



# Riparian and in-stream controls on nutrient concentrations and fluxes in a headwater forested stream

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**Abstract.** Headwater streams are recipients of water sources draining through terrestrial ecosystems. At the same time, stream biota can transform and retain nutrients dissolved in stream water. Yet studies considering simultaneously these two sources of variation in stream nutrient chemistry are rare. To fill this gap of knowledge, we analyzed stream water and riparian groundwater concentrations and fluxes as well as in-stream net uptake rates for nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ), and soluble reactive phosphorus (SRP) along a 3.7 km reach on an annual basis. Chloride concentrations (used as conservative tracer) indicated a strong hydrological connection at the riparian–stream interface. However, stream and riparian groundwater nutrient concentrations showed a moderate to null correlation, suggesting high in-stream biogeochemical processing. In-stream net nutrient uptake ( $F_{\text{sw}}$ ) was highly variable across contiguous segments and over time, but its temporal variation was not related to the vegetative period of the riparian forest. For  $\text{NH}_4^+$ , the occurrence of  $F_{\text{sw}} > 0 \mu\text{g N m}^{-1} \text{s}^{-1}$  (gross uptake > release) was high along the reach, while for  $\text{NO}_3^-$ , the occurrence of  $F_{\text{sw}} < 0 \mu\text{g N m}^{-1} \text{s}^{-1}$  (gross uptake < release) increased along the reach. Within segments and dates,  $F_{\text{sw}}$ , whether negative or positive, accounted for a median of 6, 18, and 20 % of the inputs of  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ , and SRP, respectively. Whole-reach mass balance calculations indicated that in-stream net uptake reduced stream  $\text{NH}_4^+$  flux up to 90 %, while the stream acted mostly as a source of  $\text{NO}_3^-$  and SRP. During the dormant period, concentrations decreased along the reach for  $\text{NO}_3^-$ , but increased for  $\text{NH}_4^+$  and SRP. During the vegetative period,  $\text{NH}_4^+$  decreased, SRP increased, and  $\text{NO}_3^-$  showed a U-shaped pattern along the reach. These longitudinal trends resulted from the combination of hydrological

mixing with terrestrial inputs and in-stream nutrient processing. Therefore, the assessment of these two sources of variation in stream water chemistry is crucial to understand the contribution of in-stream processes to stream nutrient dynamics at relevant ecological scales.

## 1 Introduction

Stream water chemistry integrates hydrological and biogeochemical processes occurring within its drainage area, and thus the temporal variation in stream solute concentrations at the catchment outlet is considered a good indicator of the response of terrestrial and aquatic ecosystems to environmental drivers (Bormann and Likens, 1967; Bernhardt et al., 2003; Houlton et al., 2003). Less attention has been paid to the spatial variation in water chemistry along the stream, though it can be considerably important because stream nutrient concentrations are influenced by changes in hydrological flow paths, vegetation cover, and soil characteristics (Dent and Grimm, 1999; Likens and Buso, 2006). For instance, spatial variation in nutrient concentration along the stream has been attributed to changes in soil nitrification rates (Bohlen et al., 2001), soil organic carbon availability (Johnson et al., 2000), and organic soil depth across altitudinal gradients (Lawrence et al., 2000). Moreover, nutrient cycling within the riparian zone can strongly influence stream nutrient concentrations along the stream because these ecosystems are hot spots of biogeochemical processing (McClain et al., 2003; Vidon et al., 2010). In addition, processes occurring at the riparian–stream interface have a larger influence on stream water chemistry than those occurring at catchment locations further

from the stream (Ross et al., 2012). Finally, stream ecosystems have a strong capacity to transform and retain nutrients; thus, in-stream biogeochemical processes can further influence nutrient chemistry along the stream (Peterson et al., 2001; Dent et al., 2007). Therefore, consideration of these multiple sources of variation in stream water chemistry is important to understand drivers of stream nutrient dynamics.

Our understanding of nutrient biogeochemistry within riparian zones and streams is mainly based on field studies performed at the plot scale or in small stream reaches (a few hundred meters) (Lowrance et al., 1997; Peterson et al., 2001; Sabater et al., 2003; Mayer et al., 2007; von Schiller et al., 2015). These empirical studies have widely demonstrated the potential of riparian and stream ecosystems as either sinks or sources of nutrients, which ultimately influence the transport of nutrients to downstream ecosystems. Riparian and stream biota are capable of decreasing the concentration of essential nutrients, such as dissolved inorganic nitrogen (DIN) and phosphate, especially with increasing water storage and residence time (Valett et al., 1996; Hedin et al., 1998; Peterson et al., 2001; Vidon and Hill, 2004). Conversely, riparian forests can become sources rather than sinks of nutrients when  $N_2$ -fixing species predominate (Helfield and Naiman, 2002; Compton et al., 2003), and in-stream nutrient release can be important during some periods (Bernhardt et al., 2002; von Schiller et al., 2015). Moreover, there is an intimate hydrological linkage between riparian and stream ecosystems that can result in strong biogeochemical feedbacks between these two compartments (e.g., Morrice et al., 1997; Martí et al., 2000; Bernal and Sabater, 2012). However, studies integrating biogeochemical processes of these two nearby ecosystems are rare (but see Dent et al., 2007), and the exchange of water and nutrients between stream and groundwater is unknown in most studies assessing in-stream gross and net nutrient uptake (Roberts and Mulholland, 2007; Covino et al., 2010; von Schiller et al., 2011).

There is a wide body of knowledge showing the potential of riparian and stream ecosystems to modify either groundwater or stream nutrient concentrations. However, a comprehensive view of the influence of riparian and in-stream processes on stream water chemistry at the catchment scale is still lacking (but see Meyer and Likens, 1979). This gap of knowledge mostly exists because hydrological and biogeochemical processes can vary substantially along the stream (Covino and McGlynn, 2007; Jencso et al., 2010), which limits our ability to extrapolate small plot- and reach-scale measurements to larger spatial scales. Some authors have proposed that nutrient concentrations should decline along the stream if in-stream net uptake is high enough and riparian groundwater inputs are relatively small (Brookshire et al., 2009). This declining pattern is not systematically observed in reach-scale studies, which could bring us to the conclusion that terrestrial inputs are the major driver of stream water chemistry because in-stream gross uptake and release counterbalance each other most of the time (Brookshire et al.,

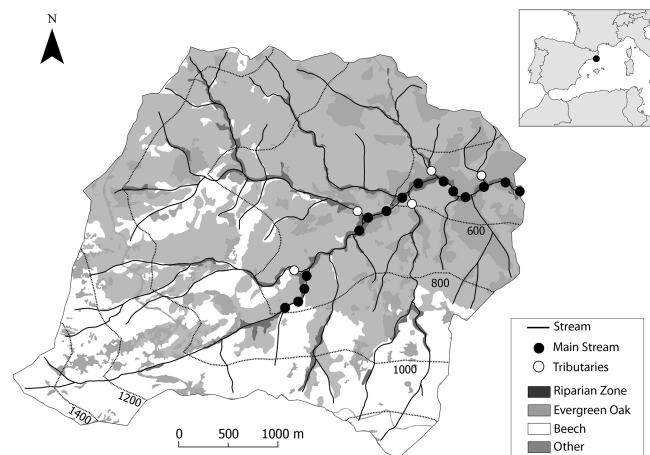
2009). However, synoptic studies have revealed that nutrient concentrations are patchy and highly variable along the stream as a result of spatial patterns in upwelling and in-stream nutrient processing (Dent and Grimm, 1999). Thus, in-stream nutrient cycling could be substantial, but it might not necessarily lead to longitudinal increases or declines in nutrient concentration, a question that probably needs to be addressed at spatial scales larger than a few hundred meters.

The goal of this study was to gain a better understanding of the influence of riparian groundwater inputs and in-stream biogeochemical processing on stream nutrient chemistry and fluxes in a headwater forested catchment. To approach this question, we explored the longitudinal pattern of stream nutrient (nitrate, ammonium, and phosphate) concentration along a 3.7 km reach over 1.5 years. We chose a headwater catchment as a model system to investigate drivers of spatial patterns in stream water chemistry because they typically show pronounced changes in riparian and stream features across relatively short distances (Uehlinger, 2000). First, we evaluated riparian groundwater inputs and in-stream nutrient processing as sources of variation in stream nutrient concentration along the reach. We expected stream and riparian groundwater nutrient concentrations to be similar and strongly correlated if riparian groundwater is a major source of nutrients to the stream. In addition, we estimated the in-stream nutrient-processing capacity for 14 contiguous segments along the reach with a mass balance approach. Second, we evaluated the relative contribution of riparian groundwater inputs and in-stream biogeochemical processing to stream nutrient fluxes at the whole-reach scale by applying a mass balance approach that included all hydrological input and output fluxes along the reach.

## 2 Study site

The research was conducted in the Font del Regàs catchment (14.2 km<sup>2</sup>) (Fig. 1), located in the Montseny Natural Park, NE Spain (41°50' N, 2°30' E; 300–1200 m a.s.l.) during the period 2010–2011. Total inorganic N deposition in this area oscillates between 15 and 30 kg N ha<sup>-1</sup> yr<sup>-1</sup> (Àvila and Rodà, 2012). The climate at the Montseny Mountains is subhumid Mediterranean. The long-term mean annual precipitation is 925 ± 151 mm and the long-term mean annual air temperature is 12.1 ± 2.5 °C (mean ± SD, period: 1940–2000; Catalan Meteorological Service: <http://www.meteo.cat/observacions/xema/>). During the study period, mean annual precipitation (975 mm) and temperature (12.9 °C) fell within the long-term average (data from a meteorological station within the study catchment). In this period, summer 2010 was the driest season (140 mm), while most of the precipitation occurred in winter 2010 (370 mm) and autumn 2011 (555 mm) (Fig. 2a).

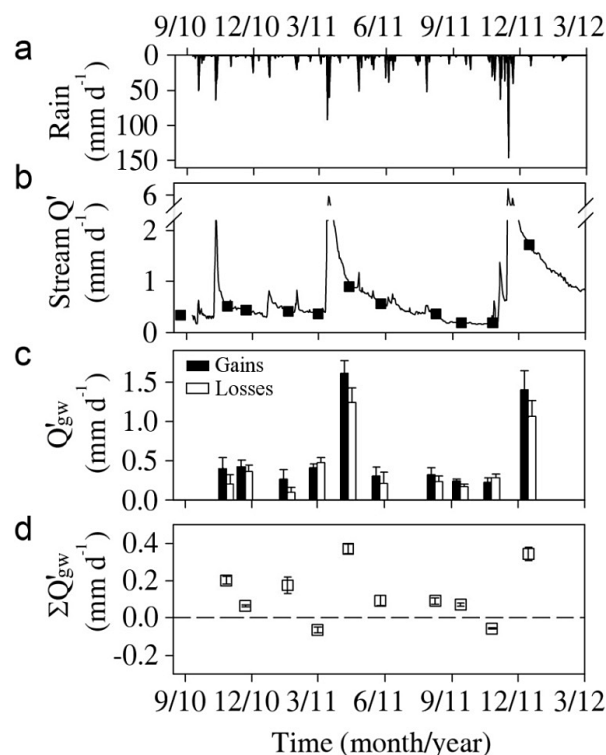
The catchment is dominated by biotitic granite (ICC, 2010) and it has steep slopes (28 %). Evergreen oak (*Quercus*



**Figure 1.** Map of the Font del Regàs catchment within the Montseny Natural Park (NE, Spain). The vegetation cover and the main stream sampling stations along the 3.7 km reach are indicated. There were 5 and 10 sampling stations along the second- and third-order sections, respectively. Four permanent tributaries discharged to the main stream from the upstream- to the downstream-most site (white circles). Additional water samples were collected from a small tributary draining through the inhabited area at the lowest part of the reach. The remaining tributaries were dry during the study period.

*ilex*) and beech (*Fagus sylvatica*) forests cover 54 and 38 % of the catchment area, respectively (Fig. 1). The upper part of the catchment (2 %) is covered by heathlands and grasslands (ICC, 2010). The catchment has a low population density ( $< 1$  person  $\text{km}^{-2}$ ) which is concentrated in the valley bottom. Hillslope soils ( $\text{pH} \sim 6$ ) are sandy, with a high content of rocks (33–36 %). Soils at the hillslopes have a 3 cm depth O horizon and a 5 to 15 cm depth A horizon (averaged from 10 soil profiles).

The riparian zone is relatively flat (slope  $< 10\%$ ), and it covers 6 % of the catchment area. Riparian soils ( $\text{pH} \sim 7$ ) are sandy loam with low rock content (13 %) and a 5 cm depth organic layer followed by a 30 cm depth A horizon (averaged from five soil profiles). Along the 3.7 km reach, the width of the riparian zone increases from 6 to 32 m, whereas the total basal area of riparian trees increases 12-fold (based on forest inventories of 30 m plots every ca. 150 m) (Fig. S1 in the Supplement). *Alnus glutinosa*, *Robinia pseudoacacia*, *Platanus hybrida*, and *Fraxinus excelsior* are the most abundant riparian tree species followed by *Corylus avellana*, *Populus tremula*, *Populus nigra*, and *Sambucus nigra*. The abundance of  $\text{N}_2$ -fixing species (*A. glutinosa* and *R. pseudoacacia*) increases from 0 to  $> 60\%$  along the longitudinal profile (Fig. S1). During base flow conditions, riparian groundwater ( $< 1.5$  m from the stream channel) flows well below the soil surface ( $0.5 \pm 0.1$  m), and thus the interaction with the riparian organic soil is minimal (averaged from 15 piezometers,



**Figure 2.** Temporal pattern of area-specific (a) rainfall, (b) stream discharge, (c) whole-reach gross hydrological gains and losses, and (d) cumulative net groundwater inputs at the downstream-most site. Black squares in (b) are dates of field campaigns. Error bars in (c) and (d) show the uncertainty associated with the empirical estimation of  $Q$  from tracer slug additions. Error bars in (b) are smaller than the symbol size.

$n = 165$ ) (Fig. S1). During the period of study, riparian groundwater temperature ranged from 5 to  $19.5^\circ\text{C}$ .

The 3.7 km study reach is a second-order stream along the first 1.5 km and a third-order stream for the remaining 63 % of its length. The geomorphology of the stream bed changes substantially with stream order. The stream bed along the second-order section is mainly composed of rocks and cobbles (70 %) with a small contribution of sand ( $\sim 10\%$ ). At the valley bottom, sands and gravels represent 44 % of the stream substrate and the presence of rocks is minor (14 %). Mean wetted width and water velocity increase between the second- and third-order section (from 1.6 to 2.7 m and from  $0.24$  to  $0.35$   $\text{m s}^{-1}$ , respectively) (Fig. S1). During the study period, stream water temperature ranged from 5 to  $18^\circ\text{C}$ . Stream discharge was low in summer ( $0.33$  mm) and peaked in spring ( $0.79$  mm).

### 3 Materials and methods

#### 3.1 Field sampling and laboratory analysis

We selected 15 sampling sites along the 3.7 km study reach. The distance between consecutive sampling sites ranged from 110 to 600 m (Fig. 1). At each sampling site, we installed a 1 m long PVC piezometer (3 cm  $\varnothing$ ) in the riparian zone at  $\sim 1.5$  m from the stream channel.

For each sampling site, we sampled stream water (from the thalweg) and riparian groundwater every 2 months from August 2010 to December 2011. We used pre-acid-washed polyethylene bottles to collect water samples after triple-rinsing them with either stream or groundwater. On each sampling date, we also measured dissolved oxygen concentration (DO, in  $\text{mg L}^{-1}$ ) and water temperature (in  $^{\circ}\text{C}$ ) with a YSI ProODO device in both stream water and riparian groundwater. We avoided sampling soon after storms to ensure that our measurements were representative of low-flow conditions, when the influence of in-stream biogeochemical processes on stream nutrient concentrations and fluxes is expected to be the highest. All field campaigns were performed at least 9 days after storm events, except in October 2011 (Fig. 2b, black squares). On each sampling date and at each sampling site, we measured groundwater table elevation (in meters below soil surface) with a water level sensor (Eijkelkamp 11.03.30) as well as wetted width (in m), stream discharge ( $Q$ , in  $\text{L s}^{-1}$ ), and water velocity ( $\text{m s}^{-1}$ ).  $Q$  and water velocity were estimated with the slug-addition technique by adding 1 L of NaCl-enriched solution to the stream (electrical conductivity =  $75 - 90 \text{ mS cm}^{-1}$ ,  $n = 11$ ) (Gordon et al., 2004). The uncertainty associated with  $Q$  measurements was calculated as the relative difference in  $Q$  between pairs of tracer additions under equal water depth conditions (difference  $< 1$  mm). The pairs of data were selected from a set of 126 slug additions and water level measurements obtained from the permanent field stations at Font del Regàs (Lupon, unpublished). The measured uncertainty was relatively small (1.9%,  $n = 11$ ). On each sampling date, we also collected stream water and measured  $Q$  at the four permanent tributaries discharging to Font del Regàs stream, which drained 1.9, 3.2, 1.8, and 1.1  $\text{km}^2$ , respectively (Fig. 1). These data were used for mass balance calculations (see below). Additional stream water samples were collected from a small permanent tributary that drained through an area ( $< 0.4 \text{ km}^2$ ) with few residences and crop fields for personal consumption.

Water samples were filtered through pre-ashed GF/F filters (Whatman<sup>®</sup>) and kept cold ( $< 4^{\circ}\text{C}$ ) until laboratory analysis ( $< 24$  h after collection). Chloride ( $\text{Cl}^{-}$ ) was used as a conservative hydrological tracer and analyzed by ionic chromatography (Compact IC-761, Methrom). Nitrate ( $\text{NO}_3^{-}$ ) was analyzed by the cadmium reduction method (Keeney and Nelson, 1982) using a Technicon autoanalyzer (Technicon, 1976). Ammonium ( $\text{NH}_4^{+}$ ) was manually an-

alyzed via the salicylate–nitroprusside method (Baethgen and Alley, 1989) using a spectrophotometer (PharmaSpec UV-1700 SHIMADZU). Soluble reactive phosphorus (SRP) was manually analyzed via the acidic molybdate method (Murphy and Riley, 1962) using a spectrophotometer (PharmaSpec UV-1700 SHIMADZU).

#### 3.2 Data analysis

The seasonality of biological activity can strongly affect both riparian groundwater chemistry and in-stream biogeochemical processes (Groffman et al., 1992; Hill et al., 2001). Therefore, the data set was separated into two groups based on sampling dates during the vegetative and dormant period (seven and four sampling dates, respectively). As a reference, we considered the vegetative period starting at the beginning of riparian leaf-out (April) and ending at the peak of leaf-litter fall (October), coinciding with the onset and offset of riparian tree evapotranspiration, respectively (Nadal-Sala et al., 2013). During the study period, rainfall was similar between the vegetative and dormant period (775 and 876 mm, respectively).

##### 3.2.1 Patterns of stream discharge, riparian groundwater inputs, and stream solute concentrations

For each period, we examined the longitudinal pattern of stream discharge, riparian groundwater inputs, and stream solute concentrations along the reach. On each sampling date, we calculated area-specific stream discharge by dividing instantaneous discharge by catchment area ( $Q'$ , in  $\text{mm d}^{-1}$ ) at each sampling site. We used  $Q'$  rather than  $Q$  to be able to compare water fluxes from the 15 nested catchments along the reach. We examined the longitudinal patterns of  $Q'$  and stream solute concentration ( $C_{\text{sw}}$ ) by applying regression models (linear, exponential, potential, and logarithmic). Model selection was performed by ordinary least squares (Zar, 2010). We referred only to the best-fit model in each case.

The contribution of net riparian groundwater inputs to surface water along each stream segment ( $Q_{\text{gw}}$ ) was estimated as the difference in  $Q$  between consecutive sampling sites (Covino et al., 2010). The empirical uncertainty associated with  $Q$  was used to calculate a lower and upper limit of  $Q_{\text{gw}}$ . We considered that  $Q_{\text{gw}}$  was representative of the net riparian groundwater flux draining to the stream within each stream segment. We acknowledge that this approach oversimplifies the complex hydrological interactions at the riparian–stream interface because it does not consider concurrent hydrological gains and losses within each segment (Payn et al., 2009), but we consider that it provides a representative estimate at the scale of this study. To investigate the longitudinal pattern of riparian groundwater inputs, we calculated the cumulative area-specific net riparian groundwater input ( $\Sigma Q'_{\text{gw}}$ , in

mm d<sup>-1</sup>) by summing up  $Q_{gw}$  from the upstream-most site to each of the downstream segments and dividing it by the cumulative catchment area.

For each sampling date, we examined whether the 3.7 km reach was either net gaining or net losing water by comparing concurrent gross hydrological gains and losses over the entire reach (Payn et al., 2009). For this spatial scale, we considered that stream segments exhibiting  $Q_{gw} > 0$  contributed to gross hydrological gains ( $\Sigma Q_{gw} > 0$ ), while segments with  $Q_{gw} < 0$  contributed to gross hydrological losses ( $\Sigma Q_{gw} < 0$ ). Note that gross riparian groundwater fluxes divided by the total catchment area are equal to  $\Sigma Q'_{gw}$  at the downstream-most site. For each sampling date, we calculated the relative contribution of different water sources to stream discharge at the downstream-most site ( $Q_{bot}$ ), with  $Q_{top}/Q_{bot}$ ,  $\Sigma Q_{ef}/Q_{bot}$ , and  $\Sigma Q_{gw}/Q_{bot}$  for upstream, tributaries and riparian groundwater, respectively.

### 3.2.2 Sources of variation in stream nutrient concentration along the reach riparian groundwater inputs

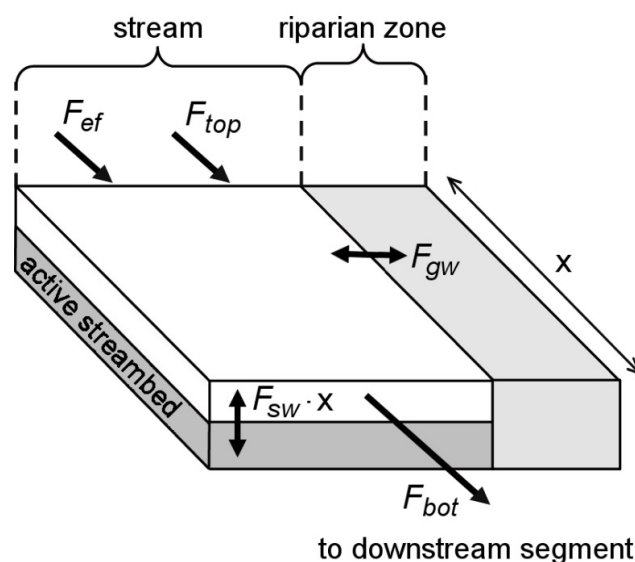
We investigated whether longitudinal patterns in stream solute concentration were driven by riparian groundwater inputs by comparing solute concentrations between stream water and riparian groundwater with a Wilcoxon paired rank sum test. A non-parametric test was used because solute concentrations were not normally distributed (Shapiro–Wilk test,  $p < 0.01$  for all study solutes) (Zar, 2010).

Moreover, we examined the degree of hydrological interaction at the riparian–stream interface by exploring the relationship between stream and riparian groundwater Cl<sup>-</sup> concentrations with a Spearman correlation. For each period, we quantified the difference between Cl<sup>-</sup> concentrations in the two water bodies by calculating divergences from the 1 : 1 line with the relative root-mean-square error (RRMSE, in %):

$$\text{RRMSE} = \frac{\sqrt{\sum_{i=1}^n (C_{sw} - C_{gw})^2}}{n \times \overline{C_{gw}}} \times 100, \quad (1)$$

where  $C_{sw}$  and  $C_{gw}$  are stream and riparian groundwater solute concentrations, respectively,  $n$  is the total number of observations, and  $\overline{C_{gw}}$  is the average of  $C_{gw}$ . A strong correlation and a low RRMSE between stream and riparian groundwater Cl<sup>-</sup> concentrations indicate a strong hydrological connection between the two water bodies. Similarly, we examined the correlation between stream and riparian groundwater nutrient concentrations. We expected a weak correlation and a high RRMSE value between nutrient concentrations measured at the two water bodies if the stream has a high nutrient processing capacity and in-stream gross uptake and release do not counterbalance each other.

*In-stream nutrient processing.* We investigated the influence of in-stream biogeochemical processes on the longitudinal pattern of stream nutrient concentrations by apply-



**Figure 3.** Conceptual representation of nutrient fluxes considered to estimate in-stream net nutrient uptake for each stream segment ( $F_{sw} \times x$ , Eq. 2). For each segment of length  $x$ , the considered nutrient input fluxes were upstream ( $F_{top}$ ) and tributaries ( $F_{ef}$ ). Nutrient fluxes exiting the stream segment ( $F_{bot}$ ) were  $F_{top}$  for the contiguous downstream segment. Riparian groundwater nutrient fluxes could either enter ( $F_{gw} > 0$ ) or exit ( $F_{gw} < 0$ ) the stream. Nutrient fluxes for each component were estimated by multiplying its water flux ( $Q$ ) by its nutrient concentration ( $C$ ). In-stream net nutrient uptake ( $F_{sw} \times x$ ) is the result of gross nutrient uptake and release by the active streambed.  $F_{sw} \times x$  can be positive (gross uptake > release), negative (gross uptake < release), or zero (gross uptake  $\sim$  release). See text for details.

ing a mass balance approach for each individual segment (Roberts and Mulholland, 2007). For each nutrient, we calculated changes in stream flux between contiguous sampling sites ( $F_{sw}$ , in  $\mu\text{g m}^{-1} \text{s}^{-1}$ ), with  $F_{sw}$  being the net flux resulting from in-stream gross uptake and release along a particular stream segment (von Schiller et al., 2011). We expressed  $F_{sw}$  by unit of stream length in order to compare net changes in stream flux between segments differing in length. For each sampling date and for each nutrient,  $F_{sw}$  was approximated with

$$F_{sw} = (F_{top} + F_{ef} + F_{gw} - F_{bot})/x, \quad (2)$$

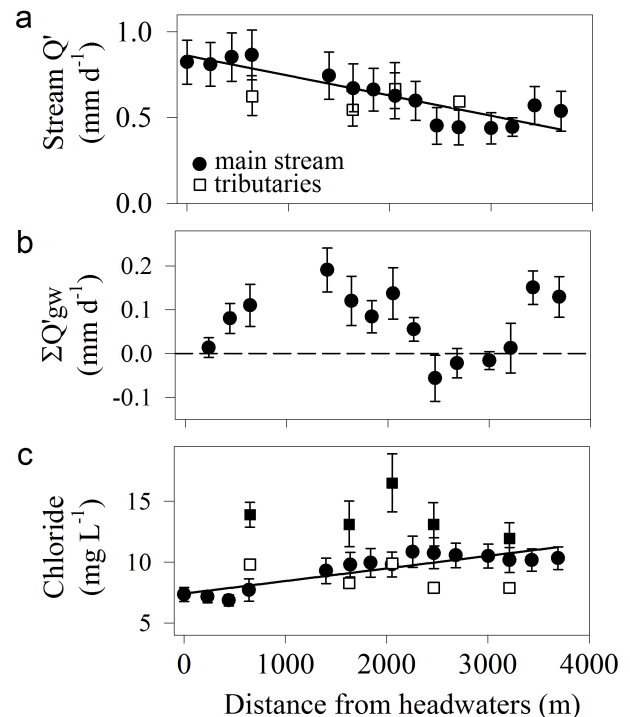
where  $F_{top}$  and  $F_{bot}$  are the nutrient flux at the top and at the bottom of each stream segment,  $F_{gw}$  is the nutrient flux from net riparian groundwater inputs, and  $F_{ef}$  is the nutrient flux from tributary inputs for those reaches including a tributary (all in  $\mu\text{g s}^{-1}$ ) (Fig. 3).  $F_{top}$  and  $F_{bot}$  were calculated by multiplying  $Q$  by  $C_{sw}$  at the top and at the bottom of the segment, respectively.  $F_{gw}$  was estimated by multiplying net groundwater inputs ( $Q_{gw}$ ) by nutrient concentration in either riparian groundwater or stream water. For net gaining segments ( $Q_{gw} > 0$ ), we assumed that the chemistry of net water inputs was similar to that measured in riparian

groundwater, and thus  $C_{gw}$  was the average between riparian groundwater nutrient concentration at the top and bottom of the reach. For net losing segments ( $Q_{gw} < 0$ ), we assumed that the chemistry of net water losses was similar to that measured in stream water and thus,  $C_{gw}$  averaged stream water concentration at the top and at the bottom of each reach segment ( $C_{top}$  and  $C_{bot}$ , respectively). For those cases in which stream segments received water from a tributary,  $F_{ef}$  was calculated by multiplying  $Q$  and  $C$  at the outlet of the tributary. We calculated an upper and lower limit of  $F_{sw}$  based on the empirical uncertainty associated with water fluxes ( $Q$  and  $Q_{gw}$ ). Finally,  $x$  (in m) is the length of the segment between two consecutive sampling sites. The same approach was applied for  $Cl^-$ , a conservative tracer that was used as a hydrological reference. For  $Cl^-$ , we expected  $F_{sw} \sim 0$  if inputs from upstream, tributaries, and riparian groundwater account for most of the stream  $Cl^-$  flux. For nutrients,  $F_{sw}$  can be positive (gross uptake > release), negative (gross uptake < release), or zero (gross uptake  $\sim$  release). Therefore, we expected  $F_{sw} \neq 0$  if in-stream gross uptake and release processes do not fully counterbalance each other (von Schiller et al., 2011). To investigate whether stream segments were consistently acting as net sinks or net sources of nutrients along the stream during the study period, we calculated the frequency of  $F_{sw} > 0$ ,  $F_{sw} < 0$ , and  $F_{sw} = 0$  for each nutrient and for each segment. We assumed that  $F_{sw}$  was undistinguishable from 0 when its upper and lower limit contained zero.

Since in-stream nutrient cycling can substantially vary with reach length (Meyer and Likens, 1979; Ensign and Doyle, 2006), we also calculated  $F_{sw}$  for the whole 3.7 km reach by including all hydrological input and output fluxes (solute fluxes from the upstream-most site, tributaries, and riparian groundwater gross gains and losses) in a mass balance at the whole-reach scale. For the two spatial scales (segment and whole reach), we examined whether  $F_{sw}$  differed among nutrients with a Mann–Whitney test.

### 3.2.3 Relative contribution of riparian groundwater and in-stream nutrient processing to stream nutrient fluxes

To assess the relevance of  $F_{sw}$  compared to input solute fluxes, we calculated the ratio between  $F_{sw} \times x$  (absolute value) and the total input flux ( $F_{in}$ ) for each solute and sampling date. For the two spatial scales (segment and whole reach),  $F_{in}$  was the sum of upstream ( $F_{top}$ ), tributaries ( $F_{ef}$ ), and net riparian groundwater inputs ( $F_{gw}$ ). The latter was included when  $Q_{gw} > 0$ . We interpreted a high  $|F_{sw} \times x / F_{in}|$  ratio as a strong potential of in-stream processes to modify input fluxes (either as a consequence of gross uptake or release). For each spatial scale, we explored whether  $|F_{sw} \times x / F_{in}|$  differed among nutrients with a Mann–Whitney test.



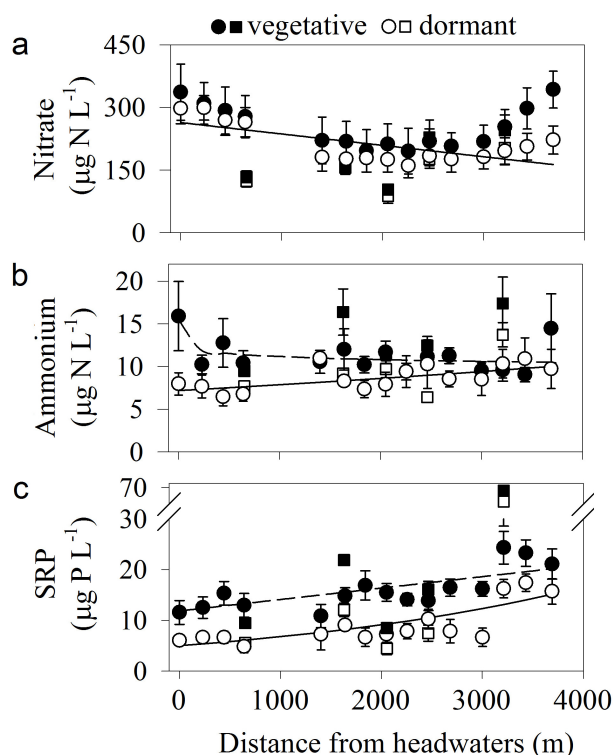
**Figure 4.** Longitudinal pattern of (a) area-specific stream discharge, (b) cumulative area-specific net groundwater inputs along the reach, and (c) stream chloride concentration. Symbols are average and standard error (whiskers) for the main stream (circles) and tributaries (squares). Stream chloride concentration in tributaries is shown separately for the dormant (white) and vegetative (black) period. Tributaries showed no differences in discharge between the two periods. Model regressions are indicated with a solid line only when significant (tributaries not included in the model).

We used a whole-reach mass balance approach to assess the relative contribution of net riparian groundwater inputs ( $(F_{gw} > 0) / F_{in}$ ) to stream solute fluxes. In addition, we calculated the contribution of upstream ( $F_{top} / F_{in}$ ) and tributary inputs ( $F_{ef} / F_{in}$ ) to stream solute fluxes. For each solute, we analyzed differences in the relative contribution of different sources to stream input fluxes with a Mann–Whitney test. Finally, when the whole reach was acting as a net sink for a particular nutrient ( $F_{sw} > 0$ ), we calculated the relative contribution of in-stream net uptake to reduce stream nutrient fluxes along the 3.7 km reach with  $F_{sw} \times x / F_{in}$ .

## 4 Results

### 4.1 Hydrological characterization of the stream reach

During the study period, mean  $Q'$  decreased from  $0.82 \pm 0.13$  [mean  $\pm$  SE] to  $0.54 \pm 0.11$   $mm\ d^{-1}$  along the reach (linear regression [l.reg],  $r^2 = 0.79$ , degrees of freedom [df] = 14,  $F = 51.4$ ,  $p < 0.0001$ ) (Fig. 4a). This pat-



**Figure 5.** Longitudinal pattern of stream nutrient concentrations for (a) nitrate, (b) ammonium, and (c) solute reactive phosphorus at Font del Regàs. Symbols are average and standard error (whiskers) for the main stream (circles) and tributaries (squares). Lines indicate significant longitudinal trends for the dormant (solid) and vegetative (dashed) period (tributaries not included in the model).

tern hold for the two seasonal periods considered (dormant and vegetative; Wilcoxon rank sum test,  $p > 0.05$ ).

On average, the stream had net water gain along the 3.7 km reach, though the hydrological interaction between the riparian zone and the stream was highly variable across contiguous segments (Fig. 4b). The stream was consistently gaining water along the first 1.5 km and the last 0.5 km, while hydrological losses were evident along the intermediate 2 km (Fig. 4b). At the whole-reach scale, gross hydrological gains exceed gross losses in 8 out of 10 field dates (Fig. 2c and d). This was especially noticeable in April and December 2011, the two sampling dates most influenced by storm events. In contrast, the whole reach was acting as net hydrological losing in March and October 2011.

Stream  $\text{Cl}^-$  concentrations showed a 40 % increase along the reach (l.reg,  $r^2 = 0.88$ ,  $df = 14$ ,  $F = 44.6$ ,  $p < 0.0001$ ), which contrasted with the longitudinal pattern exhibited by stream discharge (Fig. 4c). The two periods showed a similar longitudinal pattern, though stream  $\text{Cl}^-$  concentration was lower during the dormant than during the vegetative period (Wilcoxon rank sum test,  $Z = -6.4$ ,  $p < 0.0001$ ) (Table 1). The same seasonal pattern was exhibited by the five permanent tributaries (Fig. 4c). There was a strong correlation be-

tween stream and riparian groundwater  $\text{Cl}^-$  concentrations, which fitted well to the 1 : 1 line (low RRMSE for the two periods) (Table 2 and Fig. S2).

The median net change in  $\text{Cl}^-$  flux within individual segments was  $6 \mu\text{g m}^{-1} \text{s}^{-1}$ , which represented a small fraction of the  $\text{Cl}^-$  input flux ( $|F_{\text{sw}} \times x / F_{\text{in}}| = 3 \%$ ). Similar results were obtained when calculating  $\text{Cl}^-$  budgets for the whole-reach approach (Table 3). The stream  $\text{Cl}^-$  flux was mainly explained by inputs from tributaries followed by riparian groundwater and upstream. Similar results were obtained when calculating the relative contribution of different water sources to stream discharge at the whole-reach scale (Table 4).

#### 4.2 Longitudinal pattern of stream nutrient concentration

The longitudinal pattern of stream concentration differed between nutrients and periods. During the dormant period, stream  $\text{NO}_3^-$  concentration decreased along the reach, especially within the first 1.5 km (l.reg,  $r^2 = 0.47$ ,  $df = 15$ ,  $F = 11.4$ ,  $p < 0.005$ ) (Fig. 5a). During the vegetative period, stream  $\text{NO}_3^-$  concentration showed a U-shaped pattern: it decreased along the first 1.5 km, remained constant along the following 1 km, and increased by 60 % along the last kilometer of the reach (Fig. 5a). Despite these differences, stream  $\text{NO}_3^-$  concentration was similar between the dormant and vegetative period for both the main stream and tributaries (Wilcoxon rank sum test:  $p > 0.05$  in all cases) (Table 1).

Stream  $\text{NH}_4^+$  concentration showed an increasing longitudinal pattern during the dormant period (exponential regression [e.reg],  $r^2 = 0.45$ ,  $df = 15$ ,  $F = 10.5$ ,  $p < 0.01$ ), while concentration decreased during the vegetative period (logarithmic regression [lg.reg],  $r^2 = 0.42$ ,  $df = 15$ ,  $F = 9.6$ ,  $p < 0.01$ ) (Fig. 5b). The main stream showed higher  $\text{NH}_4^+$  concentration during the vegetative than during the dormant period (Wilcoxon rank sum test,  $Z = -3.5$ ,  $p < 0.001$ ) (Table 1). For the tributaries,  $\text{NH}_4^+$  concentration was similar between the two periods (Wilcoxon rank sum test:  $p > 0.01$  in all cases).

Stream SRP concentration increased along the reach during both the dormant (e.reg,  $r^2 = 0.59$ ,  $F = 18.5$ ,  $df = 14$ ,  $p < 0.01$ ) and vegetative period (l.reg,  $r^2 = 0.49$ ,  $F = 12.4$ ,  $df = 14$ ,  $p < 0.01$ ) (Fig. 5c). Similar to  $\text{NH}_4^+$ , the main stream showed higher SRP concentration during the vegetative than during the dormant period (Wilcoxon rank sum test,  $Z = -6.6$ ,  $p < 0.001$ ) (Table 1). For the tributaries, SRP concentration was similar between the two periods (Wilcoxon rank sum test:  $p > 0.01$  in all cases).

#### 4.3 Sources of variation in stream nutrient concentration

*Riparian groundwater inputs.* The relationship between stream and riparian groundwater concentrations differed be-

**Table 1.** Median and interquartile range [25th, 75th percentiles] of stream and riparian groundwater solute concentrations for the dormant and vegetative period. The number of cases is shown in parentheses for each group. For each variable, the asterisk indicates statistically significant differences between the two water bodies (Wilcoxon paired rank sum test, \*  $p < 0.01$ ).

		Stream	Riparian groundwater
Dormant	Cl <sup>-</sup> (mg L <sup>-1</sup> )	7.6 [6.5, 8] (60)	7.7 [7.2, 8.8] (57)*
	N-NO <sub>3</sub> <sup>-</sup> (μg L <sup>-1</sup> )	192 [159, 262] (60)	194 [109, 298] (56)
	N-NH <sub>4</sub> <sup>+</sup> (μg L <sup>-1</sup> )	8.9 [6.5, 10.3] (60)	19 [13.8, 34.2] (56)*
	SRP (μg L <sup>-1</sup> )	7.6 [4.5, 11.7] (60)	8 [6, 20] (51)
	DO (mg L <sup>-1</sup> )	12.9 [11.5, 16] (60)	3.5 [1.5, 4.6] (54)*
Vegetative	Cl <sup>-</sup> (mg L <sup>-1</sup> )	8.8 [7.9, 13.5] (100)	10.1 [8.6, 15] (98)*
	N-NO <sub>3</sub> <sup>-</sup> (μg L <sup>-1</sup> )	223 [155, 282] (102)	168 [77, 264] (98)*
	N-NH <sub>4</sub> <sup>+</sup> (μg L <sup>-1</sup> )	10 [8.7, 12.8] (103)	27 [18.2, 37.1] (101)*
	SRP (μg L <sup>-1</sup> )	16.5 [11.7, 21.3] (103)	14.1 [9.3, 23.3] (97)
	DO (mg L <sup>-1</sup> )	9.9 [9.1, 11.1] (84)	1.7 [0.8, 2.5] (98)*

**Table 2.** Spearman  $\rho$  coefficient between stream water and riparian groundwater solute concentrations for each period and for the whole data set collected at the Font del Regàs during the study period. The relative root-mean-square error (RRMSE) indicates divergences from the 1 : 1 line.  $n$  = number of cases. \*  $p < 0.01$ . ns: not significant.

		Dormant			Vegetative			All data		
	$\rho$	RRMSE (%)	$n$	$\rho$	RRMSE (%)	$n$	$\rho$	RRMSE (%)	$n$	
Cl <sup>-</sup>	0.78*	2.1	53	0.8*	2.9	98	0.84*	2.8	151	
N-NO <sub>3</sub> <sup>-</sup>	0.48*	8.1	57	0.34*	8.3	101	0.37*	6	158	
N-NH <sub>4</sub> <sup>+</sup>	ns	11.7	57	ns	9.1	101	ns	7.3	158	
SRP	ns	17.9	57	0.43*	5.5	101	0.41*	7.3	158	

**Table 3.** Median and interquartile range [25th, 75th percentile] of in-stream net nutrient uptake flux ( $F_{sw}$ ) and the potential of  $F_{sw}$  to modify solute input fluxes ( $|F_{sw} \times x / F_{in}|$ ) for the two spatial scales considered (stream segment and whole reach) during the study period.  $n = 150$  and 10 for segments and whole-reach data sets, respectively.

		By segment	By whole reach
$F_{sw}$ (μg m <sup>-1</sup> s <sup>-1</sup> )	Cl <sup>-</sup>	6 [-37, 80]	12 [2, 33]
	N-NO <sub>3</sub> <sup>-</sup>	-0.43 [-4.4, 1.3]	-0.97 [-3.4, 1.6]
	N-NH <sub>4</sub> <sup>+</sup>	0.17 [-0.06, 0.63]	0.2 [-0.02, 1.1]
	SRP	0 [-0.6, 0.21]	-0.06 [-0.21, 0.01]
$ F_{sw} \times x / F_{in} $ (%)	Cl <sup>-</sup>	3 [1, 10]	4 [2, 9]
	N-NO <sub>3</sub> <sup>-</sup>	6 [2, 14]	24 [8, 67]
	N-NH <sub>4</sub> <sup>+</sup>	18 [9.5, 35]	48 [25, 71]
	SRP	20.5 [3.4, 41]	15.5 [6, 66]

tween nutrients and periods. During the dormant period, stream and riparian groundwater NO<sub>3</sub><sup>-</sup> concentrations were similar, while the stream showed higher concentration during the vegetative period (Table 1). During the two periods, stream and riparian groundwater NO<sub>3</sub><sup>-</sup> concentrations were positively correlated and showed relatively small RRMSE (Table 2 and Fig. S2). NH<sub>4</sub><sup>+</sup> concentration in stream water was 2–3 times lower than in riparian groundwater (Table 1),

and stream and groundwater concentrations were no correlated either during the dormant or vegetative periods (Table 2). Stream and riparian groundwater SRP concentrations were similar in the two periods (Table 1). During the dormant period, SRP concentration showed a significant correlation between the two water bodies, while no correlation and relatively high RRMSE occurred during the vegetative period (Table 2). The differences in nutrient concentrations between stream and riparian groundwater in the two study periods were accompanied by consistently higher DO concentrations in the stream than in riparian groundwater (Table 1).

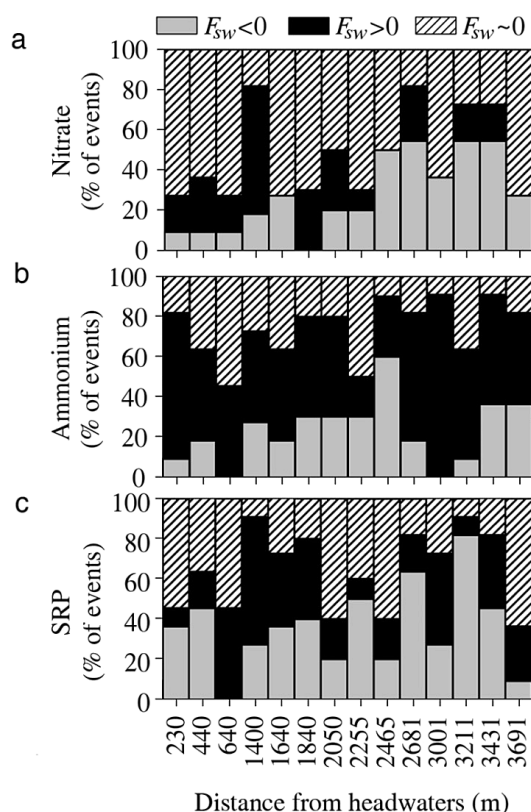
*In-stream nutrient processing.* The influence of in-stream nutrient processing on stream water chemistry differed among nutrients. During the study period, median  $F_{sw}$  was negative for NO<sub>3</sub><sup>-</sup>, positive for NH<sub>4</sub><sup>+</sup>, and close to 0 for SRP (Table 3). However, between-nutrient differences in  $F_{sw}$  were not statistically significant for either the vegetative or dormant period (for both periods: Mann–Whitney test with post hoc Tukey test,  $p > 0.05$ ). Similar  $F_{sw}$  values were obtained when calculating nutrient budgets either by segment or whole reach (Table 3).

The frequency of an individual segment to act either as a nutrient sink or source differed among nutrients and along the reach. For NO<sub>3</sub><sup>-</sup>, the frequency of  $F_{sw,NO_3} < 0$  (gross uptake < release) increased from 9 to > 50 % along



**Table 4.** Median and interquartile range [25th, 75th percentile] of the relative contribution of inputs from upstream ( $F_{\text{top}}/F_{\text{in}}$ ), net riparian groundwater ( $(F_{\text{gw}} > 0)/F_{\text{in}}$ ), tributaries ( $F_{\text{ef}}/F_{\text{in}}$ ), and in-stream release ( $(F_{\text{sw}} < 0)/F_{\text{in}}$ ) to stream solute fluxes at the whole-reach scale. Note that relative contributions from different sources do not add up to 100 % because they are medians rather than means. For each solute, different letters indicate statistically significant differences between sources (Mann–Whitney test with post hoc Tukey test,  $p < 0.01$ ).  $n = 10$  for the four solutes.

Relative contribution (%)	$\text{Cl}^-$	$\text{N-NO}_3^-$	$\text{N-NH}_4^+$	SRP
Upstream	15 [12, 17] <sup>B</sup>	22 [20, 35] <sup>A</sup>	8 [6, 13] <sup>BC</sup>	11 [6, 17] <sup>B</sup>
Riparian groundwater	28 [14, 38] <sup>B</sup>	17 [5, 47] <sup>A</sup>	63 [43, 75] <sup>A</sup>	21 [7, 38] <sup>AB</sup>
Tributaries	59 [46, 69] <sup>A</sup>	22 [19, 24] <sup>A</sup>	21 [17, 30] <sup>B</sup>	34 [26, 50] <sup>A</sup>
In-stream release	0 [0, 0.3] <sup>C</sup>	22 [0, 50] <sup>A</sup>	0 [0, 6] <sup>C</sup>	19 [0, 55] <sup>B</sup>



**Figure 6.** Frequency of dates for which  $F_{\text{sw}} < 0$  (gross uptake < release),  $F_{\text{sw}} > 0$  (gross uptake > release), and  $F_{\text{sw}} \sim 0$  (gross uptake  $\sim$  release) for (a) nitrate, (b) ammonium, and (c) soluble reactive phosphorus for the 14 contiguous segments along the study reach from August 2010 to December 2011 ( $n = 11$ ). The frequency is expressed as number of events in relative terms.

the reach (l.reg,  $r^2 = 0.55$ ,  $df = 13$ ,  $F = 14.67$ ,  $p < 0.01$ ) (Fig. 6a). For  $\text{NH}_4^+$ , the frequency of  $F_{\text{sw}, \text{NH}_4} > 0$  (gross uptake > release) was high across individual segments, ranging from 20 to 90 % (Fig. 6b). For SRP, the frequency of  $F_{\text{sw}, \text{SRP}} < 0$ ,  $> 0$ , or  $\sim 0$  did not show any consistent longitudinal pattern (Fig. 6c). Overall, the frequency of sampling dates for which in-stream biogeochemical processes were imbalanced

( $F_{\text{sw}} \neq 0$ ) was lower for  $\text{NO}_3^-$  (36 %) than for  $\text{NH}_4^+$  (80 %) and SRP (68 %) (Fig. 6).

#### 4.4 Relative contribution of riparian groundwater and in-stream processing to stream nutrient fluxes at the segment and whole-reach scale

The capacity of in-stream processes to modify stream input fluxes differed between nutrients and spatial scales. For individual segments,  $|F_{\text{sw}} \times x/F_{\text{in}}|$  was smaller for  $\text{NO}_3^-$  (6 %) than for  $\text{NH}_4^+$  and SRP ( $\sim 20$  %) (Mann–Whitney test with post hoc Tukey test,  $p < 0.01$ , Table 3). However,  $|F_{\text{sw}} \times x/F_{\text{in}}|$  increased substantially for  $\text{NO}_3^-$  and  $\text{NH}_4^+$  when nutrient budgets were calculated at the whole-reach scale (Table 3).

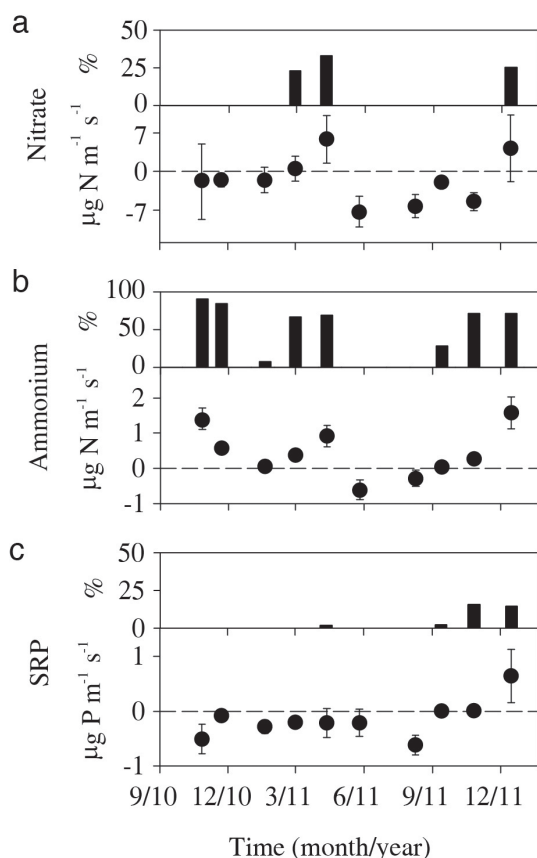
According to whole-reach mass balance calculations, the stream acted as a net source of  $\text{NO}_3^-$  on 7 out of the 10 sampling dates for which whole-reach budgets were calculated. The contribution of in-stream release to stream  $\text{NO}_3^-$  fluxes was as important as that of riparian groundwater and upstream fluxes (Table 4). In-stream net  $\text{NO}_3^-$  retention at the whole-reach scale was observed only in spring (March and April 2011) and December 2011 (Fig. 7a).

In contrast to  $\text{NO}_3^-$ , the stream generally acted as a net sink of  $\text{NH}_4^+$ , and it retained up to 90 % of the input fluxes in spring and autumn (Fig. 7b). The stream acted as a source of  $\text{NH}_4^+$  in summer (Fig. 7b), though the contribution of in-stream release to stream  $\text{NH}_4^+$  fluxes was minimal compared to that from riparian groundwater (Table 4).

The stream acted as a net source of SRP in 6 out of the 10 sampling dates. The contribution of in-stream release to stream SRP fluxes was as important as that of riparian groundwater (Table 4). In-stream net SRP retention was minimal, except in autumn 2011 (October and December 2011) (Fig. 7c).

## 5 Discussion

In terms of hydrology, the study headwater stream was a net gaining reach, though the hydrological interaction between the riparian zone and the stream was complex as in-



**Figure 7.** Temporal pattern of in-stream net nutrient uptake ( $F_{sw}$ , in  $\mu\text{g m}^{-1} \text{s}^{-1}$ ) for (a) nitrate, (b) ammonium, and (c) soluble reactive phosphorus at the whole-reach scale. Whiskers are the uncertainty associated with the estimation of stream discharge from slug tracer additions.  $F_{sw} > 0$  indicates that gross uptake prevailed over release, while  $F_{sw} < 0$  indicates the opposite. For those cases for which  $F_{sw} > 0$ , the contribution of in-stream net nutrient uptake to reduce stream nutrient fluxes ( $F_{sw} \times x / F_{in}$ , in %) is shown (black bars).

indicated by the longitudinal variation in net riparian groundwater inputs. Moreover, the longitudinal decrease in area-specific discharge suggests that hydrological retention increased at the valley bottom compared to upstream segments as reported in previous studies (Covino et al., 2010). Despite the complex hydrological processes along the reach, the strong positive correlation between stream and riparian groundwater  $\text{Cl}^-$  concentration suggests high hydrological connectivity at the riparian–stream interface (Butturini et al., 2003). In addition, we found that the permanent tributaries, which comprised  $\sim 50\%$  of the catchment area, contributed 56 % of stream discharge, and thus were an essential component for understanding stream nutrient chemistry and loads. Hydrological mixing of stream water with water from tributaries could partially explain the longitudinal increase in  $\text{Cl}^-$  because its concentration was higher at the tributaries than at the main stream, especially during the vegetative pe-

riod. In addition, riparian groundwater inputs to the stream could further contribute to the longitudinal increase in stream  $\text{Cl}^-$  concentration because they contributed 26 % of stream discharge and also exhibited higher  $\text{Cl}^-$  concentration than stream water.

Based on the strong hydrological connectivity between the stream and the riparian groundwater and the large contribution of tributaries to stream discharge, one would expect a strong influence of these water sources on the longitudinal variation in stream nutrient chemistry. However, the relationship between stream and riparian groundwater nutrient concentration was from moderate to weak for  $\text{NO}_3^-$  and SRP, and zero for  $\text{NH}_4^+$ . Further, the contribution of tributaries to stream nutrient fluxes was relatively small (from 21 to 34 %) compared to their contribution to stream  $\text{Cl}^-$  and water fluxes ( $> 50\%$ ). Together these data suggest that longitudinal patterns of stream nutrient concentration could not be explained by hydrological mixing alone, thus pointing to in-stream biogeochemical processing as a likely mechanism to modify nutrient concentrations along the study reach. In fact, the estimates of in-stream net nutrient uptake ( $F_{sw}$ ) at the different stream segments supported this idea and agreed with previous studies showing that in-stream processes can mediate stream nutrient chemistry and downstream nutrient export (McClain et al., 2003; Harms and Grimm, 2008).

Our results revealed an extremely high variability in  $F_{sw}$ , which could range by up to one order of magnitude, across individual segments and over time, which agrees with findings from other headwater streams (von Schiller et al., 2011). However, some general trends appeared when comparing patterns for the different studied nutrients. For instance, the frequency of dates for which in-stream gross uptake and release were imbalanced ( $F_{sw} \neq 0$ ) was higher for  $\text{NH}_4^+$  (80 %) and SRP (68 %) than for  $\text{NO}_3^-$  (37 %). Further, the potential of in-stream processes to modify stream fluxes within stream segments ( $|F_{sw} \times x / F_{in}|$ ) was 3-fold higher for  $\text{NH}_4^+$  and SRP than for  $\text{NO}_3^-$ . Our findings are concordant with studies performed at short stream reaches ( $< 300 \text{ m}$ ) worldwide, which show that in-stream gross uptake velocity (as a proxy of nutrient demand) is typically higher for  $\text{NH}_4^+$  and SRP than for  $\text{NO}_3^-$  (Ensign and Doyle, 2006). This difference among nutrients is commonly attributed to the higher biological demand for  $\text{NH}_4^+$  and SRP than for  $\text{NO}_3^-$ . However, we found that  $F_{sw}$  was similar among nutrients; thus, differences in  $|F_{sw} \times x / F_{in}|$  were mainly associated with differences in the concentration of the inputs, which tend to be 20-fold lower for  $\text{NH}_4^+$  and SRP than for  $\text{NO}_3^-$ . Divergences between  $F_{sw}$  and  $|F_{sw} \times x / F_{in}|$  were even more remarkable when nutrient budgets were considered at the whole-reach scale, especially for DIN forms.  $\text{NO}_3^-$  and  $\text{NH}_4^+$  showed no differences in  $F_{sw}$  between the two scales of observation; however, they showed a substantial increase in  $|F_{sw} \times x / F_{in}|$  at the whole-reach scale (length of kilometers) compared to the segment scale (length of hundreds of meters). Similarly, previous nutrient

spiraling studies have reported an increase in the proportion of nutrient removal with stream order despite no changes in gross uptake rates among stream reaches (Ensign and Doyle, 2006; Wollheim et al., 2006). This pattern has been attributed to variation in intrinsic stream characteristics, such as stream nutrient concentration, discharge, stream width, and the size of the hyporheic zone (Wollheim et al., 2006; Alexander et al., 2009), which may also hold for our study since these characteristics varied along the 3.7 km reach. However, our results also indicate that the assessment of riparian groundwater inputs is crucial to understand the contribution of in-stream processes to stream nutrient fluxes. Overall, our findings add to the growing evidence that streams are hot spots of nutrient processing (Peterson et al., 2001; Dent et al., 2007), and that in-stream processes can substantially modify stream nutrient fluxes at the catchment scale (Ensign and Doyle, 2006; Bernal et al., 2012).

The potential of in-stream processes to regulate stream nutrient fluxes was especially remarkable for  $\text{NH}_4^+$ . There was no relationship between stream and riparian groundwater  $\text{NH}_4^+$  concentrations; further, whole-reach budgets indicated that in-stream net uptake could reduce the flux of  $\text{NH}_4^+$  up to 90 % along the reach. This high in-stream bioreactive capacity could be favored by the sharp increase in redox conditions from riparian groundwater to stream water (Hill et al., 1998; Dent et al., 2007). Concordantly,  $\text{NH}_4^+$  concentrations were higher in riparian groundwater than in the stream, while the opposite occurred for  $\text{NO}_3^-$  (although only during the vegetative period). These results suggest fast nitrification of groundwater inputs within the stream as environmental conditions become well oxygenated (Jones et al., 1995). However, the marked increase in stream  $\text{NO}_3^-$  concentration observed along the last 700 m of the reach during the vegetative period could not be explained entirely by nitrification of riparian groundwater  $\text{NH}_4^+$  because this flux ( $F_{\text{gw}, \text{NH}_4} \sim 2 \mu\text{g N m}^{-1} \text{ s}^{-1}$ ) was not large enough to sustain in-stream  $\text{NO}_3^-$  release [ $F_{\text{sw}, \text{NO}_3} < 0$ ] ( $\sim 10 \mu\text{g N m}^{-1} \text{ s}^{-1}$ ). This finding suggests an additional source of N at the valley bottom. Previous studies have shown that leaf litter from riparian trees, and especially from  $\text{N}_2$ -fixing species, can enhance in-stream nutrient cycling because of its high quality and degradability (Starry et al., 2005; Mineau et al., 2011). Thus, the increase in  $\text{NO}_3^-$  and SRP concentrations and in-stream  $\text{NO}_3^-$  release observed at the lowest part of the catchment during the vegetative period could result from the combination of warmer temperatures and the mineralization of large stocks of alder and black locust leaf litter stored in the stream bed (Strauss and Lamberti, 2000; Bernhardt et al., 2002; Starry et al., 2005). Alternatively, increases in stream  $\text{NO}_3^-$  and SRP concentration could result from human activities, which were concentrated at the lowest part of the catchment. However, regarding  $\text{NO}_3^-$ , anthropogenic sources seem unlikely because DIN concentrations at the tributary draining through the inhabited area were low. In contrast, this tributary showed

high SRP concentrations (from 2- to 6-fold higher than in the main stream), though its discharge would have had to be ca. 4 times higher than expected for its drainage area ( $< 0.4 \text{ km}^2$ ) to explain the observed changes in concentration. Another possible explanation for the increase in stream N concentration at the valley bottom could be increased N fixation by stream algae (Finlay et al., 2011). However, in-stream DIN release ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) peaked in late spring and summer (May and August 2011), when light penetration was limited by riparian canopy and in-stream photoautotrophic activity was low (Lupon et al., 2015). Altogether, these data suggest that the sharp increase in nutrient availability along the last 700 m of the reach was likely related to the massive presence of the invasive black locust at the valley bottom. Black locust is becoming widespread throughout riparian floodplains in the Iberian Peninsula (Castro-Díez et al., 2014), and its potential to subsidize N to stream ecosystems via root exudates and leaf litter could dramatically alter in-stream nutrient processing and downstream nutrient export (e.g., Stock et al., 1995; Mineau et al., 2011). However, further research is needed to test the hypothesis that this invasive species can alter stream nutrient dynamics in riparian floodplains.

It is worth noting that longitudinal trends in stream nutrient concentrations showed no simple relationship to in-stream processes. This finding evidenced that other sources of variation in stream water chemistry were counterbalancing the influence of in-stream processes on stream nutrient fluxes. In this sense, results from  $\text{NH}_4^+$  were paradigmatic. The mass balance approach clearly showed that in-stream gross uptake of  $\text{NH}_4^+$  exceeded release; concordantly,  $\text{NH}_4^+$  concentration was consistently lower in the stream than in riparian groundwater. However, stream  $\text{NH}_4^+$  concentration showed small longitudinal variation likely because in-stream net uptake balanced the elevated inputs from riparian groundwater. Therefore, our results challenge the idea that stream nutrient concentration should decrease in the downstream direction when in-stream processes are efficient in taking up nutrients from receiving waters (Brookshire et al., 2009). Conversely, our findings convincingly show that in-stream processes can strongly affect stream nutrient chemistry and downstream nutrient export even in the absence of consistent longitudinal gradients in nutrient concentration. For  $\text{NO}_3^-$ , our data suggest that the marked increase in concentration along the last 700 m could be a consequence of in-stream mineralization of N-rich leaf-litter stocks. However, the observed decrease in  $\text{NO}_3^-$  concentration along the first 1.5 km of the reach could barely be explained by in-stream processing alone because its contribution to reduce stream  $\text{NO}_3^-$  fluxes was too low, even when the whole-reach budget was recalculated excluding the last 700 m of the reach ( $F_{\text{sw}} = 0.61 \mu\text{g N m}^{-1} \text{ s}^{-1}$  and  $(F_{\text{sw}} > 0)/F_{\text{in}} = 10\%$ ). Therefore, the declining pattern was likely a combination of both in-stream nutrient processing and hydrological mixing with riparian groundwater and tributary inputs. For SRP, the longitudinal increase in concentration could neither be fully ex-

plained by in-stream release because  $F_{sw, SRP} < 0$  was not widespread along the reach and the stream only contributed to input fluxes by 19 % (6 % when excluding the last 700 m). Again, stream nutrient chemistry along the reach was the combination of both in-stream nutrient processing and hydrological mixing as indicated by our whole-reach mass balance. Recent studies have concluded that riparian groundwater is a major driver of longitudinal patterns in stream nutrient concentration in headwater streams (Bernhardt et al., 2002; Asano et al., 2009; Scanlon et al., 2010). Our study adds to our knowledge of catchment biogeochemistry by showing that stream nutrient chemistry results from the combination of both hydrological mixing from the riparian zone and in-stream nutrient processing, which can play a pivotal role in shaping stream nutrient concentrations and fluxes at the catchment scale.

## 6 Conclusions

The synoptic approach adopted in this study highlighted that the Font del Regàs stream had a strong potential to transform nutrients. The longitudinal pattern in stream nutrient concentrations could not be explained solely by hydrological mixing with riparian groundwater and tributary sources because dissolved nutrients underwent biogeochemical transformation while traveling along the stream channel. Our results revealed that in-stream processes were highly variable over time and space, though in most cases this variability could not be associated with either physical longitudinal gradients or shifts in environmental conditions between the dormant and vegetative period. Nevertheless, results from a mass balance approach showed that in-stream processes contributed substantially to modify stream nutrient fluxes and that the stream could act either as a net nutrient sink (for  $\text{NH}_4^+$ ) or as a net nutrient source (for SRP and  $\text{NO}_3^-$ ) at the catchment scale. These results add to the growing evidence that in-stream biogeochemical processes need to be taken into consideration in either empirical or modeling approaches if we are to understand drivers of stream nutrient chemistry within catchments.

Recent studies have proposed that riparian groundwater is a major control of longitudinal patterns of nutrient concentration because in-stream gross nutrient uptake and release tend to counterbalance each other most of the time (Brookshire et al., 2009; Scanlon et al., 2010). Conversely, our study showed that in-stream processes can influence stream nutrient chemistry and downstream exports without generating longitudinal gradients in concentration and flux because changes in stream nutrient chemistry are the combination of both in-stream processing and nutrient inputs from terrestrial sources. Our results imply that the assessment of these two sources of variation in stream nutrient chemistry is crucial to understand the contribution of in-stream processes to stream nutrient dynamics at relevant ecological scales.

Reliable measurements of riparian groundwater inputs are difficult to obtain because spatial variability can be high (Lewis et al., 2006) and determination of the chemical signature of the groundwater that really enters the stream is still a great challenge (Brookshire et al., 2009). In this study, we installed 15 piezometers along the reach (one per sampling site), which may not be representative enough of the variation in riparian groundwater chemistry. However, and despite its limitations, riparian groundwater sampling near the stream can help to constrain the uncertainty associated with this water source and provide more reliable estimations of in-stream net nutrient uptake for both nutrient mass balance and spiraling empirical approaches (von Schiller et al., 2011).

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