrological end-members showed distinct chemical signatures. Riparian groundwater had elevated EC and significantly higher NO_3 and Ca concentrations than the other end-members, while SRP, NH_4 and DOC levels were significantly lower (Fig. 3). In contrast, overland and subsurface flows showed similar water chemistry, with higher NH_4 concentration and lower EC, NO_3 and Ca levels compared to the other end-members. Rainwater showed low nutrient and DOC levels, yet it showed the highest (but more variable) NH_4 concentration among end-members. B1 and B2 had similar levels of NO_3 and DOC; however, EC and SRP and NH_4 concentrations were significantly higher in B2 due to the point input of dairy effluents.

Nutrient and DOC concentrations in site A was typically in-between those measured in the two tributaries and in groundwater (Fig. 3 and Fig. B in Supplementary information). Mean concentrations in A were 0.37 mg SRP/l, 3.50 mg $N\text{-}NO_3$/l, 0.06 mg $N\text{-}NO_2$/l, 0.07 mg $N\text{-}NH_4$/l, and 6.42 mg DOC/l. SRP concentration was not related to stream flow in A, while it showed opposite trends in both tributaries, increasing in B1 and decreasing in B2 with increasing flow. Both NO_3 and NO_2 showed significant and negative relationships with flow in A, B1 and B2, while NH_4 was only significantly and negatively related to flow in B2. Finally, DOC concentration positively increased with flow in A and both tributaries (Table 1).

4.3. Relative contribution of the different end-members

Under baseflow conditions, 53% of stream water flow was provided by groundwater, while B1 and B2 supplied the other half with a similar contribution from each tributary (0.20 and 0.27,

respectively) (Table2). Under stormflow conditions, end-members contributing to stream flow were the same plus OF. Mean contribution of GW, B1, B2 and OF to stream flow was, respectively, 7, 44, 27% and 23% (Table 2).

Groundwater contribution ranged widely between 3% and 71% and it was inversely and significantly related to discharge ($r=0.92$, $p<0.00001$) (Fig. 4). Larger contributions (> 55%) were observed during low flow periods (discharge < 70 L/s). In contrast, groundwater contribution at high discharge episodes clearly decreased to less than 11%. With respect to the two tributaries, B1 contribution was significantly related to discharge ($r = 0.82$, $p = 0.0003$), ranging from less than 25% at baseflow to more than 35% at high discharges. On the contrary, contribution of B2 averaged 27±6% (mean±SD) and it was unrelated to discharge ($r=0.24$, $p>0.05$). Overland flow emerged as a significant water input (23±1%) only under high flow conditions (Q > 400 L/s) (Fig. 4).

4. 4. Net in-stream solutes retention/release efficiencies

Mass balance estimates revealed that the study reach acted as a source of SRP and NO_3 during base/high flow, and as a source of DOC and a sink of NH_4 during baseflow. Pattern for NO_2 was unclear and highly variable (Fig. 5). In more detail, $\eta'_{(SRP)}$ averaged 29.6 ± 23%. SRP net release was detected in 12 surveys while SRP net retention was only detected in one survey. Variability of $\eta'_{(SRP)}$ was unrelated to discharge ($r=0.039$, $p>0.05$) and positively related to temperature ($r=0.38$, $p<0.025$) and $\eta'_{(DOC)}$ ($r=0.93$, $p<0.01$).

$\eta'_{(DOC)}$ averaged 21.9 ± 31%. Net release and net retention of DOC occurred in the 70% and 30% of cases, respectively. Variability of $\eta'_{(DOC)}$ was weakly and inversely related to discharge ($r=0.46$, $p<0.044$) and weakly and positively related to temperature ($r=0.55$, $p<0.022$). A linear model that included both discharge and temperature explained the 72% ($p<0.02$) of total $\eta'_{(DOC)}$ variance.

NO_3 was released ($\eta'_{(NO3)}$=13.2 ± 16%) in the 70% of cases and its variability was unrelated to discharge and temperature. NH_4 was the most retained solute ($\eta'_{(NH4)}$=-36 ± 45%), and its variability was strongly and positively related to discharge (r=0.7, p<0.001) and unrelated to temperature (r=0.17, p>0.05).

4.5. Changes of nutrients and DOC during a storm event

A storm event was intensively monitored during 7 days in July 2015. During this episode, stream flow increased from 62 to 20000 L/s and EC decreased from 783 to 123 µS/cm. SRP concentration increased with stream flow, and the relationship between both variables showed an eight-shaped hysteric loop (Fig. 6). The hysteresis was clockwise when flow was >12000 L/s, and counterclockwise at lower flow, showing high SRP concentration in the falling limb. NO_3 showed a clockwise hysteresis with negative slope, which indicates NO_3 dilution with increasing flow (Williams, 1989). NO_3 concentration was higher in the raising limb than in the falling limb of the hydrograph (when flow <12000 L/s). DOC concentration rapidly increased at the beginning of the storm event until flow was ≈ 6000 L/s. From that point on, DOC concentration was about 12 mg/l and remained nearly constant in the rising and falling limbs of the hydrograph (Fig. 6). No clear hysteresis pattern was detected for DOC concentration.

5. Discussion

5.1. Relative contribution of end-members to stream flow

Our study showed that the hydraulic gradient between the aquifer and the stream was never reversed and that groundwater recharge still contributed to streamflow during stormflow conditions, but at a lower rate (<11%). This indicates that stream flow is maintained by groundwater inflow, especially at baseflow conditions. The unidirectional flowpath from groundwater to the stream also suggests that there is not a true hyporheic zone in the stream, giving the lack of flowpaths leaving and returning to the stream many times within the study reach

(as defined by Harvey and Wagner, 2000). The importance of local groundwater input for the maintenance of flow in perennial Pampean streams has been previously stressed (Sala et al., 1983). Our study clearly showed the maintenance of the hydraulic gradient independently of the flow regime in a Pampean stream.

Stream flow showed a strong increase during storm events that was followed by an almost instantaneous response of the groundwater level. However, this rapid elevation of groundwater table may not be attributed to the inflow of surface water into the unconfined aquifer. When rainfall seals topsoil pores, the pressure on air trapped in the zone of aeration increases and infiltrating water compresses the underlying air. This will cause an elevation of the water table that will occurs only in the piezometer and that may not represent a 'true' input from superficial waters (Todd and Mays, 2005).

Stream flow was also explained by the contribution of tributaries B1 and B2 (plus OF under stormflow conditions). Higher contribution of B1 than B2 during storm events may be explained by the higher subcatchment area of B1 compared to that of B2 and to the input of the wastewater treatment plant to B2, which could attenuate its hydrological response to rainfall.

5.2. Sources and processing of nutrients and DOC in Las Flores stream

B2 was the main contributor of SRP to the stream at baseflow conditions, likely because the effluents released by the dairy industry located upstream B2 (Danalewich et al., 1998). At high flows, SRP was mainly provided by B1 and overland flow (Table A, Supplementary information). This is supported by the elevated SRP levels in these end-members and by the positive relationship observed between flow and SRP concentration in the stream and B1. Increased SRP with flow was previously reported in Las Flores (Feijoó et al., 1999), other Pampean streams (Torti and Andriulo, 2014; Rodríguez Castro et al., 2016), and in agricultural streams (Gentry et al., 2007; Bieroza and

Heathwaite, 2015). On the contrary, B2 showed lower level of SRP at high flow, suggesting a dilution of the effluent released by the industry.

We also observed that SRP increased with flow during the monitored storm event. Some studies described clockwise or counterclockwise hysteresis for phosphorus during storms (Bieroza and Heathwaite, 2015; Rodríguez Castro et al., 2016). However, in our case SRP hysteresis showed a figure eight shape, with a counterclockwise partial loop with low flow followed by a clockwise loop with high flow (Fig. 6). Although eight-shaped hysteric curves are complex and not well understood (Williams, 1989; Seeger et al., 2004), this behavior might reflects the input of SRP from two hydrological sources. The initial rapid, but short, increase of SRP at beginning of storm episode suggests the SRP limited leaching from near stream (riparian) through rapid overland flow. On the other hand, the delayed SRP increase during the discharge recession limb might indicates the arrival of phosphorous-rich water from subsurface flow. Finally, SRP returns to the initial basal level due to dilution from groundwater. Consequently, even though the inclusion of subsurface flow did not improve predictions of the mass balance, it can be relevant during the recession of the storm.

Unlike Bernal et al. (2015), who reported a net SRP balance close to zero in a Mediterranean catchment, we observed a net release of SRP at the study reach (median = 4.52 µg SRP/(m·s; Table 2). This contrast with previous SRP addition experiments performed in Las Flores that showed high phosphorus retention at reach scale (Feijoó et al., 2007; García et al., 2017). This suggest that, even if the SRP retention is high, SRP release is even higher. Similarly, von Schiller et al. (2015) observed that streams can be highly reactive systems with high biogeochemical processing rates, while simultaneously approaching to a short-term balance between retention and release.

$\pi'_{(SRP)}$ values were significantly and positively related to water temperature and $\pi'_{(DOC)}$. This suggests that SRP may originate from decomposition of autochthonous organic matter (macrophytes,

biofilms, etc.) because this process depends on the availability of substrata (Cebrian and Lartigue, 2004) and it is accelerated at higher temperatures (Song et al., 2013). In Las Flores stream, the benthic compartment represented 99% of the total organic matter pool (with a mean value of 718 g/m^2; García et al., 2017), and can provide abundant resources for the decomposition process. Bernal et al. (2015) also observed an increase in SRP concentration along the reach of a forested stream, and attributed it to the combination of warmer temperatures and the mineralization of leaf litter stocks stored in the stream bed.

Like SRP, NH_4 may be mainly provided by B2 at baseflow, and by B1, overland flow and subsurface flow at high flows (Table A, Supplementary Information). NH_4 in B2 may originate from the input of the diary effluent (Danalewich et al., 1998). Rainwater showed high level of NH_4 and could be a potential source of this solute, but it did not appear as relevant in the solute mass balance model. In agreement with previous research in Las Flores (García et al., 2017), NH_4 was by far the most retained solute at reach scale (median net retention=1.76 μg NH_4/(m·s)).This value is one order the magnitude higher than net uptake rates reported by Bernal et al. (2015). Biological demand may explain high NH_4 retention because NH_4 uptake rates are associated to the autotrophic activity in Las Flores (García et al., 2017), as it was observed in other streams (Kaushal et al., 2014; Bernal et al., 2015). However, NH_4 was released at high flows, suggesting that uptake capacity is lost during storm events due to the detachment of autotrophic communities (Vilches and Giorgi, 2010).

Groundwater, the main contributor to baseflow, provided NO_3 to stream water (Table A, Supplementary information). This is confirmed by the dilution of NO_3 observed in the stream, B1 and B2 at high flows, and by the negative relation between NO_3 concentration and flow during the storm event. NO_3 concentration-flow hysteresis showed a clockwise pattern with higher concentration in the rising limb, similar to the hysteresis type C3 proposed by Evans and Davies

(1998). According to this, flow generation may depend on the progressive dominance of three end-members, namely groundwater, overland flow and subsurface flow. Under this scheme, NO_3 may be diluted by the input of overland flow during the rising limb of the hydrograph, then it may remain low during the falling limb due to the contribution of subsurface flow, and finally it may returned to the high basal value.

NO_3 either behaved as a conservative ion or showed a weak release at baseflow conditions. However, we observed an unexpected and anomalous net NO_3 release (≈ 40%) at discharge between 400 and 600 L/s, while at higher discharge (> 900 L/s) NO_3 behaved as a conservative solute again. Streams can act as net sources of NO_3 due to organic matter mineralization and nitrification and to NO_3 inflow from groundwater (Kaushal et al., 2014; Bernal et al., 2015). Nitrification is enhanced under oxygenated conditions (Bernot and Dodds, 2005) and can be high in eutrophic streams (Merseburger et al., 2005; Gammons et al., 2011). Accordingly, nitrification could explain 8 to 43 % of total NH_4 uptake in Las Flores (García et al., 2017). Hence, it is possible that during high flow, turbulent and oxygenated waters may intensify in-stream nitrification. However, this process should not operate at very high flow as a result of sloughing of nitrifying bacteria (Williamson and Cooke, 1985).

DOC is mainly provided by overland and subsurface flows (Suplementary information, Table A), and therefore was positively related to stream flow. During the storm event, DOC concentration rapidly increased up to flow ≈ 700 L/s, but it remained almost constant at higher flows. This suggest that DOC should mobilized at rates nearly proportional to water flux when flow >700 L/s, and that DOC sources should not be exhausted by rainfall intensity (Godsey et al., 2009; Bieroza and Heathwaite, 2015).

We observed a consistent net DOC release at baseflow and with high stream water temperature in Las Flores. This pattern contrasts with the general assumption that stream reaches behave as

reactors under low flow, promoting active DOM processing (Raymond et al., 2016; Casas-Ruiz et al., 2016). Median net release was 860 mg DOC/(m^2·d) in Las Flores, which contrast with significant DOC net retention observed in streams and rivers from other regions (Sirivichi et al., 2011; Kaushal et al., 2014; Butturini et al., 2016). Considering that DOC in Las Flores was mainly composed by protein-like compounds (Messetta et al., 2018) during baseflow, DOC release may be explained by biological in-stream generation. Low flow velocity, warm temperatures and high light inputs enhance stream primary production and thus in-stream DOC generation (Battin et al., 2008; Fasching et al., 2016; Ejarque et al., 2017; Wagner et al., 2017). These environmental conditions are present in Las Flores, where gross primary production ranges between 2.18 and 7.79 g O_2/m^2·d and P/R between 0.56 and 3.31 (García et al., 2017). Few studies have reported net releases of DOC across river networks (Casas-Ruiz et al., 2017). Kaushal et al. (2014) observed substantial organic carbon retention in streams of an urban watershed, while the main stem of the watershed acted as a net source of DOC in summer. In-stream production of DOC was also observed in lowland reaches of a Mediterranean river and in streams of USA (Kaplan and Bott, 1982: Mulholland and Hill, 1997; Ejarque et al., 2017). We also observed a weak net DOC retention at flow ≈ 400 L/s. Considering that DOC biodegradation increases with increasing water velocity (Catalán et al., 2016; De Falco et al., 2016), this suggests that DOC consumption slightly overruled DOC production at higher flows at the study reach. However, this predominance of biodegradation DOC processes was lost at flow ≈ 600 L/s, when the stream reach behaved as a passive pipe. Under extreme hydrologic events, in-stream processing may be low and large amounts of DOC may be exported, resulting in a conservative transport of terrestrially-derived DOC (Casas-Ruiz et al., 2017; Ejarque et al., 2017).

5.3. Origin of nutrients and DOC in Pampean streams

NO_3 was provided to stream water by groundwater in Las Flores. NO_3 in groundwater can result from the oxidation of nitrogenous organic material derived from agricultural practices (Carbó et al., 2009). However, considering the autochthonous imprint of groundwater DOC in Las Flores (Messetta et al., 2018), it is possible that in our case most NO_3 derived from the degradation of ancient soil organic matter within the aquifer (Fellman et al., 2014; Kaushal et al., 2014).

OF proved to be an important source of nutrients and DOC in Las Flores. The analysis of the storm event also suggested that SF could be relevant during the recession phase. When moving towards the stream, overland and subsurface flows interact with soil mobilizing soluble phosphorus and NH_4. This process is favored by the flat landscape characteristic of the Pampean region, which slows down water movement and promotes the contact between water fluxes and the different soil fractions (Giling et al., 2014). Previous studies indicated that the origin of SRP and NH_4 in Pampean streams is not related to fertilizer use. For instance, phosphorus levels in Pampean soils before the establishment of agriculture were elevated (Morrás, 1999), being one order of magnitude higher than those observed in European soils (Tóth et al., 2014). Furthermore, neither SRP nor NH_4 concentration in stream water were associated to agricultural land use in the region (Amuchástegui et al., 2016). OF and SF also provide DOC to the stream, which should originate from the leaching of grasses, leaves and root exudates in the topsoil, as other authors reported elsewhere (Yano et al., 2004; Bernal et al., 2006; Guarch-Ribot and Butturini, 2016). Consequently, decomposition of organic matter in Pampean fertile soils may be a potential source of NH_4 and DOC.

5.4. Conclusions

Stream nutrient concentrations are modulated by two ways that act at different scale (Mulholland and Hill, 1997): (1) catchment control via seasonal variation in the dominant hydrological pathways, and (2) in-stream control via nutrient uptake. However, variation of dominant pathways

in Las Flores is related not only to seasonality but also to hydrological events. Moreover, in-stream processes include not only nutrient uptake (SRP and NH_4) but also biotic production (DOC), decomposition (SRP) and nitrification (NH_4).

The stream reach behaved differently for each solute. It acted as a passive pipe for NO_3 and as a reactor for SRP, DOC and NH_4, showing a net release of SRP and DOC and a net retention of NH_4. Our results stress the relevance of generation processes within the channel in highly productive streams. High irradiance due to the lack of riparian canopy increases fluvial metabolism and thus the production of autochthonous organic matter, whose mineralization in turn releases nutrients and DOC to the water. When in-stream generation surpass retention processes (as in the case of SRP and DOC), the stream exports solutes downstream. Higher SRP and DOC levels in the streams due to agriculture intensification in the Pampean region could even exacerbate the export of these solutes, and thus alter the metabolic activity of downstream communities (Battin et al., 2008). In addition, climatic models predict an increase of runoff in the Pampean region through rainfall intensification (Milly et al., 2005). This will foster the export of SRP and DOC from terrestrial to aquatic ecosystems via overland flow and the dilution of NO_3 provided by groundwater. According to this scenario, N/P ratio should decrease, the phosphorus limitation should be alleviate, and DOC levels should increase in stream water. In addition, our results suggest that the capacity of the stream to retain NH_4 and to generate DOC will be reduced with higher flows. The consequences of all these changes on the structure and functioning of Pampean fluvial ecosystems are still open questions.

Acknowledgements

We wish to thank to Eduardo Kruse and Patricia Laurencena for providing some interesting comments on hydrological aspects of our research. The manuscript was greatly improved by the comments and suggestions of three anonymous reviewers. We thank Silvia Lendaro for the

installation of piezometers, Eduardo Zunino for the construction and installation of sampling devices and Jim Harrison for the revision of the English text. Gonzalo Paz, Jeanine Steverlitz, María Fernanda Formenti, Andrés Solá and Juan Rojas kindly provided access to the study sites and allowed the installation of sampling devices. Universidad Nacional de Luján (UNLu) provided vehicles and drivers for sampling campaigns. Cecilia Hegoburu, Laura Rigacci and Victoria García were funded through a CONICET doctoral fellowship and María Laura Messetta through UNLu and CONICET doctoral fellowships. This project was supported by the Agencia Nacional de Promoción Científica y Tecnológica (PICT-2011-0163 and PICT-2014-1017).

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Table 1. Relationships between flow (expressed as log) and several chemical variables in the stream (A) and both tributaries (B1 and B2). Pearson´s correlation coefficients, significance levels and number of cases are indicated (n.s.: not significant).

	A	B1	B2
SRP	0.367 p = 0.071 N = 25	0.651 p = 0.0003 N = 26	-0.396 p = 0.045 N = 26
NO_3	-0.888 p < 0.0001 N = 19	-0.703 p = 0.0006 N = 20	-0.650 p = 0.0026 N = 19
NO_2	-0.619 p = 0.0047 N = 19	-0.880 p < 0.0001 N = 20	-0.636 p = 0.0034 N = 19
NH_4	n.s. N = 25	n.s. N = 26	-0.414 p = 0.040 N = 25
DOC	0.819 p = 0.0006	0.779 p = 0.0002	0.488 p = 0.047

Table 2. Net in-stream retention/release efficiencies ($\eta_{(y)}$, in percentage) in the reach. Positive values indicate a net gain of the solute along the reach, while negative ones indicate a net loss. Stream water temperature, flow, and contribution of each end-member (EM, in percentage) in the different samplings are also indicated. NC: no change. - : no data.

condition	date	flow (l/s)	water temperature	$\eta_{(y)}$					EM contribution (%)
				SRP	NO_3^-	NO_2^-	NH_4^+	DOC	
baseflow	04/10/2012	22.54	20.8	40.1	-	-	-58.07	-	GW = 59.3 B1 = 22.6 B2 = 18.1
stormflow	07/12/2013	932.27	12.7	11.05	2.26	-1.81	15.17	-	OF = 23 GW = 2.6 B1 = 48 B2 = 25.9
baseflow	07/31/2013	68.97	15.8	NC	NC	NC	-80.45	-	GW = 56.4 B1 = 14.5 B2 = 29.2
baseflow	08/09/2013	57.44	11.5	22.4	17.35	0.24	-85.0	-	GW = 62.2 B1 = 13.4 B2 = 24.4
baseflow	09/10/2013	51.57	21	25.67	NC	29.34	NC	20.47	GW = 47 B1 = 21.7 B2 = 31.3
baseflow	09/25/2013	62.42	14.3	51.78	NC	21.41	-87.94	52.85	GW = 58.5 B1 = 18.9 B2 = 22.6
baseflow	10/15/2013	60.49	21.3	54.31	-	-	-99.6	57.48	GW = 64.1 B1 = 15 B2 = 20.9
baseflow	11/13/2013	66.88	22.1	63.81	NC	604.22	-73.83	52.22	GW = 70.9 B1 = 8.3 B2 = 20.7
baseflow	03/17/2014	113.32	21.3	42.72	-	-	-4.12	34.22	GW = 47.1 B1 = 28.1 B2 = 30.3
baseflow	04/28/2014	94.95	17.3	NC	NC	-96.3	-41.86	13.05	GW = 47.9 B1 = 19.3 B2 = 32.7
baseflow	05/19/2014	137.875	15.3	33.14	NC	17.56	-22.59	25.34	GW = 37.1 B1 = 33.8 B2 = 29.0
baseflow	07/02/2014	99.57	11.4	NC	13.96	73.14	-32.79	-23.33	GW = 37.4 B1 = 25.2 B2 = 40.1
stormflow	07/23/2014	410.5	12.4	20.46	41.54	-66.88	18.41	-18.5	OF = 23.6 GW = 6.7 B1 = 48.3 B2 = 27.2
stormflow	12/01/2014	594.737	16.3	46.09	40.56	-45.95	30.86	-0.25	OF = 21.2 GW = 11.6 B1 = 35 B2 = 27.8
Median net retention/release (ug/m·s)				3.58	29.91		-2.24	31.97	

Fig. 1. Location of the studied reach of Las Flores stream. A: stream sampling point. B_1 and B_2: upstream tributaries. Collectors of the different end-members (groundwater or GR, rainfall or R, subsurface flow or SF, and overland flow or OF) where located in A.

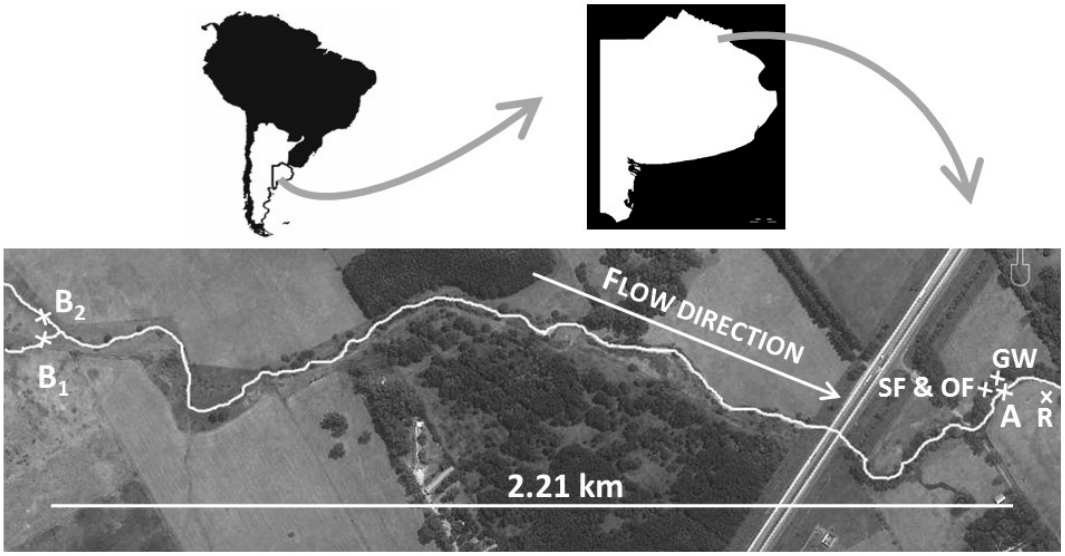
Fig 2. Temporal evolution of the hydrological variables: rainfall, stream discharge, stream and water table levels, and hydraulic gradient magnitude in the riverbank.

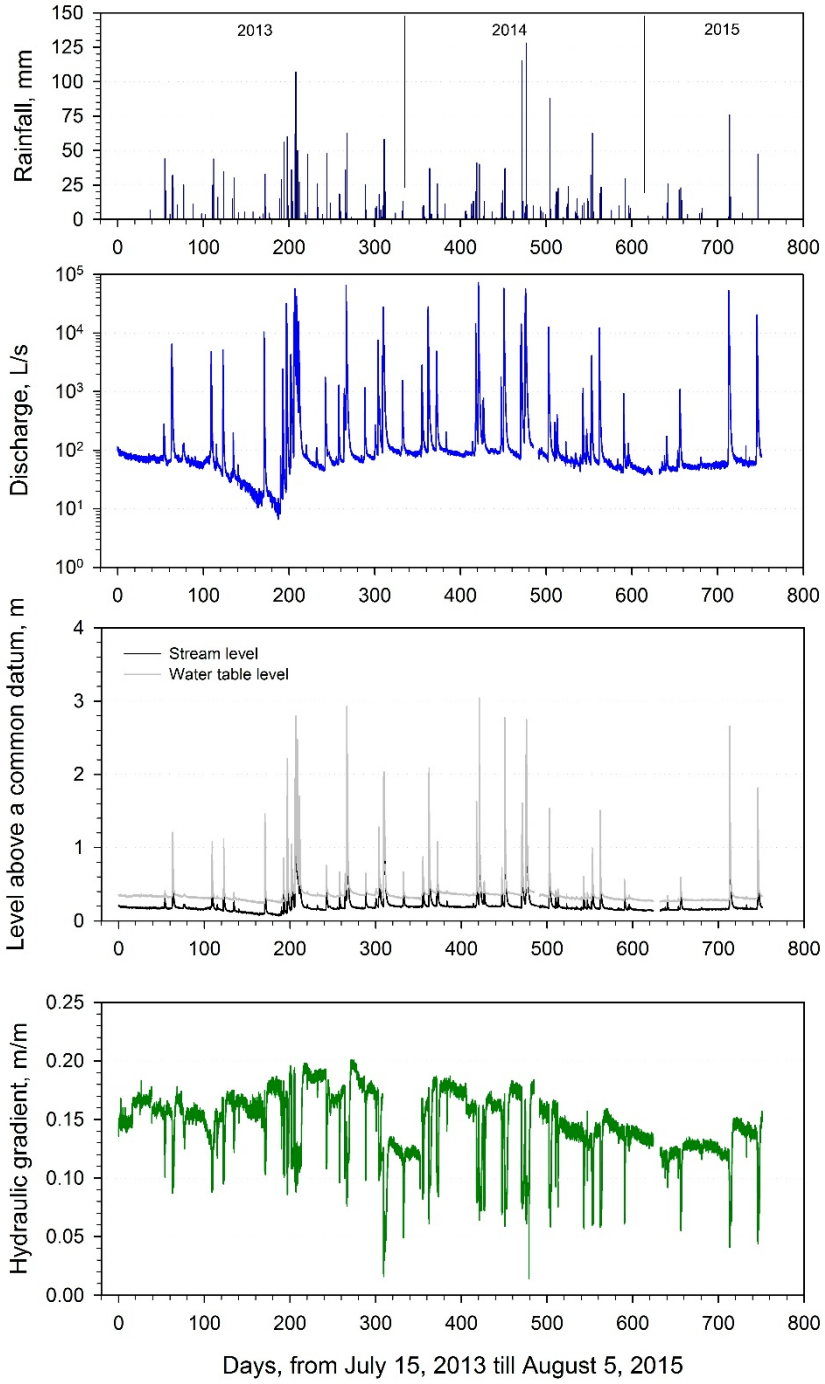
Fig. 3. Water chemistry in the stream and end-members. Bars are means values and whiskers represent SD. A: stream; OF: overland flow; SF: subsurface flow; R: rainwater; GW: phreatic groundwater; B1 and B2: upstream tributaries (note that B2 had a dairy effluent). N varied between 9 and 40 depending on the considered end-member. Letters on bars indicate significant differences among end-members for each solute ($p<0.05$).

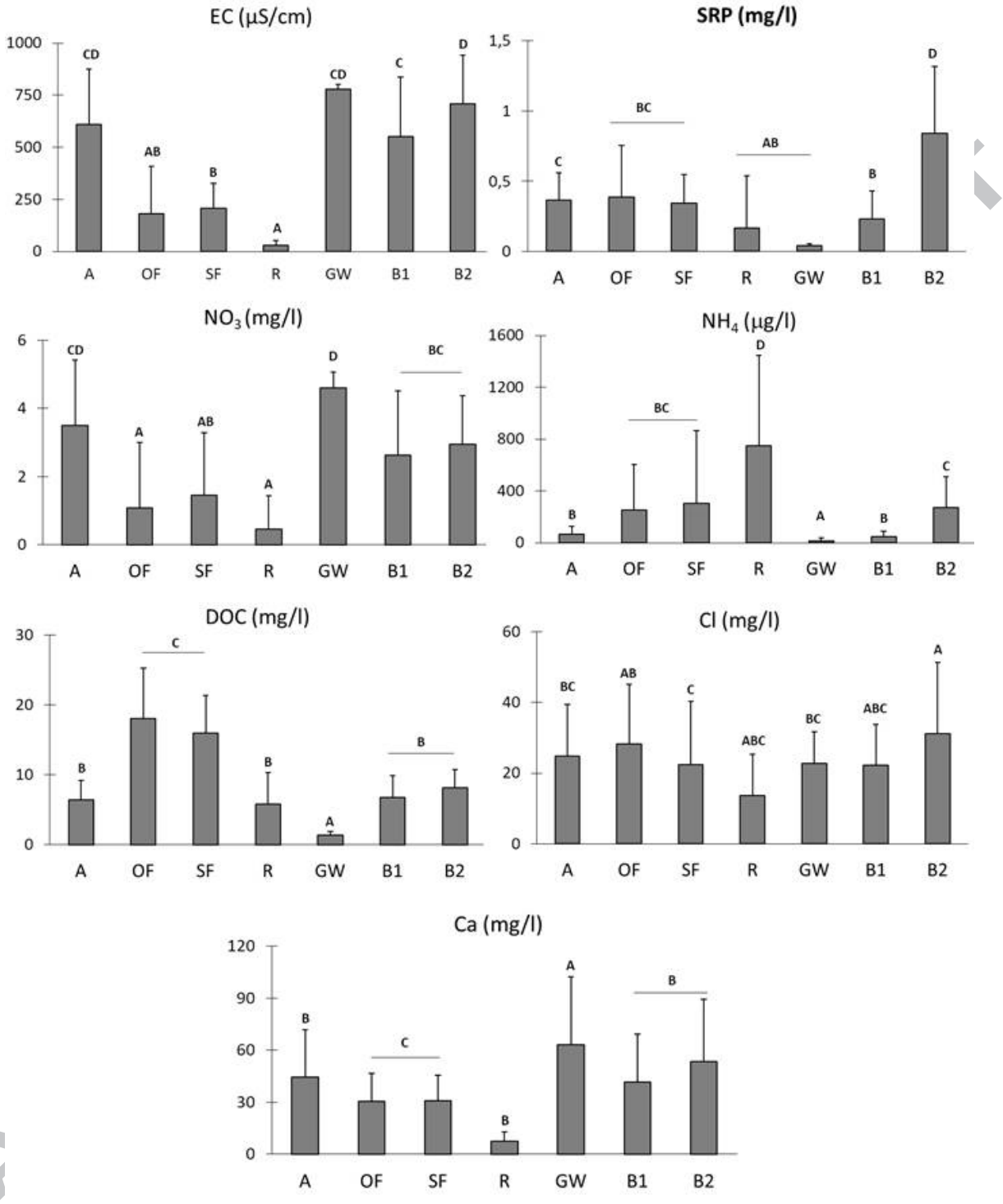
Fig. 4. Relationship between the relative contribution (in percentage) of overland flow (OF), groundwater (GW), and both tributaries (B1 and B2) and stream flow. EM: end-member.

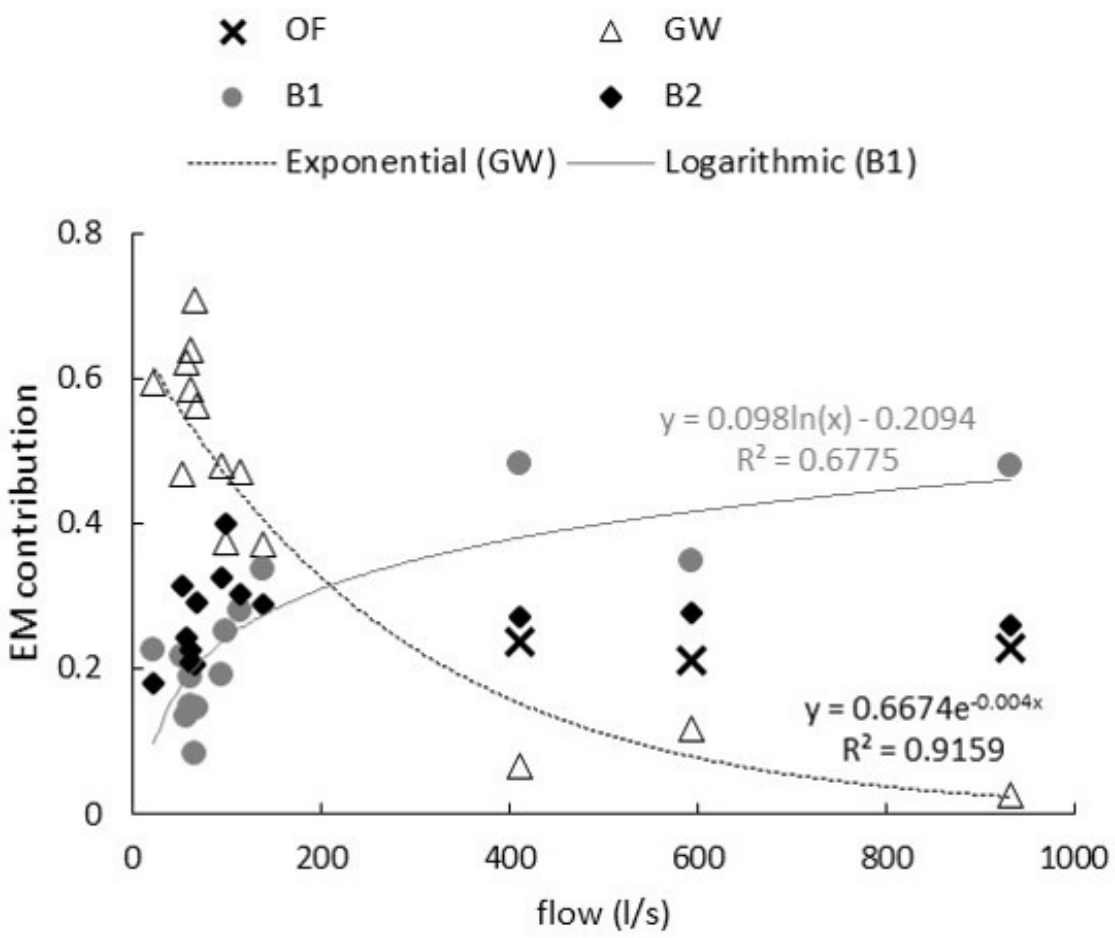
Fig. 5. Net in-stream retention/release efficiency of the different solutes. Efficiencies > 0 indicate net release, while efficiencies < 0 indicate net retention. Numbers between brackets indicate N for each solute.

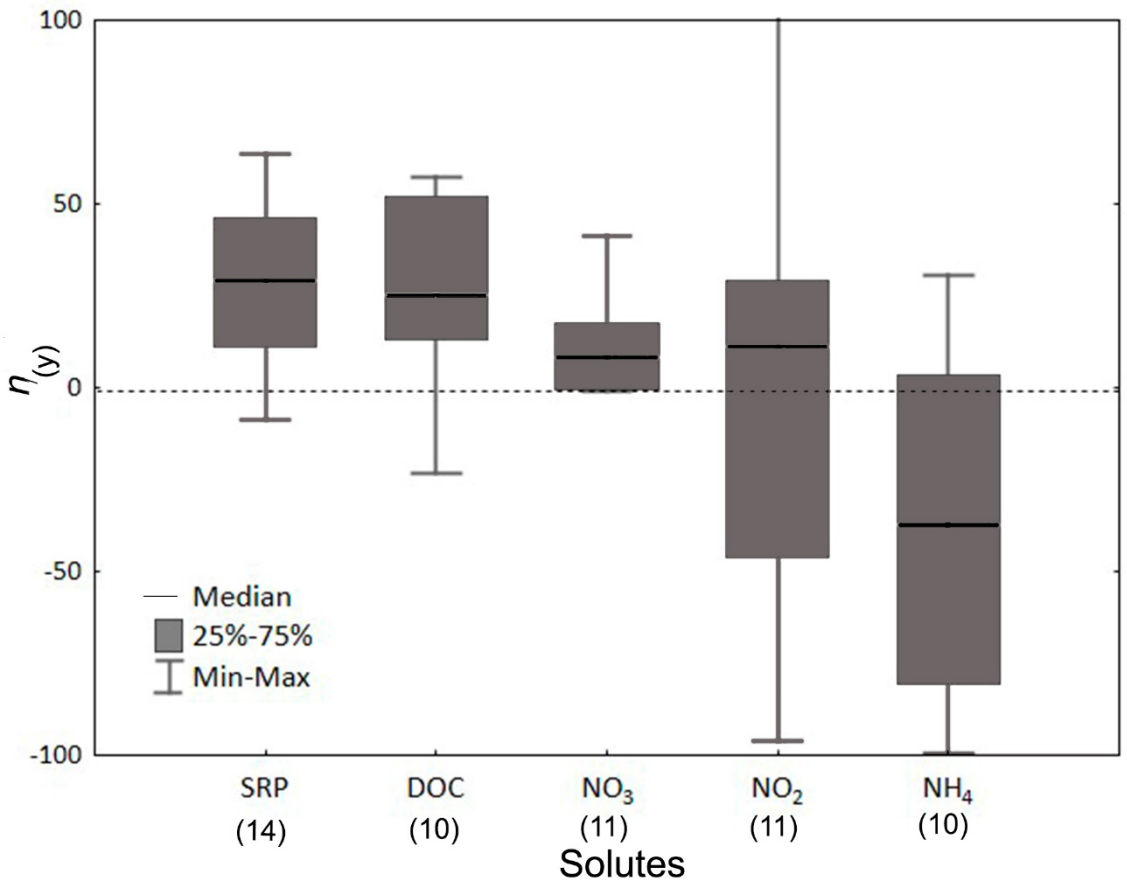
Fig. 6. Relationships between stream flow and nutrient and DOC concentrations during the storm event of July-August 2015. Arrows indicate the evolution of solute concentration through time.











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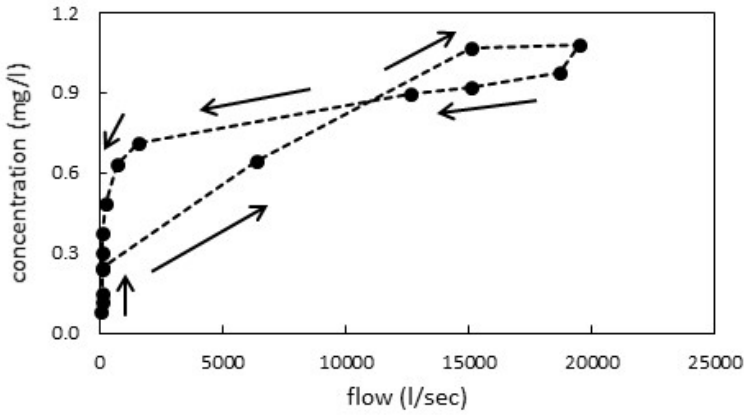
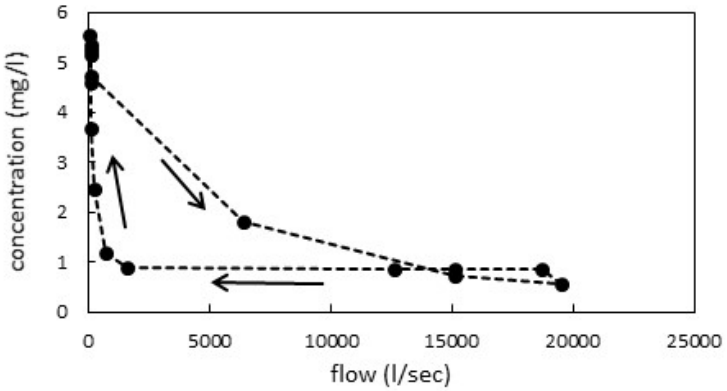
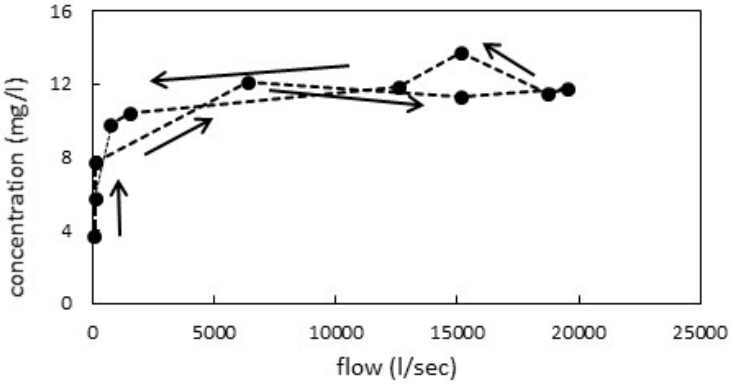


NO_3 (mg/l)



DOC



Highlights

- Origin and processing of nutrients and DOC were studied in a Pampean stream.
- NO_3 was provided to the stream by groundwater, and DOC by overland flow.
- Main sources of SRP and NH_4 were an upstream tributary and overland and subsurface flows.
- The reach acts as a source of DOC, SRP and NO_3 and a sink of NH_4.