

**AUTHOR RESPONSE.**

Ref.: Ms. Bg-2014-324. Title: *Riparian and in-stream controls on nutrient concentrations and fluxes in a headwater forested stream.*

Authors: Bernal, S., Lupon, A., Ribot, M., Sabater, F., Martí, Eugènia.

Dear Tom Battin and referees of *Biogeosciences*,

We would like to express our most sincere gratitude for your thoughtful comments and suggestions on the manuscript *Riparian and in-stream controls on nutrient concentrations and fluxes in a headwater forested stream*. We feel flattered that you consider our study *really clever* (R1), *challenging and attractive* (R2), and *pioneering* and that *it could potentially be an important contribution of our understanding of to what extent in-stream biogeochemical processes influence stream chemistry* (R3).

This is the sort of question we have been asking ourselves for a long time, and we carefully thought about how to conduct an empirical study to assess it at a relevant ecological scale. We agree with you that we have generated a “*large data set to tell a big story*” and that the paper needed to be improved and clarified in order to guide the readers through it. We have done a big effort in that sense from the introduction to the discussion following your suggestions. Moreover, we agree that the hydrological part of the study needed to be carefully reconsidered and we have reanalysed the data set thoughtfully to solve the cavities highlighted by you.

We are especially grateful to R3. First, for taking the time and patience to dive through the manuscript. Second, for the clarity in explaining what he/she felt was missing, wrong, or right. Third, for giving us clues that were essential to improve the paper and bring it, from our modest point of view, to a higher level. Our feeling is that the overall history was becoming crystal clear as we started calculating water and solute budgets at the whole-reach scale; just like if we had changed our googles. We felt a little bit overwhelmed at the beginning because this new set of results outcompeted part of the data analysis we proposed in the former version of the manuscript. Yet, we strongly believe that these new results have enormously contributed to improve the

manuscript because now we can sharply address our primeval question and further, they help us to solve several of the points highlighted not only by R3 but also by R1 and R2.

We provide below our detailed responses to the referees' comments. Your comments are listed in italics and our responses are inserted after each comment. In almost all cases, we have done what you have suggested. If we have revised things in a different way, or disagreed, we have stated why. If your suggestion was not applicable anymore in the new version of the manuscript, we have stated so. In each case, we indicate the location of the changes made, i.e. pp and lines in the final manuscript in \*.doc. We believe that the changes made are easier to follow in this way than by providing a marked-up version of the former manuscript.

We look forward hearing from you soon, and wish you a Happy New Year!

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## **Author Response to Reviewer #1**

*This manuscript uses a large synoptic stream and riparian nutrient dataset to tease out relative contribution of riparian zone and in-stream biotic processes on downstream nutrient fluxes. Very little research has addressed this question, thus the authors describe a novel finding. Their approach is really clever and the authors have a great dataset to test these ideas. However I have a lot of questions and suggestions to guide a revision.*

*This paper was a bit tough for me to follow. Part of it is that the authors are putting together a large dataset to tell a big story; I commend them for this. But this approach means that they need some sort of road map or conceptual guide for the readers to follow through the manuscript. Ways to do this may include some sort of conceptual model (see e.g. the approach taken Payn et al.), a results section that better describes the findings and how the data support these findings. I note that the discussion was much clearer in reporting the results than was the results section. It may also be possible to leave out some parts that do not add to the story the authors want to tell. E.g. the hillslope data in the discussion were not well integrated in the rest of the story.*

*The authors used linear regression and GLM to examine change along the 15 sites of the reach. That probably works well to estimate if e.g. something doubled, but tests of significance will be hampered by the fact that these sites are not independent of each other. Processes at upstream sites determine those at downstream sites. In the case of nutrients it is likely the same molecules. Statistical test should consider this fact, and if they don't then the authors need to justify that point. I regret to not being an expert in spatial statistics, but something along the line of a generalized least squares regression may help. See, e.g., Ives and Zhu, *Ecol. Apps.* 16:2-32 for possible ideas on what, if anything, to do.*

*I am having trouble with the idea of only focusing net groundwater input when in fact there may be new groundwater entering a stream reach with concomitant losses. Thus net will be zero, but there could be a lot of new solute entering the stream via ground- water. See Payn et al *WRR VOL. 45, W11427, doi:10.1029/2008WR007644.**

**Answer:** Many thanks for your positive comments! We agree with the reviewer in that we are putting together a large dataset and that we could have done a better job when explaining the results and the overall message of the study. We are happy because we

have received excellent inputs from the three reviewers and we are convinced that the new version of the manuscript reads better and more smoothly than the former one.

Following the suggestions from the three reviewers, we have included a schematic representation of our mass balance approach, where nutrient fluxes in and out a stream segment are shown (new Figure 3). Moreover, we have included a more detailed explanation of our objectives and expectations in the last paragraph of the introduction (sort of road map), so that the reader is now better guided from the very beginning (pp 5,6-lines 110-119).

In addition, we have excluded several results from the former version that were not adding much to the overall aim of the study (namely, coefficients of variation, area-specific nutrient exports, GLM model, and hillslope data). On the other hand, we have rewritten the results and discussion emphasizing the results (both former and new) that do help to understand the influence of riparian groundwater and in-stream nutrient cycling on stream nutrient chemistry (namely, comparison between stream and riparian groundwater chemistry, tributary inputs, and in-stream net nutrient uptake rates at the segment and whole-reach scale).

We agree with the reviewer that the statistical analysis of this sort of data set needs to be done carefully because contiguous points may not be independent of each other. We considered several possibilities before applying GLM models and finally, we thought that including *distance* explicitly in the model could partially deal with the problem of co-linearity between distance and other explanatory variables. We are neither experts in statistical modelling and there might be better options than this one, as we have learned from the literature you suggested. In any case, we have finally decided to exclude it from the new version of the manuscript. The main reason why we did this statistical analysis was to put into the same picture in-stream processes and riparian groundwater inputs. However, as highlighted by R3, there are other ways much more simple and elegant to do so, such as to calculate nutrient budgets at the whole reach-scale and from there, calculate the relative contribution of each component to total stream nutrient fluxes (which was also a request from R2).

Regarding hydrological groundwater inputs, we agree with the reviewer that concomitant gross hydrological gains and losses may happen within each stream segment and thus, only considering the net groundwater inputs to each segment is an oversimplification of the complex hydrological interactions that occur at the riparian-stream interface. Unfortunately, we do not have enough data to calculate gross gains

and losses as in Payn et al. (2009). Nevertheless, we do now consider this limitation within the paper and we refer to net groundwater inputs more appropriately throughout the text and figures. Moreover, we have reanalyzed our hydrological data set thoughtfully following suggestions from you and R3 in order to get a better understanding of the stream-riparian hydrology in our study site (see our responses to your specific comments).

### ***Specific comments***

598-5 unclear what “which. . .” modifies **Answer:** Clarification not needed now.

599-7 considerably important. **Answer:** OK.

25 See the now classic work of Lowrance et al. on riparian controls of watershed nutrient export. **Answer:** Thanks. Lowrance et al. 1997 included (pp 4-lines 65).

601-6 recast to downstream-most site. **Answer:** OK from here on.

No comma after both. **Answer:** OK from here on.

602-3 two separate findings in one sentence.

**Answer:** The sentence: “The riparian zone covers 6% of the catchment area on average the slope is < 10%”, reads now: “The riparian zone is relatively flat (slope < 10%), and it covers 6% of the catchment area” (pp 7-lines 141).

602-19 composed of. **Answer:** OK

602-22 wetted width. **Answer:** OK

603-22 were used. **Answer:** OK

604-4 If using the molybdate blue technique on filtered samples, then one analyzes soluble reactive P (SRP) of which phosphate is one component.

**Answer:** OK, we do now refer to SRP rather than to  $\text{PO}_4^{3-}$  throughout the text, tables, and figures.

604-20. This method assumes no loss of water along the reach, i.e., the stream is solely gaining. Is this point true for this stream?

**Answer:** As mentioned before, we agree with the reviewer that considering only net groundwater inputs to each segment is an oversimplification of the complex hydrological interactions that occur at the riparian-stream interface. We do now state this limitation in the M&M (pp 10,11-lines 235-238).

As you noted, this stream is not solely gaining water. We now address explicitly this point in two ways following suggestions from you and R3. First, we have calculated the cumulative area-specific net groundwater inputs along the reach by summing up net groundwater inputs ( $Q_{gw}$ ) from the upstream-most site to each of the downstream segments and dividing it by the cumulative catchment area (pp 10-lines 238-242; new Figure 4b).

Second, we have examined whether the reach was either a net gaining or a net losing stream by comparing concurrent hydrological gains and losses over the whole reach for each sampling date. We have considered that stream segments exhibiting  $Q_{gw} > 0$  contributed to hydrological gains ( $\Sigma Q_{gw} > 0$ ) while segments with  $Q_{gw} < 0$  contributed to hydrological losses ( $\Sigma Q_{gw} < 0$ ) (pp 11-lines 243-249). We have carefully thought about whether refer to these water fluxes as *gross* hydrological losses/gains or *whole-reach cumulative net* hydrological losses/gains. Following the suggestion from R3, we have finally considered that at this spatial scale (reach-level) these were *gross* hydrological gains and losses. Again, this approach is an oversimplification of the hydrology at the stream-riparian interface but we got to the conclusion that the question of whether water fluxes can be considered gross or net is, at the end, a question of spatial resolution. In any case, we would be happy to change the terms we are using to refer to these fluxes if you consider that some other terminology would be more accurate.

These new results indicate that, on average, the stream was a net gaining reach though gross hydrological gains exceed gross losses over the reach during two sampling dates (new Figure 2c and d). Further, the stream was a net gaining reach along the first 1.5 km and the last 0.5 km, while hydrological losses were evident along the intermediate 2 km (new Figure 4b) (pp 15-lines 347-349).

604-21. *So divided by watershed area, correct? Unclear as written.*

**Answer:** Yes. We have added “*by dividing instantaneous discharge by catchment area*” (pp 10-lines 223). Thanks.

605-9. *It seems like one could get a lot more information if the data were not averaged for each period but done separately using a multilevel approach.*

**Answer:** The GLM has been excluded from the new version of the manuscript. Thanks for the suggestion anyway.

606-4. *But Table 3 reports a p-value from the ANOVA, which is not a goodness of fit (as I understand it). I am not sure why to report a P-value between the differences in these models anyway.*

**Answer:** The GLM has been excluded from the new version of the manuscript. Thanks for the suggestion anyway.

606-10. *Up to now all of the stats are based on parametric distribution. Why a switch to a non-parametric test here?*

**Answer:** Thanks for noticing. In the former version, we forgot to include that the reason why we switch to non-parametric test is because solute concentrations were not normally distributed, as usual. We have added the following sentence in M&M: “*A non-parametric test was used because solute concentrations were not normally distributed (Shapiro-Wilk test,  $p < 0.01$  for all studied solutes) (Zar, 2010)*” (pp 11-lines 258-260).

Furthermore, we agree with the reviewer that concentrations and fluxes may or may not change linearly over space and thus, we do now consider linear and nonlinear models when examining the longitudinal pattern of discharge and nutrient concentrations (pp 10-lines 226).

606-15. *I usually think of a CI as a confidence interval. This common use of CI may be confusing when redefined as chemical index. Chemical index is by itself not very meaningful, so there may be a better phrase (and therefore acronym) to use.*

**Answer:** Agree. We were struggling for a while trying to find a better acronym and obviously, we did not succeed.

Considering comments from you and R2, we have decided to use dissolved oxygen concentration (DO) instead of the chemical index. DO is easy to interpret and it provides the same information.

608-2. *What about segments that are gaining and losing at the same time, where the nutrient concentration in the groundwater inflow is different than the outflow, which we would assume is the same concentration as the streamwater.*

**Answer:** We agree with the reviewer that some assumptions are needed when applying a mass balance approach, especially because concomitant gross gains and losses were not measured within each stream segment. Note, however, that even if we would have been able to measure those fluxes, we would have had to deal with uncertainty too. For instance, we could have never been sure if gross hydrological gains along each stream segment do actually have the same chemical signature than riparian groundwater measured in the two wells installed in each segment (at the top and at the bottom). Also, we could not know either whether the chemical signal of gross water losses is actually similar to that measured in the stream water column.

In this study, we have characterized to some extent riparian groundwater chemistry along the reach. This is one of the novelties of our design in comparison with other synoptic studies, and we believe that this information, despite being limited, is extremely helpful to constrain the uncertainty associated to the water chemistry of riparian groundwater inputs. Otherwise, we might have had to make even stronger assumptions regarding groundwater chemistry, and the uncertainty associated to  $F_{sw}$  would have been even larger. Nevertheless, we completely agree with you that we need to acknowledge the limitations of our approach and be careful when explaining and discussing these results.

In the new version of the manuscript, we are more explicit about the assumptions made when calculating nutrient groundwater fluxes ( $F_{gw}$ ) (pp 13-lines 292-298). Moreover, we now state that reliable measurements of riparian groundwater inputs are difficult to obtain and that by installing only 15 piezometers along the reach we might be missing part of the intrinsic variation of riparian groundwater chemistry along the study reach. However, and despite its limitations, we highlight that sampling near-stream riparian groundwater can help to constrain the uncertainty associated to this water source and provide more reliable estimations of in-stream net nutrient uptake (pp 26-lines 615-624).

608-18 *SE estimated by averaging over what, time? Also, what is the frequency of  $F_{sw}$  not equal to 0 by chance using this technique? It seems to me that many would be not significant, but that would require a little explanation.*



**Answer:** Yes, we agree that some additional explanation was needed. Thanks for noticing. In the new version of the manuscript, however, we have calculated the upper and lower limit of  $F_{sw}$  based on the empirical uncertainty associated with Q (please, see our responses to your specific comment on this regard). Further, we now assume that  $F_{sw}$  was undistinguishable from 0 when its upper and lower limit contained zero (pp 14-line 311)

We believe that with this approach we are more coherent because the uncertainty associated to water and nutrient fluxes is based on the same grounds throughout the study. Note that this approach is more conservative than the previous one, in the sense that the proportion of cases for which in-stream gross uptake and release counterbalance each other ( $F_{sw} = 0$ ) is higher (please compare former and new figure 6). However, the main results are the same ones: (1) the occurrence of in-stream  $\text{NO}_3^-$  release increases along the reach, (2) in-stream gross uptake tends to prevail over release for  $\text{NH}_4^+$ , and (3) the frequency of  $F_{sw} > 0$ ,  $< 0$ , and  $= 0$  does not show any clear pattern for SRP.

*608-22. But there could be groundwater input in a losing segment.*

**Answer:** Yes, that's right. However, even if we may have had data on gross hydrological gains and losses, we would have had to consider the difference between the two (that is net groundwater inputs) to calculate  $F_{in}$ . Or in other words, it may not be accurate to consider gross hydrological gains in  $F_{in}$  without simultaneously considering that part of this water is simultaneously lost towards the riparian zone and thus, does not contribute ultimately to downstream export.

Taking into account your point, we do now clearly state that  $F_{in}$  as is the sum of input fluxes from upstream, tributaries and *net* riparian groundwater inputs when  $Q_{gw} > 0$  (pp 14-lines 324).

*608-24. Putting the R code and data into an appendix will help readers to replicate this work in the future. This is a really valuable dataset.*

**Answer:** The GLM has been excluded from the new version of the manuscript and thus, this R code is not in the paper anymore. This data set is part of the grounds of the thesis of Anna Lupon who is in her last year of PhD. We have in mind to add these data as Supplementary Material or Appendix as soon as she starts publishing the chapters of her thesis. Hopefully, this will be soon. Thanks for the suggestion.

609-3 Units for this area specific discharge simplify to units of length per time (say mm/d) which is common in hydrological literature and therefore less confusing for some to read. **Answer:** Ok. We have transformed water fluxes to  $\text{mm d}^{-1}$ .

609-15 The Cl data and the Q data in this paragraph are not really well linked, i.e. I am not sure what collective finding they support.

**Answer:** Agree. We have reorganized this section of the results. In the new version, we introduce first results related to water fluxes and then, those related to  $\text{Cl}^-$  concentrations and fluxes. Thanks.

609-20 What is the error in measuring Q via using dilution gaging? Given that there is always some measurement error, streams will always be gaining or losing when calculated as a strict difference. How much does a stream have to gain or lose to detect a difference above the measurement error?

**Answer:** This is a good point. Thanks. We have calculated the uncertainty associated to our empirical estimates of  $Q$  by selecting pairs of slug additions performed under similar water depth conditions (difference in water level at the thalweg  $< 1$  mm). To do so, we have benefited of a data set with 126 slug additions used to calibrate the water level sensor from a parallel study at the Font del Regàs stream. We have considered the relative difference in  $Q$  between these pairs of data as an empirical uncertainty estimator. There were 11 pairs of data that fall within our criterion, and the relative difference in  $Q$  between them was pretty small (1.9%).

In the new version of the manuscript, we have included the estimation of this uncertainty (pp 8,9-lines 186-191). Moreover, we have considered this uncertainty for calculating the upper and lower limits of water fluxes and posteriorly, the uncertainty of nutrient fluxes. The uncertainty associated to stream and groundwater fluxes is now included in the results (see new Figure 2 and 7).

610-23. I assume that these predictor variables do not covary among themselves?

**Answer** The GLM has been excluded from the new version of the manuscript.

610-27. ok so then why fit a straight line to the data?

**Answer:** Agree. We do now describe the longitudinal pattern of  $\text{NO}_3^-$  concentrations more accurately: “During the vegetative period, stream  $\text{NO}_3^-$  showed a U-shaped

*pattern: concentrations decreased along the first 1.5 km, remained constant along the following 1 km, and increased by 60% along the last km of the reach” (pp 16-line 374).*

*612-9 Makes sense given the chemical index uses NH<sub>4</sub> as part of its calculation.*

**Answer:** After taking into account comments from you and R2, we have decided to use dissolved oxygen concentration as a proxy of the redox conditions in stream water and groundwater rather than the chemical index (pp 17-line 407).

*613-1 This paragraph is problematic in that it is repeating things from the introduction. Then it describes the significance of the findings before stating what the main findings are. I note that the results were a difficult place for me to understand the main finding that was mostly a description of the data. In any case this paragraph can be safely deleted. Better, given the results section, would be to summarize the main findings.*

**Answer:** Thanks for the suggestion. This paragraph has been deleted and we have rewritten the discussion to start going directly to the point.

*614 8-12. This clear statement of the findings are restating results.*

**Answer:** We agree with the three reviewers about that. The reanalysis of the hydrological data set motivated by your comments and suggestions has shed new light on what drives the longitudinal pattern of stream Cl<sup>-</sup> concentration. Briefly, we have found that Cl<sup>-</sup> concentration was higher at the tributaries than at the main stream, especially during the vegetative period. Permanent tributaries comprised ca. 50% of the catchment area and were the main contributors to stream discharge (56%). Therefore, hydrological mixing with water from tributaries could partially explain the 40% increase in stream Cl<sup>-</sup> concentrations observed along the reach.

In addition, riparian groundwater inputs could also explained the longitudinal pattern exhibited by stream Cl<sup>-</sup> concentration because this compartment contributed substantially to stream discharge (26%) and also exhibited higher Cl<sup>-</sup> concentration than stream water during the two periods.

These new results and the associated discussion are included in the new version of the manuscript (results section 4.1 and pp 20-line 457-465)

615-6 By saying .."where the N<sub>2</sub> fixers are highest" implies that the N<sub>2</sub> fixer may control NO<sub>3</sub>, but later in the paragraph we are reminded that there was no relationship with N<sub>2</sub> fixers. I would recast to avoid creating confusion here and instead simply state the most plausible mechanism up front, and not one found to be not plausible.

**Answer:** Agree. We have rewritten this part of the discussion that is now focused on the empirical evidences we have. First, and following the suggestion from R3, we highlight that the flux of NH<sub>4</sub><sup>+</sup> from riparian groundwater is not large enough to sustain in-stream NO<sub>3</sub><sup>-</sup> release during the vegetative period along the last 0.7 km of the reach (pp 22-lines 524-527). Then, we discuss that an additional source of N could be increased in-stream nitrification at the valley bottom based on previous studies showing that leaf litter from riparian trees, and especially from N<sub>2</sub>-fixing species, can enhance in-stream nutrient cycling. Thus, we propose that large stocks of leaf litter from alder and black locust combined with warm temperatures could enhance in-stream mineralization and nitrification during the vegetative period. This process could explain the marked increase in stream NO<sub>3</sub><sup>-</sup> release observed at the lowest part of the catchment (pp 22,23-lines 528-535).

In addition, we consider other alternative explanations such as anthropogenic sources and autotrophic N fixation as suggested by R3, which seem not likely given the empirical evidences we have. Finally, we end up concluding that although the sharp increase in NO<sub>3</sub><sup>-</sup> availability observed at the end of the reach could be related to the presence of black locust, further research is needed to test this hypothesis (pp 23,24-lines 536-555).

615-16 lowest part of the catchment. **Answer:** OK.

616-18 New results in the discussion?

**Answer:** Following R1 and R3 suggestions, we have deleted this part of the discussion as well as former Figure 8.

617-20. Is that because NO<sub>3</sub> concentrations are higher than SRP and NH<sub>4</sub>?

**Answer:** Good point. To answer your question, we have analyzed whether differences in  $F_{sw}$  and  $|F_{sw} \cdot x / F_{in}|$  were statistically different among nutrients. Differences were not statistically significant for  $F_{sw}$ , but they were significant for  $|F_{sw} \cdot x / F_{in}|$ . These results

suggest that differences in  $|F_{sw} \cdot x / F_{in}|$  among nutrients were mainly because input fluxes showed large differences in concentrations that tend to be 20-fold lower for  $\text{NH}_4^+$  and SRP than  $\text{NO}_3^-$ , as you pointed out (pp 21-lines 493-496).

617-22. *What is meant by “cycled more efficiently” Longer uptake length? Lower  $v_f$ ?*

**Answer:** Thanks. We now refer to *in-stream gross uptake velocity* (pp 21-line 490).

618-21 *“if we are to understand”?* **Answer:** Yes. Thanks.

618-23. *This sentence says that instream processing is important but manifests itself at a small spatial scale than riparian processes?*

**Answer:** We have been thinking thoroughly about the implications of our results, and we believe that the new version of the manuscript states more clearly the take home message that emerges from this study (new conclusions section).

Briefly, our study highlights that the stream had a strong potential to transform nutrients and that in-stream processes were substantially contributing to either increase or decrease stream nutrient export to downstream ecosystems. However, the influence of in-stream processes on stream nutrient concentration and fluxes did not systematically translate into longitudinal patterns because changes in nutrient concentration along the reach were the combination of both in-stream cycling and nutrient inputs from terrestrial sources (pp 25,26-lines 590-614).

619-1. *Ok a great way to end, but I could use bit more explanation here.*

**Answer:** Agree. We have carefully thought about the bottom line and implications derived from our results, as mentioned in our earlier response. One of the major implications of our study is that the assessment of both in-stream cycling and terrestrial nutrient sources is crucial if we are to understand the contribution of in-stream processes to stream nutrient dynamics at relevant ecological scales. Please read the new conclusions section for more detailed explanations.

*Table 1. Reporting an SE implies normally distributed data, yet there is a non-parametric test used. The equation for CI is not how it is described in the text.*

**Answer:** Following the reviewer suggestion, we do now provide the median and the interquartile range in Tables 1, 3, and 4. The chemical index has been substituted by dissolved oxygen concentration.

*Table 3. Likelihood is relative likelihood, correct?*

**Answer:** The GLM along with former Table 3 have been excluded from the new version of the manuscript.

*Fig 2. The X-axis looks like it is plotted categorically vs. numerically as a function of distance. It seems to me that plotting numerically would be clearer because the sites are not equally spaced. Stream width and % sand should be on separate plots.*

**Answer:** Following the reviewer suggestion, the X-axis in Figure S1 (former Figure 2) is now plotted numerically, and stream wetted width and the percentage of sand in streambed are plotted in two separated plots. In alias of simplicity and to condense the study, note that this figure is now in Supplementary Materials.

*Fig. 5. A straight line seems to be a poorly fitting model for a U-shaped pattern of nitrate concentration and flux.*

**Answer:** Following the reviewer suggestion, we do now use lineal and nonlinear models. Moreover, we refer specifically to a U-shaped pattern when describing the longitudinal pattern of stream nitrate concentrations in the results section.

*Fig 7 legend, bottom “post-hoc”.* **Answer:** OK. In any case, this figure has been excluded.

## **Author Response to Reviewer #2**

*Presented data and descriptions include interesting and important information. The aim and approach to evaluate the riparian and in-stream controls presented in this manuscript is challenging and attractive. Therefore, the document itself is valuable, even though this is a report of a case study at particular middle scale catchments. However, there are several weaknesses for considering a publication as an original article in the current version. I hope following comments will help the authors to improve the contents and descriptions.*

**Answer:** Thanks for your positive comments. Yes, this study only reports results for a particular headwater catchment. Yet, as pointed out by R3, it is worth noticing that the experimental design we have conducted is not commonly found in the literature and in this sense, our study can provide valuable information to the reader of *Biogeosciences*. In this new version of the manuscript, we have worked throughout the results and the text in order to highlight the novelties and uniqueness of our study. We hope that you find the paper improved.

### ***Comments:***

*1. As the authors stated in the first paragraph of the discussion section, the novel point of this paper was statistically quantitative analysis of sources of nutrients in the stream water. However, in the conclusion section and abstract, their descriptions on relative contribution of riparian and in-stream processes was still very qualitative. More quantitative expressions on relative contributions were favorable.*

**Answer:** Thanks for noticing, this is a very important point. Following the suggestion made by you and R3, we now quantify the relative contribution of riparian groundwater and in-stream cycling to stream nutrient fluxes for the whole reach. By going that, we are able to provide quantitative estimates of the contribution of riparian groundwater, in-stream release, upstream, and tributaries to stream inputs fluxes for each sampling date. Moreover, we are able to quantify in-stream net nutrient retention at the whole-reach scale (see new Table and Figure 7, and associated results).

*2. P11600, L17-20: This type of hypothesis has been commonly recognized by many researchers in the field of river ecosystems. Not only your proposal.*

**Answer:** That's right. Following your suggestion, we now indicate that some authors have proposed that nutrient concentrations should decline in the downstream direction if in-stream net uptake is high enough and changes in riparian groundwater inputs are relatively small (Brookshire et al., 2009), while other have shown 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) (pp 5-lines 91-102).

3. *In the methods section, it would be helpful for readers to show a conceptual diagram or picture examples expressing the spatial distributions and scales of riparian forests (zone), riparian-stream interface (zone?).*

**Answer:** Thanks for your suggestion. As already mentioned in our general response to R1, we have added a schematic representation of our mass balance approach. This scheme is intended to clearly define fluxes in and out a stream segment (new Figure 3). Following the suggestion from R3, we have also simplified our conceptualization of the stream-riparian interface. As depicted in the new Figure 3, there are only two compartments: the stream and the riparian zone, and by definition biogeochemical processes can only occur either inside (stream) or outside (riparian zone) the boundary.

4. *How does the climatic seasons correspond to the vegetated/dormant seasons? Is the vegetative season rainy season or high flow season? And also, doesn't the seasonal variation in discharge rate affect those of the nutrient concentrations and fluxes?*

**Answer:** In order to answer your question as well as those from R3, we have included more information regarding the seasonality of precipitation and discharge during the study period.

First, the temporal pattern of P and Q can be seen in new Figure 2 (panels a and b). These data comes from our permanent sampling station which is equipped with a water sensor and an automatic sampler. Second, we do now provide information about the seasonality of rainfall during the study period which was minima in summer and highest in spring (pp 6-lines 130-132). Moreover, we specify that rainfall was similar between the dormant and vegetative period (pp 10-lines 216). This was so because the spring 2011 was rainy and this rain compensate the following dry summer.



Finally, we also provide information about the seasonality of stream discharge at base flow conditions which was low in summer and peaked in spring (pp 8-lines 163). High-temporal resolution data showed that stream discharge was higher during the dormant than during the vegetative (Table R1). However, this seasonality was not reflected in our synoptic study likely because our sample size was too low. In addition, the vegetative period included dates with high flow (spring 2011) and low flow (summer 2011) which likely balanced each other (Table R1).

**Table R1.** Median and interquartile range [25th, 75th percentile] of area-specific discharge (mm) at the downstream-most site. Data from this study and from the permanent sampling station installed at the valley bottom is shown. The number of cases is shown in parenthesis for each group. Different letters indicate statistically significant differences between either calendar seasons or periods (Wilcoxon paired rank sum test,  $p < 0.01$ ).

	This study	Permanent Station
Autumn 2010	0.46 (2)	0.41 [0.28, 0.73] <sup>B</sup> (91)
Winter 2011	0.36 (2)	0.44 [0.40, 0.57] <sup>B</sup> (91)
Spring 2011	0.88 (2)	0.79 [0.62, 1.07] <sup>A</sup> (91)
Summer 2011	0.26 (2)	0.33 [0.20, 0.38] <sup>C</sup> (91)
Autumn 2011	0.70 (2)	0.42 [0.17, 1.88] <sup>A</sup> (91)
Vegetative	0.41 [0.24, 0.59] <sup>A</sup> (7)	0.37 [0.20, 0.62] <sup>B</sup> (214)
Dormant	0.43 [0.35, 0.64] <sup>A</sup> (4)	0.46 [0.40, 1.55] <sup>A</sup> (151)

Regarding your last question, our data showed no seasonality in stream nutrient concentrations, at least for nitrate which was the nutrient exhibiting the highest concentrations (Table R2).

**Table R2:** Mean and standard deviation of nitrate concentration at the downstream-most site. Data is from this study (only mean) and from our permanent station. The number of cases is shown in parenthesis for each group. No differences were found between calendar seasons for the permanent station data set (Wilcoxon paired rank sum test,  $p < 0.01$ ).

	Nitrate ( $\text{mg N l}^{-1}$ )	
	This study	Permanent Station
Autumn 2010	0.27 (2)	0.17±0.03 (20)
Winter 2011	0.22 (2)	0.21±0.09 (71)
Spring 2011	0.13 (2)	0.17±0.02 (80)
Summer 2011	0.17 (2)	0.17±0.02 (89)
Autumn 2011	0.22 (2)	0.23±0.12 (89)

5. P11602, L14-16: *In the discussion section, the authors discussed about the influences of the N<sub>2</sub> fixing trees and the N dynamics of soil microbes on the nutrient input from the riparian zone to the stream. Those processes are usually most active at the organic horizon and the near surface part of the mineral soils. Was the sampling conditions which made the influences from those soil horizons minimal appropriate to investigate the direct effect of those N dynamics? Related to this question, in order to elucidate the controls by the biogeochemical processes of riparian zones comprehensively, relative degree of impact under the low flow condition should be estimated compare to that under the high flow or storm conditions.*

**Answer:** Thanks for your suggestion. The reviewer is right in that the contribution of different water sources may change between base flow and storm flow conditions. However, the present study was designed to understand in-stream nutrient cycling during base flow conditions, when its contribution could be more relevant and thus easier to tease out from other factors as indicated in the M&M section.

As we already mentioned in our specific responses to R1, we have rewritten this part of the discussion to avoid creating confusion. We have focused on the most plausible mechanisms that could explain the observed increase in stream NO<sub>3</sub><sup>-</sup> concentration and the high frequency of in-stream NO<sub>3</sub><sup>-</sup> release at the valley bottom during the vegetative period. We still invoke the potential role of black locust, but mostly as a source of edible leaf litter to the stream, which could enhance in-stream nitrification during warm periods. We have been more cautious on our rationale and we do now explicitly say that further research is needed to test the hypothesis that black locust alter stream nutrient dynamics (pp 22,24-lines 524-555).

6. *What was the specific benefit to use the CI value (considering the NH<sub>4</sub> + concentration) for evaluating redox condition instead of DO?*

**Answer:** Following your suggestion, we do now use dissolved oxygen concentration (DO) instead of the chemical index.

7. *A table for summarizing definition of each variable (Q<sub>gw</sub>, Q<sub>spf</sub>, F<sub>spf</sub>, C<sub>sw</sub>, etc.) would be very helpful for readers.*

**Answer:** As we already mentioned in our earlier responses to your point #3, we have included a schematic representation of the mass balance (new Figure 3). The

conceptual model indicates the two compartments considered: the stream and the riparian zone, as well as fluxes in and out the segment ( $F_{ef}$ ,  $F_{top}$ ,  $F_{bot}$ ,  $F_{gw}$ ,  $F_{sw}$ ). In addition, we have indicated in the caption how nutrient fluxes were calculated (Q x C). We believe that in this manner the reader can get a quick idea of the variables considered in the mass balance, which is more illustrative than a glossary table.

8. *More detailed explanations of stream water sampling should be needed. Because, solute concentrations are often different with parts of cross section. Effect of the riparian groundwater might be evaluated differently at the bank side and the center of the stream.*

**Answer:** In the new version of the manuscript, we have specified that stream water samples were collected at the thalweg of the watercourse (pp 8-line 171). Moreover, we acknowledge that our characterization of riparian groundwater, despite valuable, is limited because riparian groundwater inputs can be highlight variable over space and it is complicate to determine the chemical signature of the groundwater that really enters the stream (pp 26-lines 615-624).

9. *Sub-chapter 4.3 included some discussions based on observed results. Some contents in this section might be categorized into the discussion chapter.*

**Answer:** After rereading carefully this subsection, I'm afraid to say that we do not really get which particular contents you are referring to.

Our impression is that we are not including any discussion into the results section, though at some point we add some clarifications such as that the stream was acting as a net source or sink for a particular nutrient. Not sure if this is what you meant, but in any case we believe that this is a result in itself as the considered variables were already defined using these terms in the M&M section.

10. *The first paragraph of the Chapter 5 was redundant, and some contents overlapped with the introductory section.*

**Answer:** Agree. Thanks for noticing. This paragraph has been deleted.

11. *P11613, L25- P11614, L1: The authors cited the paper Asano et al. (2009) to show an example indicating a reduction in the variability of solute concentrations as catchment size increases. Then, they stated that their case did not show the decrease*

*in the CV of stream solute concentration along the reach. However, the discussions in Asano et al. (2009) did not tell the variability of solute concentrations decrease with the distance from the headwater in a SINGLE PARTICULAR stream. The “variability” in their paper indicated a variability of solute concentrations AMONG THE MULTIPLE DIFFERENT STREAMS WITH SAME CATCHMENT SCALE. The discussion was totally different with that in this manuscript.*

**Answer:** Thanks for noticing. We do not refer to this paper anymore in the new version of the manuscript because, as suggested by R1, we should focus on the results that really contribute to solve our question, and the longitudinal patterns of CV of nutrient concentrations were not very helpful in this sense.

12. *P11614, L407: It is generally accepted that a major source of phosphate in stream is chemical weathering of the bedrocks. And also, phosphate in the vegetated floor and surface soils are usually recycled tightly within the plant-soil internal cycling. If the authors want to conclude that the controlling factor for stream phosphate concentration was the “hill slope water source”, they had to explain how different the hill slope water (groundwater?) input was from the water input from the riparian zones. Citations of Asano et al. (2009) was not appropriate, because they did not discuss the sources of solutes using riparian-hill slope comparison. Their concept was that the stream solute concentrations could be explained the mixing of shallow and deep groundwaters both from hillslopes.*

**Answer:** Thanks for your comment. See our previous response.

Following suggestions by the three reviewers, we do now focus on whole-reach mass balance calculations to examine the relative contribution of different sources to stream nutrient fluxes. Regarding SRP, our calculations indicate that tributaries and riparian groundwater were the major contributors of this nutrient to the stream. In-stream processes mostly contributed to increase rather than decrease stream fluxes of SRP (see new Table 4 and new Figure 7).

13. *P11614, L8-14: The authors concluded that the drop in the specific discharge and the Cl<sup>-</sup> concentration along the reach were due to increase of evapotranspiration. This explanation might be doubtful. Could the evapotranspiration rate vary significantly within a few-km scale? Did vegetation type change from headwater to valley bottom significantly?*

**Answer:** We agree with the three reviewers that this point needed to be better discussed.

As already mentioned to R1, a more careful look to our data set highlighted that the longitudinal increase in  $\text{Cl}^-$  concentrations could be explained by hydrological mixing of stream water with tributaries and riparian groundwater. These two sources contributed substantially to stream discharge (> 50% and 26%, respectively), and both exhibited higher  $\text{Cl}^-$  concentrations than the stream, in particular during the vegetative period. Please, see more details about the changes made in our response to R1, as well as in results section 4.1 and [pp 20-line 457-465](#).

14. *P11615, L4-L28: The discussions of this part were confusing. It seemed that the longitudinal pattern of  $\text{NO}_3^-$  concentration had two phases: decrease from (0-3000 m) and increase (3000-3700 m). But, the ratio of the  $\text{N}_2$ -fixing trees increased monotonically from headwater to valley bottom. To explain the longitudinal pattern of  $\text{NO}_3^-$  concentration sufficiently, they should show the more consistent logic to apply to both decreasing and increasing phases with certain evidences. Probably, there might be two different controllers*

**Answer:** Agree. Thanks for noticing. As already mentioned in our responses to R1, we now describe the U-shaped pattern of stream  $\text{NO}_3^-$  concentrations ([pp 16-lines 374-376](#)). Moreover, we now acknowledge 2 different drivers of stream  $\text{NO}_3^-$  concentrations. First, as included in our responses to your comment #5, we have rewritten this discussion paragraph and now we refer specifically to the increase in stream  $\text{NO}_3^-$  concentrations observed at the lowest part of catchment. We now consider several alternative explanations that could be behind such pattern, being one of them the predominance of  $\text{N}_2$ -fixing species along the last 0.7 km ([pp 22,24-lines 524-555](#)). Second, we discuss that the decreasing pattern exhibited by  $\text{NO}_3^-$  concentrations along the first 1.5 km could not be explained by in-stream processes alone because its contribution to reduce stream  $\text{NO}_3^-$  fluxes was too low. Thus, the observed longitudinal pattern resulted from the combination of both in-stream nutrient cycling and hydrological mixing with riparian groundwater and tributary inputs ([pp 24,25-lines 570-588](#)).

15. *P11615, L17-19: On English expression at the following part: “...we could not establish any positive relationship between the proportion of  $\text{N}_2$ -fixing tree species*

*and stream NO<sub>3</sub>- concentrations along the reach.” Any relationships can not be “established” by researchers. They can only observe and find the relationships.*

**Answer:** We have substituted “*establish*” by “*find*”

### **Author Response to Reviewer #3**

*This is a pioneering and potentially an important contribution to the long standing question of whether, how, and to what extent in-stream biogeochemical processes influence stream chemistry. The question has sparked debate over whether in-stream processes alter chemistry enough to compromise the interpretation of stream exports as a measure of terrestrial watershed losses, and therefore whether inferences based on the small watershed concept need to be reassessed. It has been suggested that if in-stream processes are important enough to make a difference, then this should be revealed by longitudinal concentration gradients reflecting the removal or addition of nutrients to the water column. And, in fact, a number of studies have linked net uptake to declining downstream concentrations. But because nutrients enter (and leave) the stream laterally along its length, the notion a net uptake necessarily generates a declining concentration gradient is a simplistic fallacy. (See Eq. 4 of Brookshire et al. 2009 Ecol. 90:293, which is correct but misinterpreted by those same authors).*

*The work under review demonstrates (1) that in-stream processes may strongly affect stream chemistry and yet leave concentrations longitudinally uniform (or otherwise varying), as did Meyer and Likens (1979 Ecol. 60:1255), and (2) the magnitude of biotic effects can be inferred using a mass balance approach that fully accounts for lateral inputs and losses. The authors estimated the effect on stream chemistry of uptake or release by taking the difference between inputs and outputs assessed on each of 15 segments of a 3700 m reach of reach. The assessments were synoptic, repeated 11 times over a year and a half. No one has done this before, at least on this scale. The approach has limitations. We do not get an annual budget and a “sink” is simply the instantaneous difference of inputs and outputs: We do not see whether the nutrient is accumulating, being transformed, or being lost to the atmosphere. However, we do see detailed spatial resolution, revealing a surprising degree of spatial heterogeneity and, wherever there are lateral losses as well as gains, mass balance even at the whole-reach scale, cannot be assessed without it.*

*I use the caveat “potentially” because I think the presentation is weak in several ways and falls far short of what this amazing data set could support. The major problem, I think, is that the authors are trapped by the misconception that in-stream processes should produce longitudinal gradients. They often do, but they don’t necessarily and there are good reasons why they wouldn’t. I think this could be a very*

*powerful paper if directly challenged the misconception, and showed how streams can strongly affect concentrations without generating longitudinal gradients. Much more on this below. I have trouble understanding the basic message or messages of the paper, find that important information is missing, see a number of inconsistencies in presentation, and suspect that there may be some major errors. I hope all these can be resolved because this is important work.*

**Answer:** Many thanks for your constructive comments! Your review is plenty of good advices and helpful suggestions. Overall, we have learned and enjoyed while working on it. As we mentioned in our letter to the editor, it has been like changing googles!

We have done a great effort to clarify the message, we have added the information that was missing and we believe that the inconsistencies are resolved (see our detailed responses below). Considering how fast and mercantilist is becoming science in the last years, at least, at this end, we sincerely appreciate the time you have spent to get through the paper and explain us how to improve it. For us it has been one of those rare and beautiful moments, in which doing science and the whole peer review process is worth and meaningful. Thank you.

First, here is what I consider missing:

*(1) A whole reach budget, or budgets (by period, or sampling date). The whole-reach budget summarizes the big picture and will, in the long-run, be an essential, citable result of the paper. Moreover, what happens segment by segment must add up to the whole reach, so it provides closure. A budget would consist of upstream inputs, tributary inputs, groundwater inputs, groundwater losses, instream source-or-sink, and downstream export, i.e., the same as your segment (Eq. 2) budgets, for water, chloride, and nutrients, reported in mass/time (not area-specific).*

**Answer:** Great suggestion! The paper now includes water and nutrient budgets at the whole-reach scale for each sampling date. These calculations are now an essential result of the paper because they allow quantifying the relative contribution of different sources to either increase or decrease stream nutrient fluxes, which was one of our aims. See new Table 4 and Figure 7 for a quick overview.

The consequences of following your advice have cascaded down because these results outcompete an important part of the analysis proposed in our former version, such as the examination of the CV of stream nutrient concentration, the longitudinal



pattern of area-specific nutrient fluxes, and the GLM. These results did not help much to address our objectives as pointed out by R1. We have rewritten the M&M, results and discussion section according to these changes (see more detailed changes below).

Overall, calculating the budgets at the catchment scale is a beautiful idea, and we feel that these new results bring the paper to a higher level.

*(2) The map shows many unmeasured tributaries that account for roughly half as much contributing area as the measured tributaries (a table of sub-basin areas would be helpful). The way inputs from the unmeasured tributaries were incorporated into the mass balance should be described. If they were dry, this should be so stated.*

**Answer:** Yes, thanks for noticing. You are correct: we only collected water samples from the four tributaries carrying water, at least during the dates we went to the field.

In addition, and motivated by some of your comments below, we have added a fifth tributary in this new version of the manuscript. This was a small effluent (catchment area  $< 0.4 \text{ km}^2$ ) that drained through the inhabited area at the valley bottom. It was covered with bushes which prevented us from successfully conducting slug additions (and thus include it in our budgets). This was the reason why we did not include these data in the former version. However, stream chemistry from this tributary can be valuable to evaluate the potential influence of human impacts on stream water chemistry (see our responses to your comment in this regard).

This info together with their drainage area is now included in M&M (pp 9-lines 191-197) See also improvements in the caption of Figure 1.

*(3) The seasonality of rainfall and flow should be provided. Knowing that dormant and vegetative season flows were similar is not sufficient because we would expect increasing baseflows throughout the dormant season and declining flows throughout the vegetative season.*

**Answer:** Following suggestions by you and R2, we have added more detailed information about the seasonality of rainfall and discharge during the study period (please see our responses to R2 in this regard).

Briefly, high-temporal resolution data from our permanent sampling station showed that stream discharge at base flow conditions was lower in summer than in winter/spring. Yet, this was not reflected in our synoptic study likely because the sampling size was too low and high discharge in spring compensate low summer flows

(see Table R1). Please, see more details in our response to your *temporal pattern* comment.

*(4) We need more information on groundwater gains and losses, especially the gross inputs and losses over the reach. The assessment of groundwater contribution requires both inputs and losses, not simply a net inflow. Also needed is an explanation of the groundwater input shown in Fig 3. c. If it is cumulative with watershed area, as is  $Q_{spf}$  in Fig. 3a, then Fig. 3 c tells us that, over the whole reach, there was no net gain from groundwater, yet the longitudinal increase in flow (L/s, not area-specific) was substantially greater than explained by measured tributary inputs.*

**Answer:** As already mentioned in our responses to R1, we have followed your suggestion and calculated gross hydrological gains and losses over the entire reach for each sampling date (see new Figure 2 and related results in section 4.1). Please, find more details in our specific responses to R1.

The former Figure 3c showed mean net groundwater inputs for each segment, no cumulative inputs. We agree, however, that plotting cumulative area-specific net groundwater inputs along the reach (new Figure 4b) is more helpful and easy to compare to area-specific discharge in Figure 4a (which is a cumulative variable by definition).

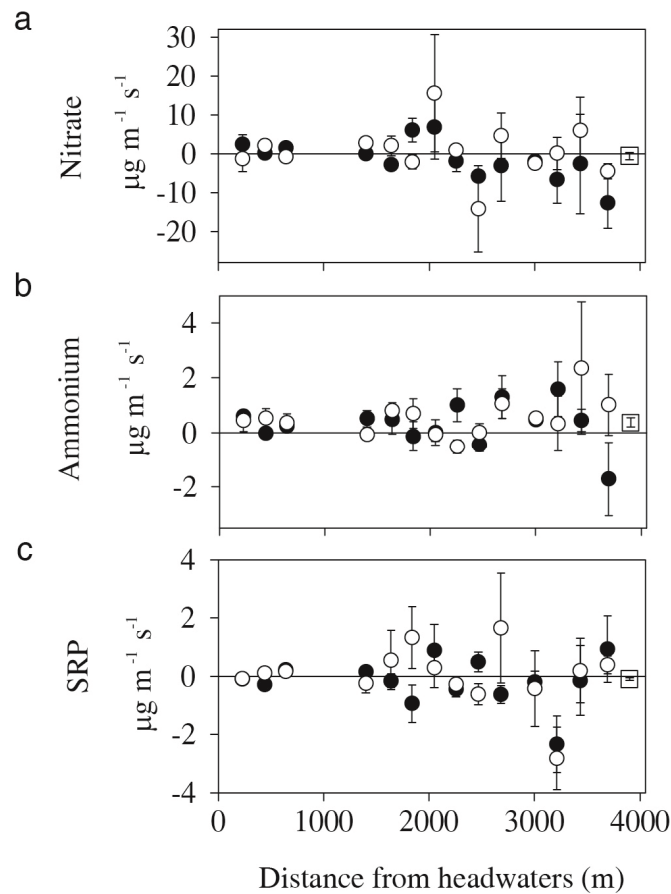
*(5) We need a more complete presentation of the  $F_{sw}$  data, longitudinal and temporal. These are the real contribution of the paper, but we see only the whole-study averages, standard errors, and a graph of the frequency of positive and negative estimates.*

**Answer:** That's right. Thanks for your suggestion. These results have been emphasized in the new version of the manuscript. We do now show:

- (1) medians and IQR of  $F_{sw}$  at two spatial scales (segment and whole reach) (new Table 3),
- (2) medians and IQR relative contribution of different sources (upstream, riparian groundwater, tributaries, and in-stream release) to stream solute fluxes (new Table 4),
- (3) the longitudinal variation of the frequency of  $F_{sw} > 0$ ,  $< 0$ , and  $= 0$  (Figure 6), and
- (4) the temporal pattern of  $F_{sw}$  (whole reach-scale) together with its contribution to reduce stream nutrient fluxes (new Figure 7).

In addition, here we include the longitudinal variation of  $F_{sw}$  for individual segments (Figure R1). For the three nutrients,  $F_{sw}$  shows high spatial variation and

longitudinal patterns, if any, are difficult to visualize. This is why we believe that showing the longitudinal variation of the frequencies (sort of “stream modes” as used in animal ecology) could be more informative to the reader.



**Figure R1.** In-stream net nutrient uptake ( $F_{sw}$ ) for individual segments along the reach for (a) nitrate, (b) ammonium, and (c) SRP during the study period. Circles are means and whiskers are standard error for the dormant (white) and vegetative (black) period. The square at the right end of each plot is the mean  $F_{sw}$  calculated for the whole reach.  $F_{sw} > 0$  indicates that gross uptake prevail over release.  $F_{sw} < 0$  indicates the opposite. The zero line is shown as a reference.

(6) *We need an acknowledgment of the human activity in the lower part of the catchment. I see buildings and agricultural or horticultural activity on Google Earth. As much as 15 hectares of riparian area may be disturbed. This much activity could account for the higher N and P concentrations in the lower-most segments. If you are sure that the activity is inconsequential, you should explain this.*

**Answer:** Right. Thanks for raising this point. The catchment has a low population density ( $< 1$  person/km<sup>2</sup>) which is concentrated at the valley bottom (info now included in [pp 6-line 136](#)). Human settlements consist in few summer residences with some orchards, and one permanent residence with few field crops for personal consumption

(pp 9-lines 195-197). There is also a small water bottling plant between the last sampling sites (this is spring water that emerges from deep, old groundwater layers, which are disconnected from the annual hydrological water cycle).

As you point out, the potential influence of human activity on stream nutrient chemistry cannot be rule out. As already mentioned in our responses, your comment has motivated us to rescue some stream water samples collected from a small tributary that drained though the inhabited area of the catchment. We could not measure stream discharge at this site but we have characterized stream water chemistry which can now be seen in Figures 4 and 5. These data suggest that anthropogenic sources were unlikely behind the increase in nitrate concentrations observed at the valley bottom because DIN concentrations at this human-altered tributary were low. In contrast, this tributary showed high SRP concentrations (multiple-fold higher than in the main stream), and thus could partially explain the increase in SRP observed at the valley bottom. However, its discharge should 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 (pp 23-lines 536-542).

In addition, we have checked out some isotopic N data available from past studies performed at this site. In particular, data from a survey carried out in 2010 at the downstream-most site of the reach showed that N isotopic signatures ( $\delta^{15}\text{N}$  in ‰) were  $-0.44 \pm 3.70$  and  $0.30 \pm 0.54$  for  $^{15}\text{N-NH}_4^+$  and  $^{15}\text{N-NO}_3^-$ , respectively (mean  $\pm$  SE,  $n = 4$  for each DIN species). These values are well bracketed by data reported for pristine streams, including upstream reaches at our study site and other streams worldwide (Pastor et al. 2013; Pastor et al. 2014; Peipoch et al. 2012). Thus, these data do not support either that the increase in DIN concentration at the downstream reaches was a consequence of human activities at the valley bottom.

*(7) Fig. 5 shows no tributary data for the vegetative season. Tributaries were the major fluvial input, so these data are critical to understanding what was influencing concentrations.*

**Answer:** You're right! The water budgets at the whole reach-scale indicate that tributaries were contributing  $> 50\%$  to stream discharge. In addition, tributaries showed contrasting  $\text{Cl}^-$  concentration between the dormant and vegetative period. This info is crucial to understand the longitudinal pattern of this hydrological tracer.

In the former version, plotted data from tributaries were not separated by seasons. Now, we do so for Cl<sup>-</sup> (Figure 4c) and nutrients (Figure 5). In addition, we pay more attention to tributaries' stream water chemistry in the results section (sections 4.1 and 4.2).

(8) *Correct units for  $|F_{sw}/F_{in}|$ . These would be inverse meters (like a longitudinal loss rate) except that the values are so high that I assume that  $F_{sw}$  is actually  $F_{sw} \cdot x$ .*

**Answer:** That's right. Thanks for noticing! We have corrected this mistake throughout the text, tables, and figures.

**The message:** *I see two contradictory messages emerging from this paper. One is that in-stream biogeochemical processes can be evaluated from mass balance considerations and are large. The other is that the signal from these processes is overwhelmed by other factors—inputs, losses, riparian processes, and landscape features—so that the stream chemistry doesn't reveal what the biogeochemical processes are doing. Both can't be true. Your equation (2)-the statement of mass balance—says that the stream chemistry does reveal biogeochemical processes. It does not make sense to say that “other factors...may overcome the effect of...processing” (598:6), or that “longitudinal trends...were not consistent with...biogeochemical processes” (598:19, 618:25), or that “sources...can offset the effect of nutrient cycling on stream water chemistry” (619:5). If an in-stream process removes some nutrient then it unequivocally affects stream concentration, and the effect can be measured (the point of Eq. 2). It is not overcome, made inconsistent, or offset by other factors. The problem is the implicit equating of an effect on concentration with the presence of a longitudinal gradient. This shows up most clearly in the first sentence of the abstract and as a “hypothesis” in the discussion (614:22). More careful wording would resolve the problem: Clearly state that you are not talking about an effect on concentration but about the expectation that an effect shows up as a longitudinal gradient. Your work shows that a longitudinal gradient does not equate to an effect on concentration. It also shows that biogeochemical processes have a strong effect on concentration—that require careful mass balance to see.*

*One way to clarify the message might be to focus on how in-stream processes can be large without creating a concentration gradient. The basis for this lies in*

*Brookshire et al's (2009) equation 4: Biological uptake draws the concentration groundwater that enters the stream laterally (presuming that it is higher than the streamwater concentration) down to the concentration of the streamwater that entered the reach. Thus the streamwater concentration shows no gradient, but is maintained lower than the concentration of its sources. Below I question whether your reported in-stream processes ( $F_{sw}$ ) are too high, but regardless, it would be instructive to drill down on the high values, asking whether these were associated with segment-specific concentration declines or high groundwater inputs.*

*The confusion in message extends to the treatment of riparian and landscape factors as variables that explain stream concentrations. The glm model leaves out  $F_{sw}$  despite your finding that in-stream processes affect ammonium and phosphate fluxes by 29 and 30% in a single segment. Instead it includes  $D$  (the gradient) making the implicit assumption that in-stream processes can only be represented by a gradient. The image I get is that of two demons—in-stream processes and riparian characteristics—competing for control of stream concentrations. But you already know how much each is controlling, from the mass balance calculations, and don't need statistics for that. The riparian and landscape variables can affect concentrations either through affecting inputs (tributary and groundwater fluxes) or by affecting in-stream processes (e.g., wider stream increases uptake per unit distance, or opens the canopy to primary production). Because the inputs have been measured, the analysis should separate these pathways: one analysis for riparian concentrations (or inputs) and one for in-stream processing ( $F_{sw}$ ). For example, terrestrial nitrogen-fixation should be related to its effects on riparian concentrations.*

**Answer:** We agree that we had to improve the bottom line of the paper and explain clearly what our results mean. Your comments in this regard are smart and have been very helpful. Following your suggestion, we have reconsidered the wording throughout the text to avoid creating confusion and unclear messages.

In the introduction, we now explicitly address that some authors have proposed that nutrient concentrations should decline in the downstream direction if in-stream net uptake is high enough (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 may counterbalance each other most of the time (Brookshire et al., 2009). However, previous 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 not necessarily lead to longitudinal increases or declines in nutrient concentration, a question that probably needs to be addressed at spatial scales larger than few hundred meters (pp 5-lines 91-102).

The new version of the manuscript emphasizes the results (both former and new) showing that in-stream processes are relevant and contribute substantially to stream nutrient fluxes. Namely, (1) poor correlations between stream and riparian groundwater nutrients, (2) large  $|F_{sw} \cdot x/F_{in}|$  values at both segment and whole-reach scales, and (3) relative contribution of in-stream processes to either increase (i.e.,  $|F_{sw} \cdot x < 0|/F_{in}$ ) or decrease (i.e.  $F_{sw} \cdot x > 0/F_{in}$ ) stream nutrient fluxes at the whole-reach scale.

The comparison of these results with the longitudinal patterns in stream nutrient concentrations highlights that in-stream processes can be important without generating longitudinal gradients in nutrient concentration, as you point out. The case of  $\text{NH}_4^+$  is paradigmatic: concentrations were consistently lower in the stream than in riparian groundwater and  $F_{sw} > 0$  predominated (gross uptake > release). Yet, stream  $\text{NH}_4^+$  concentrations showed small longitudinal variation likely because in-stream net uptake balances the elevated inputs from riparian groundwater. Thus, our results challenge the idea that stream nutrient concentrations should decrease in the downstream direction when in-stream processes are efficient in taking up nutrient from receiving waters, as highlighted by you (pp 24-lines 556-570).

In the conclusions section, we now remark that dissolved nutrients underwent biogeochemical transformation while travelling along the stream and further, in-stream processes contributed substantially to modify stream nutrient fluxes. In addition, we found that the influence of in-stream processes on stream nutrient concentrations and fluxes did not translate into gradual changes in nutrient concentration along the reach because the longitudinal pattern in nutrient concentration resulted from the combination of both in-stream cycling and nutrient inputs from terrestrial sources. Thus, one of the implications of our study is that the assessment of these two sources of variation of stream nutrient chemistry is crucial to understand the contribution of in-stream

processes to stream nutrient dynamics at relevant ecological scales (pp 25,26-lines 590-614).

Finally, as mentioned in our previous responses, we have decided to eliminate the GLM analysis from the paper. As you suggest, calculating whole-reach budgets is a more simple and elegant way to put in-stream cycling and riparian groundwater inputs into the same picture and thus, we believe that these new results are more helpful to address the overall aim of the study.

***In-stream process estimates:** The  $F_{sw}$  and  $|F_{sw}/F_{sin}|$  values for ammonium and phosphate are surprisingly large (except, perhaps for nitrate), so large that I suspect an error. What follows is an explanation of why I suspect an error. I may be wrong, but either way, I think the paper should provide the data and considerations needed to answer the questions I raise here—because other readers would surely raise them as well.*

*$F_{sw}$  for ammonium is reported to average  $0.6 \pm 0.2$  (SE)  $\mu\text{g}/\text{m}/\text{s}$  which, for the 3700-m reach is a net uptake of 2220  $\mu\text{g}/\text{s}$ . The inputs to the reach from upstream are roughly 200  $\mu\text{g}/\text{s}$  and the downstream export is about 800  $\mu\text{g}/\text{s}$ , for a net export of 600  $\mu\text{g}/\text{s}$ . Thus an input of 2800  $\mu\text{g}/\text{s}$  from lateral (tributary and groundwater) sources is needed to balance the budget. The net inflow of water from these sources is about 70 L/s, so the average concentration of the lateral sources would have to be about 40  $\mu\text{g}/\text{L}$ . This is far higher than the reported concentrations for both tributary and groundwater, especially tributary which supplies most of the water. The needed concentration may be lower than 40  $\mu\text{g}/\text{L}$  if there is a large exchange of groundwater but this exchange would have to be quite large. Thus I suspect that the  $F_{sw}$  of 0.6  $\mu\text{g}/\text{m}/\text{s}$  is erroneously high. Based on the data available in the paper I calculate that the average  $F_{sw}$  could be no higher than 0.2  $\mu\text{g}/\text{m}/\text{s}$ , even allowing for substantial additional gains through groundwater exchange. (This conclusion could change if vegetative-season tributary inputs, missing from Fig. 5, were much higher than in the dormant season). Also, the mean  $F_{sw}$  of 0.6  $\mu\text{g}/\text{m}/\text{s}$  translates to an areal net uptake (U) of about 0.27  $\mu\text{g}/\text{m}^2/\text{s}$ , which seems implausibly high, at least for a mean. It is far greater than the median of 0.03  $\mu\text{g}/\text{m}^2/\text{s}$  reported by von Shiller et al. 2011. And it is about half the gross uptake typical of second and third order reaches (Ensign and Doyle 2006 JGR 111:G04009),*



*which we expect to be much higher than net uptake because retention in stream biota occurs on a timescale of days or weeks.*

*My concerns are similar for phosphorus, for which the mean  $F_{sw} = 0.2 \pm 0.4$  ug/m/s. In this case the large SE indicates that the net uptake is not statistically significant, yet a net uptake is claimed (598:16). Using the whole reach mass balance approach that I used for ammonia above, I find that the average FSW of 0.2 ug/m/s is reasonable only for the dormant period, and only if the high downstream concentrations are ignored (in which case P concentrations are longitudinally uniform). If the downstream concentrations are included, then I estimate FSW at -0.20, i.e., a net P release. This matches your lower error bound, but because this is simple arithmetic and not statistical sampling, your average should be correct. The large apparent release of P comes from the lower 3-4 stations. If we consider only the reach upstream of 3000 m, then I estimate a net uptake of about 0.1 ug/m/s. I did not check the vegetative period, but unless the tributary concentrations were much higher than in the dormant period, there is no way that there could be a significant net uptake in the vegetative period, over the whole reach or even the upper 3000 m.*

*For nitrate, I'd say that your negative  $F_{sw}$  is entirely driven by the high downstream concentrations in the vegetative period. I get a net uptake for the dormant period, and a net uptake for both periods in the upper 3000 m.*

*One source of error may be in the measurements. The mass balance calculations depend on accurate measures of both concentration and flow and depend on small differences in sometimes small numbers. The errors are made worse by multiplying these estimates together. Some evaluation of the role such errors should be included. Error may also come from averaging  $F_{sw}$  (assuming you have divided by  $x$ ). The segments are unequally weighted with the fourth segment contributing only one-quarter as much as it should.*

**Answer:** Tones of thanks for taking the time and patience to calculate the nutrient budgets. Your exercise has been extremely useful for us and we have enjoyed and learn while following your rationale.

Your back-of-the-envelope calculations have helped us to sort out why inputs and outputs were not matching. Our main mistake was using an erroneous set of distances between sampling sites. In the former version, all segments summed up to 2400 m but the reach is 3691 m long! It's a pity we did not realize before, and it's great that you highlight this issue. This mistake would have been obvious to us if we would

have calculated budgets for the whole reach from the very beginning. Now, we have rechecked the whole data base and recalculated nutrient budgets for individual segments. Further, we have calculated budgets for whole reach; these new results are included in the manuscript. As shown in Table R3, there is a good match between inputs and outputs.

**Table R3.** Mean values of whole-reach nutrient fluxes for the study period. Nutrient fluxes are as in new Figure 3. Nutrient inputs to the reach are upstream sources ( $F_{top}$ ), net riparian groundwater inputs ( $F_{gw}$ ) and tributaries ( $F_{ef}$ ). Nutrient downstream export is  $F_{bot}$ . In-stream processes can either contribute to input ( $F_{sw} \cdot x < 0$ ) or output ( $F_{sw} \cdot x > 0$ ) stream nutrient fluxes. For the three nutrients, mismatches between mean inputs and outputs were < 4%.

	Flux ( $\mu\text{g/s}$ )		
	N-NH <sub>4</sub> <sup>+</sup>	N-NO <sub>3</sub> <sup>-</sup>	SRP
$F_{top}$	237	6863	170
$F_{bot}$	1058	23633	1530
$F_{sw} \cdot x$	1362	-2045	-337
$F_{gw}$	1719	9122	476
$F_{ef}$	556	6261	609
Inputs	2512	24291	1592
Outputs	2420	23633	1530
dif (%)	3.7	2.7	3.9

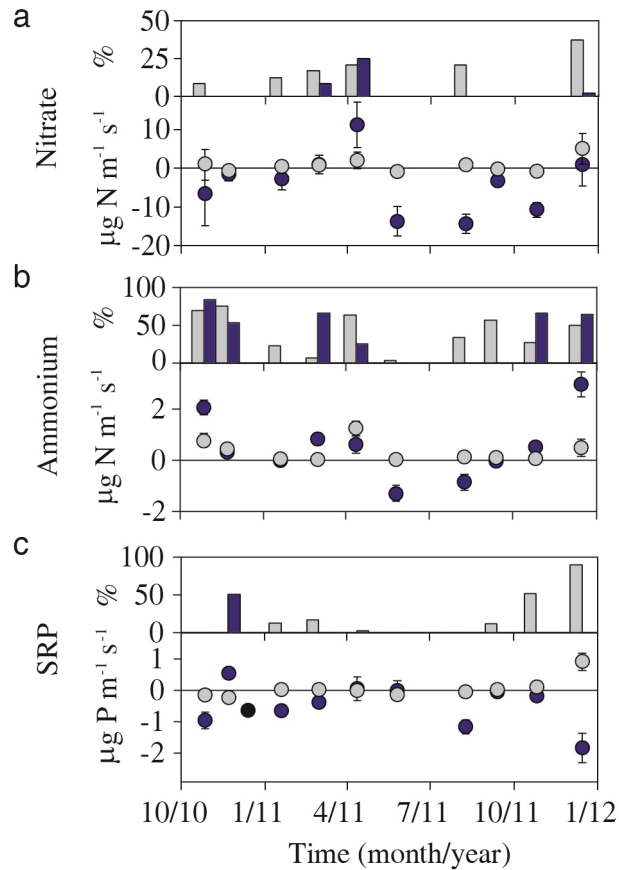
Overall, the recalculated in-stream net nutrient fluxes are close to your back-of-the-envelope calculations. Regarding NH<sub>4</sub><sup>+</sup>, median  $F_{sw}$  during the study period was 0.17 and 0.2  $\mu\text{g N m}^{-1} \text{s}^{-1}$  by segment and whole reach, respectively (new Table 3). If we consider a mean wetted of 240 m and a reach length of 3691 m (8860 m<sup>2</sup> of streambed area), the estimated in-stream net uptake areal rate is 0.03  $\mu\text{g N m}^{-2} \text{s}^{-1}$  which is in the range of values reported for streams nearby our study site (-0.4 to 0.5  $\mu\text{g N m}^{-2} \text{s}^{-1}$ , von Schiller et al., 2011).

Regarding SRP, median  $F_{sw}$  was -0.06 and 0  $\mu\text{g P m}^{-1} \text{s}^{-1}$  by segment and whole reach, respectively (new Table 3). This result suggests that in-stream gross uptake and release tended to counterbalanced each other, or at least that positive and negative values of  $F_{sw}$  were of similar magnitude. We have calculated budgets separately for the first 3 km (sampling sites from 1 to 10) and the last 0.7 km (sampling sites from 11 to 15). This last 0.7 km is the section that could be most influenced by human activities and its riparian zone is the most affected by the invasion of black locust. As you pointed out, these factors could be behind the increased stream NO<sub>3</sub><sup>-</sup> and SRP concentrations at

end of the reach. As required, here we show  $F_{sw}$  separately for these two sections of the reach (Figure R2). If nutrient budgets are calculated in this way, the obtained values follow your predication in that  $F_{sw,SRP}$  was higher for the upper than for the lower section of the reach (0.005 vs -0.28  $\mu\text{g P m}^{-1} \text{s}^{-1}$ ), though differences were not significant (Wilcoxon paired rank sum test,  $p = 0.1$ ).

Regarding  $\text{NO}_3^-$ , median  $F_{sw}$  was -0.43 and -0.97  $\mu\text{g N m}^{-1} \text{s}^{-1}$  by segment and whole-reach, respectively. This rate equals to -0.07  $\mu\text{g N m}^{-2} \text{s}^{-1}$ , a value within the range reported by von Schiller et al. (2011) (-3.5 to 5  $\mu\text{g N m}^{-2} \text{s}^{-1}$ ). As you predicted,  $F_{sw,NO3}$  was higher for the upper than for the lower section of the reach (0.61 vs. -3  $\mu\text{g N m}^{-1} \text{s}^{-1}$ ). In this case, differences were statistically significant, at least for a  $p < 0.05$  (Wilcoxon signed-rank test, signed-rank = 19.5,  $df = 9$ ,  $p = 0.049$ ). These differences occurred mainly during the vegetative period, and no statistical differences were found for the dormant period, as you expected. Thus, you were correct in that in-stream net uptake for  $\text{NO}_3^-$  prevailed over release for the upper-most segments, though its contribution to reduce stream  $\text{NO}_3^-$  flux was relatively small 10% [0%, 20%] (median [IQR]) compared to that of  $\text{NH}_4^+$  42% [24%, 62%] (Figure R2). We have we decided not to include all these results in the paper to avoid redundancies, though we refer to these values when discussing about the influence of in-stream processes on stream  $\text{NO}_3^-$  concentrations and fluxes (pp 24-lines 575-578).

Finally, we agree that we should take into account the uncertainty associated to these measurements. Following suggestions by you and R1, we have calculated the empirical uncertainty associated to stream discharge (see more detailed explanations in our responses to R1), and we have considered it when calculating water and nutrient fluxes and the frequency of cases for which  $F_{sw} > 0$ ,  $< 0$ , and  $= 0$ . In addition, we have analyzed whether differences in  $F_{sw}$  and  $|F_{sw} \cdot x / F_{IN}|$  were statistically significant between nutrients which is also a way to consider the uncertainty associated to these estimations. In any case, we would like to highlight that differences in  $F_{sw}$  are pretty similar when calculated either by segment or whole reach, indicating that our estimates are reliable despite the fact that  $F_{sw}$  for individual segments may be unequally weighted.



**Figure R2.** Whole-reach in-stream nutrient uptake ( $F_{sw}$ ) for the first 3-km (gray) and the last 0.7-km section of the reach (blue) for (a) nitrate, (b) ammonium, and (c) SRP. Whiskers are the uncertainty associated with the estimation of stream discharge from slug tracer additions.  $F_{sw} > 0$  indicates that gross uptake prevail over release.  $F_{sw} < 0$  indicates the opposite. For those cases for which  $F_{sw} > 0$ , the contribution of in-stream net nutrient uptake to reduce stream nutrient fluxes ( $F_{sw} \cdot x > 0/F_{in}$ , in %) is shown (same color code as for  $F_{sw}$ ).

**Groundwater and the riparian interface:** *The conceptual framework is not entirely clear. The use of mass balance requires a boundary within which processes are measured (as  $F_{sw}$ ). For clarity I would suggest the following: The boundary is the riparian-stream edge, the stream channel is inside, and the riparian zone is outside. Fluxes pass through the stream edge, but no processes can occur “at the stream edge” (cf.617:3), only on one side or the other. In re-reading, I see that your suggestion that the apparent downstream nitrate source represents nitrification of riparian ammonium that occurs “at the riparian-stream edge” (617:14). From the standpoint of the Eq. 2, this process occurs inside the boundary, i.e., in the stream channel, the nitrogen having crossed the boundary as ammonium. So you could define “stream-edge” this way, clarifying that it is inside the riparian-stream boundary. If this nitrification actually*

*explains the downstream increase in nitrate concentration, you should be able to demonstrate that F<sub>gw</sub> of ammonium was sufficient to account for the negative F<sub>sw</sub> of nitrate in those segments.*

*As you formulated the mass balance, all groundwater fluxes to and from the stream pass through the riparian zone. If hillslope groundwater is to explain phosphorus concentrations in the stream (616:1-20), it must pass through the riparian and would have been measured there. The possibility that hillslope water flows bypasses the riparian, e.g., via underflow, to reach the stream is not consistent with Eq. 2. That is, to invoke hillslope groundwater you must acknowledge that the mass balance is incorrect. Regardless of how you handle the hillslope question, you should explicitly address the question of whether the riparian well samples correctly characterized the groundwater that reached the stream.*

*Is there an explanation as to why the chloride differed between periods even though the flow did not? At 614:13 you suggest that evapotranspiration (ET) may explain the higher chloride in the vegetative period, which makes sense except that the greater ET should also have reduced flow. Higher precipitation during the vegetative season, if it occurs, could compensate for the additional ET, explaining the similar flows.*

*The 40% longitudinal increase in chloride seems higher than can be explained by the decrease in area specific discharge and hence by ET. In support of the ET suggestion you state that chloride was higher in the main stream than in the tributaries (614:11). But Fig. 3b shows the opposite: Tributary chloride was higher than the mainstream and this, rather than ET, appears to explain much of the increase.*

**Answer:** As mentioned in our earlier responses, we agree that the conceptualization adopted in this study needs to be clearly defined. Consequently, we have included a schematic representation of our mass balance approach (new Figure 3). Following your suggestion, we have kept our conceptualization as clear and simple as possible, defining two compartments: the stream and the riparian zone. Moreover, we have removed ambiguous wording throughout the manuscript such as referring to biogeochemical transformation at the riparian-stream edge, which does not match with our conceptual model, as highlighted by you.

Following your suggestion, we have calculated whether increased stream NO<sub>3</sub><sup>-</sup> concentration at the valley bottom during the vegetative period could be explained by nitrification of NH<sub>4</sub><sup>+</sup> entering from riparian groundwater. Our results suggest additional

sources of nitrate within the stream because the flux of  $\text{NH}_4^+$  from riparian groundwater,  $F_{gw,NH4/x} \sim 2 \mu\text{g N m}^{-1} \text{ s}^{-1}$ , was not large enough to sustain in-stream  $\text{NO}_3^-$  release,  $|F_{sw,NO3} < 0| \sim 10 \mu\text{g N m}^{-1} \text{ s}^{-1}$  (pp22-lines 524-527). Discussion about these potential additional sources can be read in pp 22,24-lines 528-555 (see below our responses to your specific comments in this regard).

We agree with you, as well as with R1 and R2, that considering the influence of hillslope groundwater on stream water chemistry independently of riparian groundwater is confusing and further it violates the assumptions underlying the mass balance. In the new version of the manuscript, we have deleted this part from the discussion as well as former Figure 8. Further, the whole reach-scale nutrient budgets have shed new light about catchment sources contributing to stream SRP. According to our mass balance calculations, tributaries and riparian groundwater were the major contributors to the stream flux of SRP. In-stream processes further contributed to increase stream SRP fluxes (new Table 4 and Figure 7).

As already mentioned, the concluding remarks do now explicitly address the issue of whether water samples from riparian wells correctly characterized the groundwater that reached the stream (pp 26-lines 615-624).

As mentioned in our responses to R1 and R2, the easiest explanation for the increase in stream  $\text{Cl}^-$  during the vegetative period is that concentrations were higher in tributaries than at the main stream (the former interpretation was wrong). According to whole reach budgets, permanent tributaries were the major contributor to stream discharge (56%) and therefore, hydrological mixing with water from tributaries could partially explain the 40% increase in stream  $\text{Cl}^-$  concentrations observed along the reach as well as the higher concentrations during the vegetative period. In addition, riparian groundwater inputs could also contribute to these patterns because this compartment was an important source of water to the stream (26%) and it exhibited higher  $\text{Cl}^-$  concentration than stream water during through the year. These new results and the associated discussion are included in the new version of the manuscript (results section 4.1 and pp 20-lines 457-465).

Regarding the seasonality in P and Q, a more detailed look to data collected from our permanent sampling station has highlighted that both P and Q followed a seasonal pattern with minima in summer 2011, and further that Q at base flow conditions was higher during the dormant than during the vegetative period (Table R1). Yet, P was similar between the vegetative and dormant period mainly because spring

2011 was rainy and this rain counterbalanced the following dry summer. In addition, similar Q between the dormant and vegetative period for the synoptic survey were likely a consequence of the small sample size and the fact that the highest (April 2011) and the lowest flows (September 2011) fall within the vegetative period.

**Temporal variation:** *The absence of a temporal perspective (beyond the dormant/vegetative classification) is surprising. Most streams exhibit distinct seasonal variations in both flow and nutrient chemistry, and these patterns have proven critical to understanding biotic influences on stream chemistry (e.g., Mulholland et al.'s many papers on Walker Branch). If neither precipitation nor flow vary with season, this should be clearly stated. Otherwise we should at least be informed of the seasonal patterns and, ideally, your analysis would make use of them. You do state that dormant and vegetative flows were similar. However, if precipitation is uniform we would expect flow to reach a minimum at the end of the vegetative period and a maximum at the end of the dormant period, with the result that the average of the two seasons is the same.*

**Answer:** Right. We split the data in two groups (dormant and vegetative seasons) because the synoptic survey was not large enough to evaluate seasonal changes by calendar seasons. The consequence of doing so is that the seasonality was screened in some cases, such as indicated for stream discharge.

As mentioned in p-#4 (R2), our high-temporal resolution data indicated that stream discharge was highest in spring and autumn 2011 and lowest in summer 2011 (Table R1). Following the suggestion of both reviewers, we now provide information about the temporal variation in precipitation and stream discharge in the text as well as in new Figure 2. Please see our response to R2 in this regard.

**Page:line-referenced comments:**

610:22. *“According to the glm results, this decrease was positively related to basal area...” etc. These are the opposite of what is given in Table 3, which shows how these factors would relate to an increase, not a decrease. According to Table 3, basal area increases nitrate, but % N-fixers decreases it. In the GLM the independent variables clearly suffer from collinearity, as they all vary consistently with downstream direction. Collinearity leads to erratic regression coefficients, which may explain some of the seemingly odd relationships.*

**Answer:** Yes, right. As pointed out by R1, the statistical analysis of this sort of data set requires very careful consideration. This has been one of the reasons why we have decided to delete the GLM analysis from the new version of the manuscript.

614:17. “...suggesting...a gaining reach”? You should know from your flow measurements whether it was or was not gaining.

**Answer:** Yes, that’s right. As already mentioned, we have reanalyzed our data set in order to improve the hydrological characterization of the study reach. Please, see our previous responses to you and R1 in this regard. New results are shown in Figure 2c and d, and Figure 4b. The new discussion on these results can be read in [pp 19,20-lines 449-457](#)).

614:23. ” We found a decreasing longitudinal pattern of stream NO<sub>3</sub><sup>-</sup> concentrations, though only during the dormant period.” Fig. 5 clearly shows that nitrate decreased in both seasons above 3000 m. Don’t let the regression model obscure reality.

**Answer:** That’s right. As aforementioned, we now clearly state the U-shaped pattern exhibited by stream NO<sub>3</sub><sup>-</sup> concentrations in the results section. In the discussion, we indicate that stream NO<sub>3</sub><sup>-</sup> concentrations showed a decreasing longitudinal pattern along the first 1.5 km (regardless of the period) ([pp24 –lines 571-575](#)).

614:27-615:2 I disagree. It makes little sense to ignore the clear change in nitrate gradient that occurred at 3000 m, in both periods. It looks to me like in-stream processes were important for the downstream decline above 3000 m. I would say  $|F_{sw}/F_{in}|$ , at 11% (per segment, right?), is large. Positive values were much more frequent than negative values in the 11 segments above 3000 m. Tributary concentrations were low, so also contributed to the decline. You should calculate how much was instream and how much was dilution.

**Answer:** Thanks for raising this point. We agree that some further work was needed. Following the suggestions from the three reviewers, the U-shaped pattern exhibited by stream NO<sub>3</sub><sup>-</sup> concentrations is now clearly stated ([pp 16-line 374](#)).

We have calculated separately nutrient budgets for the upper and lower sections of the reach (first 3 km vs. last 0.7 km, respectively). As included in our earlier responses,  $F_{sw,NO_3}$  was higher for the upper than for the lower section of the reach (0.61 vs. -3  $\mu\text{g N m}^{-1}\text{s}^{-1}$ ) (Wilcoxon signed-rank test, Signed-rank = 19.5, df = 9, p = 0.049).



Thus, you are essentially right in that in-stream net uptake prevailed over release for  $\text{NO}_3^-$  along the upper section and in-stream processes could contribute to reduce stream nitrate flux. According to our calculations, this contribution could explain 10% [0%, 20%] of the reduction in stream  $\text{NO}_3^-$  flux along the first 3 km of the reach.

In addition, hydrological mixing with water from tributaries could also contribute to decrease stream  $\text{NO}_3^-$  concentrations because  $\text{NO}_3^-$  concentration was lower in the tributaries. We have calculated the expected stream  $\text{NO}_3^-$  concentration if the upstream-most tributary would have explained the change in stream  $\text{NO}_3^-$  concentration between the 4<sup>th</sup> and 5<sup>th</sup> sampling site. For the 11 sampling dates, the relative difference between measured and expected stream  $\text{NO}_3^-$  concentration ranges from -45 to +18% (mean = -12%). Once the uncertainty associated to these calculations is taken into account, there are only 2 out of 11 dates for which hydrological mixing could fully explain the change in  $\text{NO}_3^-$  concentration between these two sampling sites. In the other 9 cases, the combination of in-stream net uptake and dilution were likely responsible for the observed longitudinal decrease. We have not included all these calculations in the manuscript, though we now clearly state in the discussion that both in-stream cycling and hydrological mixing could explain the observed declining pattern for  $\text{NO}_3^-$  (pp 24-lines 57-581).

*615: 18 The lack of correlation with N<sub>2</sub>-fixing species does not rule out their possible influence because there was an opposite nitrate gradient in the region upstream of 3000 that would have obscured the correlation in the lower reaches.*

**Answer:** Yes, right. Following suggestions from the three reviewers, we have rewritten this part of the discussion. In particular, we highlight that the flux of  $\text{NH}_4^+$  from riparian groundwater is not large enough to sustain in-stream  $\text{NO}_3^-$  release during the vegetative period along the last 700 m of the reach (pp 22-lines 524-527). Among several potential explanations for such additional source of stream N, we pointed out leaf litter from riparian trees, and especially from  $\text{N}_2$ -fixing species, that combined with warm temperatures could enhance in-stream mineralization and nitrification during the vegetative period (pp 22,23-lines 528-535).

*615: 22 Release of nitrate from the streambed raises the question of the ultimate source of the nitrogen. Simple storage and release cannot produce a net release; the high concentrations at one time would have to be balanced by low concentrations at another.*

*On 616:14-18 your suggest that the source may be nitrified ammonium. Perhaps that should be mentioned here. Another possibility is instream N fixation. See Finley et al. 2011 Ecol. 92:140 who saw a sharp downstream increase due to Nfixation. Also, as mentioned above, I am concerned about human sources.*

**Answer:** Right. We have reorganized the discussion section as suggested. After noticing that the flux of  $\text{NH}_4^+$  from riparian groundwater is not high enough to sustain in-stream  $\text{NO}_3^-$  release along the last 700 m, we propose several additional sources: (1) in-stream nitrification enhanced by large stocks of riparian leaf litter (from  $\text{N}_2$ -fixing species in particular), (2) human sources of dissolved N (3) autotrophic N fixation. The feasibility of these different sources is discussed in light of the evidences we have (pp22,24-lines 515-555).

*616:1 Stream fluxes did not decrease downstream; what decreased was area-specific flux. The absolute nutrient fluxes increased greatly.*

**Answer:** Right, thanks. This sentence has been deleted from the discussion, though.

*616:3 "...concentrations increased from the top to the bottom of the reach for all nutrients (except for  $\text{NO}_3$  during the dormant period." Nitrate did not increase in the vegetative period either, and ammonium did not increase in the vegetative period.*

**Answer:** Right, thanks. This sentence has been deleted from the discussion.

*617:13 "especially during the vegetative period" should read "although only in the vegetative period".*

**Answer:** Yes. Thanks (pp22-line 520).

*617:25-28. This sentence does not quite make sense. The first part refers to "predominant" in-stream processes while the second part effectively states that there was not a net uptake or release along the reach, i.e., that there was no "predominant" process. As I have argued, it is fallacious to say that a longitudinal trend is consistent or in-consistent with in-stream processing, as it is a matter of balance with lateral inputs. I think that for nitrate above 3000 m, you had both net uptake and a declining trend although low tributary concentrations may also have contributed to the decline. For ammonium and phosphate above 3000 m you had net uptake and no decline because the uptake drew down the concentrations of lateral inputs, i.e., uptake balanced*

*lateral inputs. In my view this is a common case in streams where there is a net uptake. However, this is hard to document from the literature, Meyer and Likens (1979) and Alexander et al. (2009 Biogeochemistry 93:91) being the only two examples I can readily find. This is why your study is so important.*

**Answer:** Yes, thanks. We have rewritten this paragraph following several of the points highlighted in your review. As already included in earlier parts of this letter, (1) we don't refer to "predominant" in-stream processes because  $F_{sw}$  was highly variable as it is now explicitly included in the discussion (pp 21-line 481), (2) we have calculated nutrient budgets separately for the upper and lower part of the reach, (3) we have calculated the contribution of tributaries to stream nutrient fluxes for the whole reach, and (4) we have calculated whether riparian groundwater fluxes of  $\text{NH}_4^+$  at the valley bottom could sustain the measured in-stream  $\text{NO}_3^-$  release.

With these new pieces of information, we now discuss that the declining pattern exhibited by  $\text{NO}_3^-$ , especially along the first 1.5 km could be explained by the combination of in-stream net  $\text{NO}_3^-$  uptake (ca. 10%) and hydrological mixing with water from tributaries (pp 24,25-lines 572-581). Conversely, the marked increase in stream  $\text{NO}_3^-$  along the 700 km may be explained by in-stream mineralization and nitrification of organic matter accumulated within the stream. Human sources of DIN and N fixation by algae seem not plausible explanations (pp 23-lines 536-547).

As you pointed out, our results for  $\text{NH}_4^+$  indicated that in-stream net uptake balanced elevated inputs from riparian groundwater. Thus, the case of  $\text{NH}_4^+$  is paradigmatic because it clearly shows that in-stream processes can strongly affect stream nutrient chemistry and downstream nutrient export without generating longitudinal gradients (pp 24-lines 560-570).

For SRP, the importance of in-stream net uptake above 3 km is not so clear. If nutrient budgets are calculated separately for the upper and lower sections,  $F_{sw,SRP}$  is higher for the first 3 km than for the last 0.7 km (0.005 vs -0.28  $\mu\text{g P m}^{-1} \text{s}^{-1}$ ). However, these differences are not significant (Wilcoxon paired rank sum test,  $p = 0.1$ ), and  $F_{sw,SRP}$  is close to zero even for the first 3 km of the reach. Along the last 0.7- km, mixing with the downstream-most tributary (the human-altered one) could partially explain the increase in stream SRP concentrations. Yet, on average, the discharge at this tributary should be 4 folds higher than expected for its drainage area to fully explain the observed increase in SRP (pp 23-lines 539-542).

618:14 delete “through” (an apparent typo) and “profound” (exaggeration).

**Answer:** Ok. Thanks.

619:19 “can offset the effect of” should read “must be taken into account when interpreting longitudinal gradients”.

**Answer:** Agree. Thanks. We have reworded the text to avoid this sort of confusion in our message.

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1 **Riparian and in-stream controls on nutrient concentrations and fluxes in a**  
2 **headwater forested stream.**

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12 variation, riparian groundwater, in-stream net nutrient cycling.

13

14 **Abstract**

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

38 chemistry is crucial to understand the contribution of in-stream processes to stream  
39 nutrient dynamics at relevant ecological scales.

## 40 **1. Introduction**

41 Stream water chemistry integrates hydrological and biogeochemical processes  
42 occurring within its drainage area and thus, the temporal variation of stream solute  
43 concentrations at the catchment outlet is considered a good indicator of the response of  
44 terrestrial and aquatic ecosystems to environmental drivers (Bormann and Likens, 1967;  
45 Bernhardt et al., 2003; Houlton et al., 2003). Less attention has been paid to the spatial  
46 variation of water chemistry along the stream, though it can be considerably important  
47 because stream nutrient concentrations are influenced by changes in hydrological flow  
48 paths, vegetation cover, and soil characteristics (Dent and Grimm, 1999; Likens and  
49 Buso, 2006). For instance, spatial variation in nutrient concentration along the stream  
50 has been attributed to changes in soil nitrification rates (Bohlen et al., 2001), soil  
51 organic carbon availability (Johnson et al., 2000), and organic soil depth across  
52 altitudinal gradients (Lawrence et al., 2000). Moreover, nutrient cycling within the  
53 riparian zone can strongly influence stream nutrient concentrations along the stream  
54 because these ecosystems are hot spots of biogeochemical processing (McClain et al.,  
55 2003; Vidon et al., 2010). In addition, processes occurring at the riparian-stream  
56 interface have a major influence on stream water chemistry than those occurring at  
57 catchment locations further from the stream (Ross et al., 2012). Finally, stream  
58 ecosystems have a strong capacity to transform and retain nutrients; and thus, in-stream  
59 biogeochemical processes can further influence nutrient chemistry along the stream  
60 (Peterson et al., 2001; Dent et al., 2007). Therefore, consideration of these multiple  
61 sources of variation of stream water chemistry is important to understand drivers of  
62 stream nutrient dynamics.

63           Our understanding of nutrient biogeochemistry within riparian zones and  
64 streams is mainly based on field studies performed at the plot-scale or in small stream  
65 reaches (few hundred meters) (Lowrance et al., 1997; Peterson et al., 2001; Sabater et  
66 al., 2003; Mayer et al., 2007; von Schiller et al., 2015). These empirical studies have  
67 widely demonstrated the potential of riparian and stream ecosystems as either sinks or  
68 sources of nutrients, which ultimately influence the transport of nutrients to downstream  
69 ecosystems. Riparian and stream biota are capable to decrease the concentration of  
70 essential nutrients, such as dissolved inorganic nitrogen (DIN) and phosphate,  
71 especially with increasing water storage and residence time (Valett et al., 1996; Hedin et  
72 al., 1998; Peterson et al., 2001; Vidon and Hill, 2004). Conversely, riparian forests can  
73 become sources rather than sinks of nutrients when N<sub>2</sub>-fixing species predominate  
74 (Helfield and Naiman, 2002; Compton et al., 2003), and in-stream nutrient release can  
75 be important during some periods (Bernhardt et al., 2002; von Schiller et al., 2015).  
76 Moreover, there is an intimate hydrological linkage between riparian and stream  
77 ecosystems that can result in strong biogeochemical feedbacks between these two  
78 compartments (e.g., Morrice et al., 1997; Martí et al., 2000; Bernal and Sabater, 2012).  
79 However, studies integrating biogeochemical processes at these two nearby ecosystems  
80 are rare (but see Dent et al., 2007), and the exchange of water and nutrients between  
81 stream and groundwater is unknown in most studies assessing in-stream gross and net  
82 nutrient uptake (Roberts and Mulholland, 2007; Covino et al., 2010; von Schiller et al.,  
83 2011).

84           There is a wide body of knowledge showing the potential of riparian and stream  
85 ecosystems to modify either groundwater or stream nutrient concentrations. Yet, a  
86 comprehensive view of the influence of riparian and in-stream processes on stream  
87 water chemistry at the catchment scale is still lacking (but see Meyer and Likens, 1979).



88 This gap of knowledge mostly exists because hydrological and biogeochemical  
89 processes can vary substantially along the stream (Covino and McGlynn, 2007; Jencso  
90 et al., 2010), which limits our ability to extrapolate small plot- and reach- scale  
91 measurements to larger spatial scales. Some authors have proposed that nutrient  
92 concentrations should decline along the stream if in-stream net uptake is high enough  
93 and riparian groundwater inputs are relatively small (Brookshire et al., 2009). This  
94 declining pattern is not systematically observed in reach-scale studies, which could  
95 bring us to the conclusion that terrestrial inputs are the major driver of stream water  
96 chemistry because in-stream gross uptake and release counterbalance each other most of  
97 the time (Brookshire et al., 2009). However, synoptic studies have revealed that nutrient  
98 concentrations are patchy and highly variable along the stream as a result of spatial  
99 patterns in upwelling and in-stream nutrient processing (Dent and Grimm, 1999). Thus,  
100 in-stream nutrient cycling could be substantial, but not necessarily lead to longitudinal  
101 increases or declines in nutrient concentration, a question that probably needs to be  
102 addressed at spatial scales larger than few hundred meters.

103         The goal of this study was to gain a better understanding of the influence of  
104 riparian groundwater inputs and in-stream biogeochemical processing on stream  
105 nutrient chemistry and fluxes in a headwater forested catchment. To approach this  
106 question, we explored the longitudinal pattern of stream nutrient (nitrate, ammonium,  
107 and phosphate) concentration along a 3.7-km reach during 1.5 years. We choose a  
108 headwater catchment as a model system to investigate drivers of spatial patterns in  
109 stream water chemistry because they typically show pronounce changes in riparian and  
110 stream features across relatively short distances (Uehlinger, 2000). First, we evaluated  
111 riparian groundwater inputs and in-stream nutrient processing as sources of variation of  
112 stream nutrient concentration along the reach. We expected stream and riparian

113 groundwater nutrient concentrations to be similar and strongly correlated if riparian  
114 groundwater is a major source of nutrients to the stream. In addition, we estimated the  
115 in-stream nutrient processing capacity for 14 contiguous segments along the reach with  
116 a mass balance approach. Second, we evaluated the relative contribution of riparian  
117 groundwater inputs and in-stream biogeochemical processing to stream nutrient fluxes  
118 at the whole-reach scale by applying a mass balance approach that include all  
119 hydrological input and output fluxes along the reach.

## 120 **2. Study Site**

121 The research was conducted in the Font del Regàs catchment (14.2 km<sup>2</sup>) (Figure  
122 1), located in the Montseny Natural Park, NE Spain (41°50'N, 2°30'E, 300-1200 m  
123 a.s.l.) during the period 2010-2011. Total inorganic N deposition in this area oscillates  
124 between 15-30 kg N ha<sup>-1</sup> year<sup>-1</sup> (Àvila and Rodà 2012). The climate at the Montseny  
125 Mountains is subhumid Mediterranean. The long-term mean annual precipitation is  
126 925±151 mm and the long-term mean annual air temperature is 12.1±2.5 °C (mean ±  
127 SD, period: 1940-2000, Catalan Metereologic Service:  
128 <http://www.meteo.cat/servmet/index.html>). During the study period, mean annual  
129 precipitation (975 mm) and temperature (12.9 °C) fall within the long-term average  
130 (data from a meteorological station within the study catchment). In this period, summer  
131 was the driest season (140 mm) while most of the precipitation occurred in winter 2010  
132 (370 mm) and autumn 2011 (555 mm) (Figure 2a).

133 The catchment is dominated by biotitic granite (ICC, 2010) and it has steep  
134 slopes (28%). Evergreen oak (*Quercus ilex*) and beech (*Fagus sylvatica*) forests cover  
135 54% and 38% of the catchment area, respectively (Figure 1). The upper part of the  
136 catchment (2%) is covered by heathlands and grasslands (ICC, 2010). The catchment  
137 has a low population density (< 1 person km<sup>-2</sup>) which is concentrated in the valley

138 bottom. Hillslope soils (pH ~ 6) are sandy, with high content of rocks (33-36%). Soils at  
139 the hillslopes have a 3-cm depth O-horizon and a 5- to 15-cm depth A-horizon  
140 (averaged from 10 soil profiles).

141 The riparian zone is relatively flat (slope < 10%), and it covers 6% of the  
142 catchment area. Riparian soils (pH ~ 7) are sandy-loam with low rock content (13%)  
143 and a 5-cm depth organic layer followed by a 30-cm depth A-horizon (averaged from 5  
144 soil profiles). Along the 3.7-km reach, the width of the riparian zone increases from 6 to  
145 32 m, whereas the total basal area of riparian trees increases by 12 folds (based on forest  
146 inventories of 30-m plots every ca. 150 m) (Figure S1). *Alnus glutinosa*, *Robinia*  
147 *pseudoacacia*, *Platanus hybrida*, and *Fraxinus excelsior* are the most abundant riparian  
148 tree species followed by *Corylus avellana*, *Populus tremula*, *Populus nigra*, and  
149 *Sambucus nigra*. The abundance of N<sub>2</sub>-fixing species (*A. glutinosa* and *R.*  
150 *pseudoacacia*) increases from 0% to > 60% along the longitudinal profile, (Figure S1).  
151 During base flow conditions, riparian groundwater (< 1.5 m from the stream channel)  
152 flows well below the soil surface ( $0.5 \pm 0.1$  m) and thus, the interaction with the riparian  
153 organic soil is minimal (averaged from 15 piezometers, n = 165) (Figure S1). During  
154 the period of study, riparian groundwater temperature ranged from 5 to 19.5 °C.

155 The 3.7-km study reach is a 2<sup>nd</sup> order stream along the first 1.5 km and a 3<sup>rd</sup>  
156 order stream for the remaining 63% of its length. The geomorphology of the stream bed  
157 changes substantially with stream order. The stream bed along the 2<sup>nd</sup> order section is  
158 mainly composed of rocks and cobbles (70%) with a small contribution of sand (~  
159 10%). At the valley bottom, sands and gravels represent 44% of the stream substrate  
160 and the presence of rocks is minor (14%). Mean wetted width and water velocity  
161 increase between the 2<sup>nd</sup> and 3<sup>rd</sup> order section (from 1.6 to 2.7 m and from 0.24 to 0.35  
162 m s<sup>-1</sup>, respectively) (Figure S1). During the study period, stream water temperature

163 ranged from 5 to 18°C. Stream discharge was low in summer (0.33 mm) and peaked in  
164 spring (0.79 mm).

### 165 **3. Materials and Methods**

#### 166 *3.1. Field sampling and laboratory analysis*

167 We selected 15 sampling sites along the 3.7-km study reach. The distance  
168 between consecutive sampling sites ranged from 110 to 600 m (Figure 1). At each  
169 sampling site, we installed a 1-m long PVC piezometer (3-cm Ø) in the riparian zone at  
170 ~ 1.5 m from the stream channel.

171 For each sampling site, we sampled stream water (from the thalweg) and riparian  
172 groundwater every 2 months from August 2010 to December 2011. We used pre-acid  
173 washed polyethylene bottles to collect water samples after triple-rinsing them with  
174 either stream or groundwater. On each sampling date, we also measured dissolved  
175 oxygen concentration (DO, in mg l<sup>-1</sup>) and water temperature (T, in °C) with an YSI  
176 ProODO device in both stream water and in riparian groundwater. We avoid sampling  
177 soon after storms to ensure that our measurements were representative of low flow  
178 conditions, when the influence of in-stream biogeochemical processes on stream  
179 nutrient concentrations and fluxes is expected to be the highest. All field campaigns  
180 were performed at least 9 days after storm events, except in October 2011 (Figure 2b,  
181 black squares). On each sampling date and at each sampling site, we measured  
182 groundwater table elevation (in m below soil surface) with a water level sensor  
183 (Eijkelkamp 11.03.30) as well as wetted width (in m), stream discharge ( $Q$ , in l s<sup>-1</sup>), and  
184 water velocity (m s<sup>-1</sup>).  $Q$  and water velocity were estimated with the slug-addition  
185 technique by adding 1 l of NaCl-enriched solution to the stream (electrical conductivity  
186 = 75-90 mS cm<sup>-1</sup>, n = 11) (Gordon et al., 2004). The uncertainty associated with  $Q$   
187 measurements was calculated as the relative difference in  $Q$  between pairs of tracer

188 additions under equal water depth conditions (difference < 1 mm). The pairs of data  
189 were selected from a set of 126 slug additions and water level measurements obtained  
190 from the permanent field stations at Font del Regàs (Lupon, unpublished). The  
191 measured uncertainty was relatively small (1.9%, n = 11). On each sampling date, we  
192 also collected stream water and measured  $Q$  at the four permanent tributaries  
193 discharging to Font del Regàs stream, which drained 1.9, 3.2, 1.8, and 1.1 km<sup>2</sup>,  
194 respectively (Figure 1). These data were used for mass balance calculations (see below).  
195 Additional stream water samples were collected from a small permanent tributary that  
196 drained through an area (< 0.4 km<sup>2</sup>) with few residences and crop fields for personal  
197 consumption.

198 Water samples were filtered through pre-ashed GF/F filters (Whatman®) and  
199 kept cold (< 4°C) until laboratory analysis (< 24h after collection). Chloride (Cl<sup>-</sup>) was  
200 used as a conservative hydrological tracer and analyzed by ionic chromatography  
201 (Compact IC-761, Methrom). Nitrate (NO<sub>3</sub><sup>-</sup>) was analyzed by the cadmium reduction  
202 method (Keeney and Nelson 1982) using a Technicon Autoanalyzer (Technicon, 1976).  
203 Ammonium (NH<sub>4</sub><sup>+</sup>) was manually analyzed by the salicilate-nitropruside method  
204 (Baethgen and Alley 1989) using a spectrophotometer (PharmaSpec UV-1700  
205 SHIMADZU). Soluble reactive phosphorus (SRP) was manually analyzed by the acidic  
206 molybdate method (Murphy and Riley, 1962) using a spectrophotometer (PharmaSpec  
207 UV-1700 SHIMADZU).

### 208 3.2. Data analysis

209 The seasonality of biological activity can strongly affect both riparian  
210 groundwater chemistry and in-stream biogeochemical processes (Groffman et al., 1992;  
211 Hill et al., 2001). Therefore, the data set was separated in two groups based on sampling  
212 dates during the vegetative and dormant period (7 and 4 sampling dates, respectively).

213 As a reference, we considered the vegetative period starting at the beginning of riparian  
214 leave out (April) and ending at the peak of leave-litter fall (October), coinciding with  
215 the onset and offset of riparian tree evapotranspiration, respectively (Nadal-Sala et al.,  
216 2013). During the study period, rainfall was similar between the vegetative and dormant  
217 period (775 and 876 mm, respectively).

218

### 219 *3.2.1. Patterns of stream discharge, riparian groundwater inputs, and stream solute* 220 *concentrations*

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

230 The contribution of net riparian groundwater inputs to surface water along each  
231 stream segment ( $Q_{gw}$ ) was estimated as the difference in  $Q$  between consecutive  
232 sampling sites (Covino et al., 2010). The empirical uncertainty associated with  $Q$  was  
233 used to calculate a lower and upper limit of  $Q_{gw}$ . We considered that  $Q_{gw}$  was  
234 representative of the net riparian groundwater flux draining to the stream within each  
235 stream segment. We acknowledge that this approach oversimplifies the complex  
236 hydrological interactions at the riparian-stream interface because it does not consider  
237 concurrent hydrological gains and losses within each segment (Payn et al., 2009), but

238 we consider that it provides a representative estimate at the scale of this study. To  
239 investigate the longitudinal pattern of riparian groundwater inputs, we calculated the  
240 cumulative area-specific net riparian groundwater input ( $\Sigma Q'_{gw}$ , in  $\text{mm d}^{-1}$ ) by summing  
241 up  $Q_{gw}$  from the upstream-most site to each of the downstream segments and dividing it  
242 by the cumulative catchment area.

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

253

### 254 3.2.2. Sources of variation of stream nutrient concentration along the reach

255 *Riparian groundwater inputs.* We investigated whether longitudinal patterns in  
256 stream solute concentration were driven by riparian groundwater inputs by comparing  
257 solute concentrations between stream water and riparian groundwater with a Wilcoxon  
258 paired sum rank test. A non-parametric test was used because solute concentrations  
259 were not normally distributed (Shapiro-Wilk test,  $p < 0.01$  for all study solutes) (Zar,  
260 2010).

261 Moreover, we examined the degree of hydrological interaction at the riparian-  
262 stream interface by exploring the relationship between stream and riparian groundwater

263 Cl<sup>-</sup> concentrations with a Spearman correlation. For each period, we quantified the  
 264 difference between Cl<sup>-</sup> concentrations in the two water bodies by calculating  
 265 divergences from the 1:1 line with the relative root mean square error (RRMSE, in %):

$$266 \quad RRMSE = \frac{\sqrt{\sum_{i=1}^n (C_{sw} - C_{gw})^2}}{n \cdot \overline{C_{gw}}} \cdot 100 \quad (1)$$

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

276 *In-stream nutrient processing.* We investigated the influence of in-stream  
 277 biogeochemical processes on the longitudinal pattern of stream nutrient concentrations  
 278 by applying a mass balance approach for each individual segment (Roberts and  
 279 Mulholland, 2007). For each nutrient, we calculated changes in stream flux between  
 280 contiguous sampling sites ( $F_{sw}$ , in  $\mu\text{g m}^{-1} \text{s}^{-1}$ ), being  $F_{sw}$  the net flux resulting from in-  
 281 stream gross uptake and release along a particular stream segment (von Schiller et al.,  
 282 2011). We expressed  $F_{sw}$  by unit of stream length in order to compare net changes in  
 283 stream flux between segments differing in length. For each sampling date and for each  
 284 nutrient,  $F_{sw}$  was approximated with:

$$285 \quad F_{sw} = (F_{top} + F_{ef} + F_{gw} - F_{bot}) / x, \quad (2)$$



286 where  $F_{top}$  and  $F_{bot}$  are the nutrient flux at the top and at the bottom of each stream  
287 segment,  $F_{gw}$  is the nutrient flux from net riparian groundwater inputs, and  $F_{ef}$  is the  
288 nutrient flux from effluent inputs for those reaches including a tributary (all in  $\mu\text{g s}^{-1}$ )  
289 (Figure 3).  $F_{top}$  and  $F_{bot}$  were calculated by multiplying  $Q$  by  $C_{sw}$  at the top and at the  
290 bottom of the segment, respectively.  $F_{gw}$  was estimated by multiplying net groundwater  
291 inputs ( $Q_{gw}$ ) by nutrient concentration in either riparian groundwater or stream water.  
292 For net gaining segments ( $Q_{gw} > 0$ ), we assumed that the chemistry of net water inputs  
293 was similar to that measured in riparian groundwater and thus,  $C_{gw}$  was the average  
294 between riparian groundwater nutrient concentration at the top and bottom of the reach.  
295 For net losing segments ( $Q_{gw} < 0$ ), we assumed that the chemistry of net water losses  
296 was similar to that measured in stream water and thus,  $C_{gw}$  averaged stream water  
297 concentration at the top and at the bottom of each reach segment ( $C_{top}$  and  $C_{bot}$ ,  
298 respectively). For those cases in which stream segments received water from a tributary,  
299  $F_{ef}$  was calculated by multiplying  $Q$  and  $C$  at the outlet of the tributary. We calculated  
300 an upper and lower limit of  $F_{sw}$  based on the empirical uncertainty associated with water  
301 fluxes ( $Q$  and  $Q_{gw}$ ). Finally,  $x$  (in m) is the length of the segment between two  
302 consecutive sampling sites. The same approach was applied for  $\text{Cl}^-$ , a conservative  
303 tracer that was used as a hydrological reference. For  $\text{Cl}^-$ , we expected  $F_{sw} \sim 0$  if inputs  
304 from upstream, tributaries, and riparian groundwater account for most of the stream  $\text{Cl}^-$   
305 flux. For nutrients,  $F_{sw}$  can be positive (gross uptake  $>$  release), negative (gross uptake  $<$   
306 release) or nil (gross uptake  $\sim$  release). Therefore, we expected  $F_{sw} \neq 0$  if in-stream  
307 gross uptake and release processes do not fully counterbalance each other (von Schiller  
308 et al., 2011). To investigate whether stream segments were consistently acting as net  
309 sinks or net sources of nutrients along the stream during the study period, we calculated  
310 the frequency of  $F_{sw} > 0$ ,  $F_{sw} < 0$ , and  $F_{sw} = 0$  for each nutrient and for each segment..

311 We assumed that  $F_{sw}$  was undistinguishable from 0 when its upper and lower limit  
312 contained zero.

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

319

### 320 *3.2.3. Relative contribution of riparian groundwater and in-stream nutrient processing* 321 *to stream nutrient fluxes*

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

330 We used a whole-reach mass balance approach to assess the relative contribution  
331 of net riparian groundwater inputs ( $F_{gw} > 0 / F_{in}$ ) and in-stream release ( $|F_{sw} < 0 / F_{in}|$ ) to  
332 stream solute fluxes. In addition, we calculated the contribution of upstream ( $F_{top} / F_{in}$ )  
333 and tributary inputs ( $F_{ef} / F_{in}$ ) to stream solute fluxes. For each solute, we analyzed  
334 differences in the relative contribution of different sources to stream input fluxes with a  
335 Mann Whitney test. Finally, when the whole reach was acting as a net sink for a

336 particular nutrient ( $F_{sw} > 0$ ), we calculated the relative contribution of in-stream net  
337 uptake to reduce stream nutrient fluxes along the 3.7-km reach with  $F_{sw} \cdot x / F_{in}$ .

## 338 4. Results

### 339 4.1. Hydrological characterization of the stream reach

340 During the study period, mean  $Q'$  decreased from  $0.82 \pm 0.13$  [mean  $\pm$  SE] to  
341  $0.54 \pm 0.11$  mm d<sup>-1</sup> along the reach (linear regression [l.reg],  $r^2 = 0.79$ , degrees of  
342 freedom [df] = 14,  $F = 51.4$ ,  $p < 0.0001$ ) (Figure 4a). This pattern hold for the two  
343 seasonal periods considered (dormant and vegetative; Wilcoxon rank sum test,  $p >$   
344  $0.05$ ).

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

354 Stream Cl<sup>-</sup> concentrations showed a 40% increase along the reach (l.reg,  $r^2 =$   
355  $0.88$ ,  $df = 14$ ,  $F = 44.6$ ,  $p < 0.0001$ ), which contrasted with the longitudinal pattern  
356 exhibited by stream discharge (Figure 4c). The two periods showed a similar  
357 longitudinal pattern, though stream Cl<sup>-</sup> concentration was lower during the dormant than  
358 during the vegetative period (Wilcoxon rank sum test,  $Z = -6.4$ ,  $p < 0.0001$ ) (Table 1).  
359 The same seasonal pattern was exhibited by the five permanent tributaries (Figure 4c).

360 There was a strong correlation between stream and riparian groundwater Cl<sup>-</sup>  
361 concentrations, which fitted well to the 1:1 line (low RRMSE for the two periods)  
362 (Table 2 and Figure S2).

363 The mean net change in Cl<sup>-</sup> flux within individual segments was  $0.4 \pm 0.03$  mg  
364 m<sup>-1</sup> s<sup>-1</sup>, which represented a small fraction of the Cl<sup>-</sup> input flux ( $|F_{sw} \cdot x / F_{in}| < 6\%$ ).  
365 Similar results were obtained when calculating Cl<sup>-</sup> budgets for the whole-reach  
366 approach (Table 3). The stream Cl<sup>-</sup> flux was mainly explained by inputs from tributaries  
367 followed by riparian groundwater and upstream (Table 4). Similar results were obtained  
368 when calculating the relative contribution of different water sources to stream discharge  
369 at the whole-reach scale.

#### 370 *4.2. Longitudinal pattern of stream nutrient concentration*

371 The longitudinal pattern of stream concentration differed between nutrients and  
372 periods. During the dormant period, stream NO<sub>3</sub><sup>-</sup> concentration decreased along the  
373 reach especially within the first 1.5 km (l.reg,  $r^2 = 0.47$ ,  $df = 15$ ,  $F = 11.4$ ,  $p < 0.005$ )  
374 (Figure 5a). During the vegetative period, stream NO<sub>3</sub><sup>-</sup> concentration showed a U-  
375 shaped pattern: it decreased along the first 1.5 km, remained constant along the  
376 following 1 km, and increased by 60% along the last km of the reach (Figure 5a).  
377 Despite these differences, stream NO<sub>3</sub><sup>-</sup> concentration was similar between the dormant  
378 and vegetative period for both the main stream and tributaries (in all cases, Wilcoxon  
379 rank sum test,  $p > 0.05$ ) (Table 1).

380 Stream NH<sub>4</sub><sup>+</sup> concentration showed an increasing longitudinal pattern during the  
381 dormant period (exponential regression [e.reg],  $r^2 = 0.45$ ,  $df = 15$ ,  $F = 10.5$ ,  $p < 0.01$ ),  
382 while concentration decreased during the vegetative period (logarithmic regression  
383 [lg.reg],  $r^2 = 0.42$ ,  $df = 15$ ,  $F = 9.6$ ,  $p < 0.01$ ) (Figure 5b). The main stream showed

384 higher  $\text{NH}_4^+$  concentration during the vegetative than during the dormant period  
385 (Wilcoxon rank sum test,  $Z_{\text{NH}_4} = -3.5$ ,  $p < 0.001$ ) (Table 1). For the tributaries,  $\text{NH}_4^+$   
386 concentration was similar between the two periods (in all cases, Wilcoxon rank sum  
387 test,  $p > 0.01$ ).

388 Stream SRP concentration increased along the reach during both the dormant  
389 (e.reg,  $r^2 = 0.59$ ,  $F = 18.5$ ,  $df = 14$ ,  $p < 0.01$ ) and vegetative period (l.reg,  $r^2 = 0.49$ ,  $F =$   
390  $12.4$ ,  $df = 14$ ,  $p < 0.01$ ) (Figure 5c). Similarly to  $\text{NH}_4^+$ , the main stream showed higher  
391 SRP concentration during the vegetative than during the dormant period (Wilcoxon rank  
392 sum test,  $Z_{\text{SRP}} = -6.6$ ,  $p < 0.001$ ) (Table 1). For the tributaries, SRP concentration was  
393 similar between the two periods (in all cases, Wilcoxon rank sum test,  $p > 0.01$ ).

#### 394 *4.3. Sources of variation of stream nutrient concentration*

395 *Riparian groundwater inputs.* The relationship between stream and riparian  
396 groundwater concentrations differed between nutrients and periods. During the dormant  
397 period, stream and riparian groundwater  $\text{NO}_3^-$  concentrations were similar, while the  
398 stream showed higher concentration during the vegetative period (Table 1). During the  
399 two periods, stream and riparian groundwater  $\text{NO}_3^-$  concentrations were positively  
400 correlated and showed relatively small RRMSE (Table 2 and Figure S2).  $\text{NH}_4^+$   
401 concentration in stream water was 2-3 folds lower than in riparian groundwater (Table  
402 1), and stream and groundwater concentrations were no correlated either during the  
403 dormant or vegetative periods (Table 2). Stream and riparian groundwater SRP  
404 concentrations were similar in the two periods (Table 1). During the dormant period,  
405 SRP concentration showed a significant correlation between the two water bodies, while  
406 no correlation and relatively high RRMSE occurred during the vegetative period (Table  
407 2). The differences in nutrient concentrations between stream and riparian groundwater

408 in the two study periods were accompanied by consistently higher DO concentrations in  
409 the stream than in riparian groundwater (Table 1).

410 *In-stream nutrient processing.* The influence of in-stream nutrient processing on  
411 stream water chemistry differed among nutrients. During the study period, median  $F_{sw}$   
412 was negative for  $\text{NO}_3^-$ , positive for  $\text{NH}_4^+$ , and close to 0 for SRP (Table 3). Yet,  
413 differences in  $F_{sw}$  were not statistically significant among nutrients for either the  
414 vegetative or dormant period (for both periods: Mann Whitney test with post-hoc Tukey  
415 test,  $p > 0.05$ ). Similar  $F_{sw}$  values were obtained when calculating nutrient budgets  
416 either by segment or whole reach (Table 3).

417 The frequency of an individual segment to act either as a nutrient sinks or source  
418 differed among nutrients and along the reach. For  $\text{NO}_3^-$ , the frequency of  $F_{sw, \text{NO}_3} < 0$   
419 (gross uptake < release) increased from 9 to > 50% along the reach (l.reg,  $r^2 = 0.55$ ,  $df =$   
420 13,  $F = 14.67$ ,  $p < 0.01$ ) (Figure 6a). For  $\text{NH}_4^+$ , the frequency of  $F_{sw, \text{NH}_4} > 0$  (gross  
421 uptake > release) was high across individual segments, ranging from 20 to 90% (Figure  
422 6b). For SRP, the frequency of  $F_{sw, \text{SRP}} < 0$ ,  $> 0$ , or  $\sim 0$  did not show any consistent  
423 longitudinal pattern (Figure 6c). Overall, the frequency of sampling dates for which in-  
424 stream biogeochemical processes were imbalanced ( $F_{sw} \neq 0$ ) was lower for  $\text{NO}_3^-$  (36%)  
425 than for  $\text{NH}_4^+$  (80%) and SRP (68%) (Figure 6).

#### 426 *4.4. Relative contribution of riparian groundwater and in-stream processing to stream* 427 *nutrient fluxes at the segment and whole-reach scale*

428 The capacity of in-stream processes to modify stream input fluxes differed  
429 between nutrients and spatial scales. For individual segments,  $|F_{sw} \cdot x / F_{in}|$  was smaller for  
430  $\text{NO}_3^-$  (6%) than for  $\text{NH}_4^+$  and SRP (~20%) (Mann Whitney test with post-hoc Tukey

431 test,  $p < 0.01$ , Table 3). However,  $|F_{sw} \cdot x / F_{in}|$  increased substantially for  $\text{NO}_3^-$  and  $\text{NH}_4^+$   
432 when nutrient budgets were calculated at the whole-reach scale (Table 3).

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

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

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

## 448 **5. Discussion**

449 In terms of hydrology, the study headwater stream was a net gaining reach,  
450 though the hydrological interaction between the riparian zone and the stream was  
451 complex as indicated by the longitudinal variation in net riparian groundwater inputs.  
452 Moreover, the longitudinal decrease in area-specific discharge suggests that  
453 hydrological retention increased at the valley bottom compared to upstream segments as  
454 reported in previous studies (Covino et al., 2010). Despite the complex hydrological

455 processes along the reach, the strong positive correlation between stream and riparian  
456 groundwater  $\text{Cl}^-$  concentration suggests high hydrological connectivity at the riparian-  
457 stream interface (Butturini et al., 2003). In addition, we found that the permanent  
458 tributaries, which comprised  $\sim 50\%$  of the catchment area, contributed 56% of stream  
459 discharge; and thus, they were an essential piece to understand stream nutrient  
460 chemistry and loads. Hydrological mixing of stream water with water from tributaries  
461 could partially explain the longitudinal increase in  $\text{Cl}^-$  because its concentration was  
462 higher at the tributaries than at the main stream, especially during the vegetative period.  
463 In addition, riparian groundwater inputs to the stream could further contribute to the  
464 longitudinal increase in stream  $\text{Cl}^-$  concentration because they contributed 26% of  
465 stream discharge and also exhibited higher  $\text{Cl}^-$  concentration than stream water.

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



479 chemistry and downstream nutrient export (McClain et al., 2003; Harms and Grimm,  
480 2008).

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

504 attributed to variation in intrinsic stream characteristics, such as stream nutrient  
505 concentration, discharge, stream width, and the size of the hyporheic zone (Wollheim et  
506 al., 2006; Alexander et al., 2009), which may also hold for our study since these  
507 characteristics varied along the 3.7-km reach. However, our results also indicate that the  
508 assessment of riparian groundwater inputs is crucial to understand the contribution of  
509 in-stream processes to stream nutrient fluxes. Overall, our findings add to the growing  
510 evidence that streams are hot spots of nutrient processing (Peterson et al., 2001; Dent et  
511 al., 2007), and that in-stream processes can substantially modify stream nutrient fluxes  
512 at the catchment scale (Ensign and Doyle, 2006; Bernal et al., 2012).

513         The potential of in-stream processes to regulate stream nutrient fluxes was  
514 especially remarkable for  $\text{NH}_4^+$ . There was no relationship between stream and riparian  
515 groundwater  $\text{NH}_4^+$  concentrations; and further, whole-reach budgets indicated that in-  
516 stream net uptake could reduce the flux of  $\text{NH}_4^+$  up to 90% along the reach. This high  
517 in-stream bioreactive capacity could be favored by the sharp increase in redox  
518 conditions from riparian groundwater to stream water (Hill et al., 1998; Dent et al.,  
519 2007). Concordantly,  $\text{NH}_4^+$  concentrations were higher in riparian groundwater than in  
520 the stream, while the opposite occurred for  $\text{NO}_3^-$  (although only during the vegetative  
521 period). These results suggest fast nitrification of groundwater inputs within the stream  
522 as environmental conditions become well oxygenated (Jones et al., 1995). Supporting  
523 this idea, we found that in-stream gross  $\text{NH}_4^+$  uptake prevailed over release along the  
524 reach. However, the marked increase in stream  $\text{NO}_3^-$  concentration observed along the  
525 last 700 m of the reach during the vegetative period could not only be explained by  
526 nitrification of riparian groundwater  $\text{NH}_4^+$  because this flux ( $F_{gw,NH4} \sim 2 \mu\text{g N m}^{-1} \text{ s}^{-1}$ )  
527 was not large enough to sustain in-stream  $\text{NO}_3^-$  release  $|F_{sw,NO3} < 0|$  ( $\sim 10 \mu\text{g N m}^{-1} \text{ s}^{-1}$ ).  
528 This finding suggests an additional source of N at the valley bottom. Previous studies

529 have shown that leaf litter from riparian trees, and especially from N<sub>2</sub>-fixing species,  
530 can enhance in-stream nutrient cycling because of its high quality and edibility (Starry  
531 et al., 2005; Mineau et al., 2011). Thus, the increase in NO<sub>3</sub><sup>-</sup> and SRP concentrations  
532 and in-stream NO<sub>3</sub><sup>-</sup> release observed at the lowest part of the catchment during the  
533 vegetative period could result from the combination of warmer temperatures and the  
534 mineralization of large stocks of alder and black locust leaf litter stored in the stream  
535 bed (Strauss and Lamberti, 2000; Bernhardt et al., 2002; Starry et al., 2005).

536 Alternatively, increases in stream NO<sub>3</sub><sup>-</sup> and SRP concentration could result from human  
537 activities, which were concentrated at the lowest part of the catchment. However,  
538 regarding NO<sub>3</sub><sup>-</sup>, anthropogenic sources seem unlikely because DIN concentrations at the  
539 tributary draining through the inhabited area were low. In contrast, this tributary showed  
540 high SRP concentrations (from 2 to 6 folds higher than in the main stream), though its  
541 discharge should have had to be ca. 4 times higher than expected for its drainage area (<  
542 0.4 km<sup>2</sup>) to explain the observed changes in concentration. Another possible explanation  
543 for the increase in stream N concentration at the valley bottom could be increased N  
544 fixation by stream algae (Finlay et al., 2011). However, in-stream DIN release (NO<sub>3</sub><sup>-</sup>  
545 and NH<sub>4</sub><sup>+</sup>) peaked in late spring and summer (May and August 2011), when light  
546 penetration was limited by riparian canopy and in-stream photoautotrophic activity was  
547 low (Lupon et al., 2014). Altogether, these data suggest that the sharp increase in  
548 nutrient availability along the last 700 m of the reach was likely related to the massive  
549 presence of the invasive black locust at the valley bottom. Black locust is becoming  
550 widespread throughout riparian floodplains in the Iberian Peninsula (Castro-Díez et al.,  
551 2014) and its potential to subsidize N to stream ecosystems via root exudates and leaf  
552 litter could dramatically alter in-stream nutrient processing and downstream nutrient  
553 export (e.g., Stock et al., 1995; Mineau et al., 2011). However, further research is

554 needed to test the hypothesis that this invasive species can alter stream nutrient  
555 dynamics in riparian floodplains.

556         It is worth noting that longitudinal trends in stream nutrient concentration were  
557 not always pointing towards the same direction than estimates based on in-stream  
558 processes. This divergence evidenced that other sources of variation of stream water  
559 chemistry were counterbalancing the influence of in-stream processes on stream nutrient  
560 fluxes. In this sense, results from  $\text{NH}_4^+$  were paradigmatic. The mass balance approach  
561 clearly showed that in-stream gross uptake of  $\text{NH}_4^+$  exceeded release; and concordantly,  
562  $\text{NH}_4^+$  concentration was consistently lower in the stream than in riparian groundwater.  
563 Yet, stream  $\text{NH}_4^+$  concentration showed small longitudinal variation likely because in-  
564 stream net uptake balanced the elevated inputs from riparian groundwater. Therefore,  
565 our results challenge the idea that stream nutrient concentration should decrease in the  
566 downstream direction when in-stream processes are efficient in taking up nutrients from  
567 receiving waters (Brookshire et al., 2009). Conversely, our findings convincingly show  
568 that in-stream processes can strongly affect stream nutrient chemistry and downstream  
569 nutrient export despite this may not result in consistent longitudinal gradients in nutrient  
570 concentration. For  $\text{NO}_3^-$ , we found that the marked increase in concentration along the  
571 last 700 m could be attributed to an increase in in-stream nitrification. However, the  
572 observed decrease in  $\text{NO}_3^-$  concentration along the first 1.5 km of the reach could be  
573 barely explained by in-stream processing alone because its contribution to reduce  
574 stream  $\text{NO}_3^-$  fluxes was too low, even when the whole-reach budget was recalculated  
575 excluding the last 700 m of the reach ( $F_{sw} = 0.61 \mu\text{g N m}^{-1} \text{ s}^{-1}$  and  $F_{sw} > 0/F_{in} = 10\%$ ).  
576 For SRP, the longitudinal increase in concentration could neither be fully explained by  
577 in-stream release because  $F_{sw,SRP} < 0$  was not widespread along the reach and the stream  
578 only contributed to input fluxes by 19% (6% when excluding the last 700 m). In fact,

579 our whole-reach mass balance indicated that stream nutrient chemistry along the reach  
580 resulted from the combination of both in-stream nutrient processing and hydrological  
581 mixing with riparian groundwater and tributary inputs. Recent studies have concluded  
582 that riparian groundwater is a major driver of longitudinal patterns in stream nutrient  
583 concentration in headwater streams (Bernhardt et al., 2002; Asano et al., 2009; Scanlon  
584 et al., 2010). Our study adds to our knowledge of catchment biogeochemistry by  
585 showing that stream nutrient chemistry results from the combination of both  
586 hydrological mixing from the riparian zone and in-stream nutrient processing, which  
587 can play a pivotal role on shaping stream nutrient concentrations and fluxes at the  
588 catchment scale.

## 589 **6. Conclusions**

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

603 modeling approaches if we are to understand drivers of stream nutrient chemistry within  
604 catchments.

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

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

625 **Author contribution**

626 S.B., F.S., and E.M. designed the experiment. S.B, A.L., M.R., and F.S. carried  
627 them out. A.L. performed all laboratory analysis. S.B. analyzed the data set and  
628 prepared the manuscript with contributions from A.L., M.R., and E.M.

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828 **Tables**

829 **Table 1.** Median and interquartile range [25<sup>th</sup>, 75<sup>th</sup> percentiles] of stream and riparian  
 830 groundwater solute concentrations for the dormant and vegetative period. The number  
 831 of cases is shown in parenthesis for each group. For each variable, the asterisk indicates  
 832 statistically significant differences between the two water bodies (Wilcoxon paired rank  
 833 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 N L <sup>-1</sup> )	192 [159, 262] (60)	194 [109, 298] (56)
	N-NH <sub>4</sub> <sup>+</sup> (µg N L <sup>-1</sup> )	8.9 [6.5, 10.3] (60)	19 [13.8, 34.2] (56)*
	SRP (µg P 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 N L <sup>-1</sup> )	223 [155, 282] (102)	168 [77, 264] (98)*
	N-NH <sub>4</sub> <sup>+</sup> (µg N L <sup>-1</sup> )	10 [8.7, 12.8] (103)	27 [18.2, 37.1] (101)*
	SRP (µg P 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)*

834  
835



836 **Table 2.** Spearman  $\rho$  coefficient between stream water and riparian groundwater solute  
 837 concentrations for each period and for the whole data set collected at the Font del Regàs  
 838 during the study period. The relative root mean square error (RRMSE) indicates  
 839 divergences from the 1:1 line. The number of cases is shown in parenthesis for each  
 840 variable. ns, no 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

841 \*p<0.001

842

843

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

		By segment	By whole reach
$F_{sw}$ ( $\mu\text{g m}^{-1} \text{s}^{-1}$ )	$\text{Cl}^-$	6 [-37, 80]	12 [2, 33]
	N- $\text{NO}_3^-$	-0.43 [-4.4, 1.3]	-0.97 [-3.4, 1.6]
	N- $\text{NH}_4^+$	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} \cdot x / F_{in} $ (%)	$\text{Cl}^-$	3 [1, 10]	4 [2, 9]
	N- $\text{NO}_3^-$	6 [2, 14]	24 [8, 67]
	N- $\text{NH}_4^+$	18 [9.5, 35]	48 [25, 71]
	SRP	20.5 [3.4, 41]	15.5 [6, 66]

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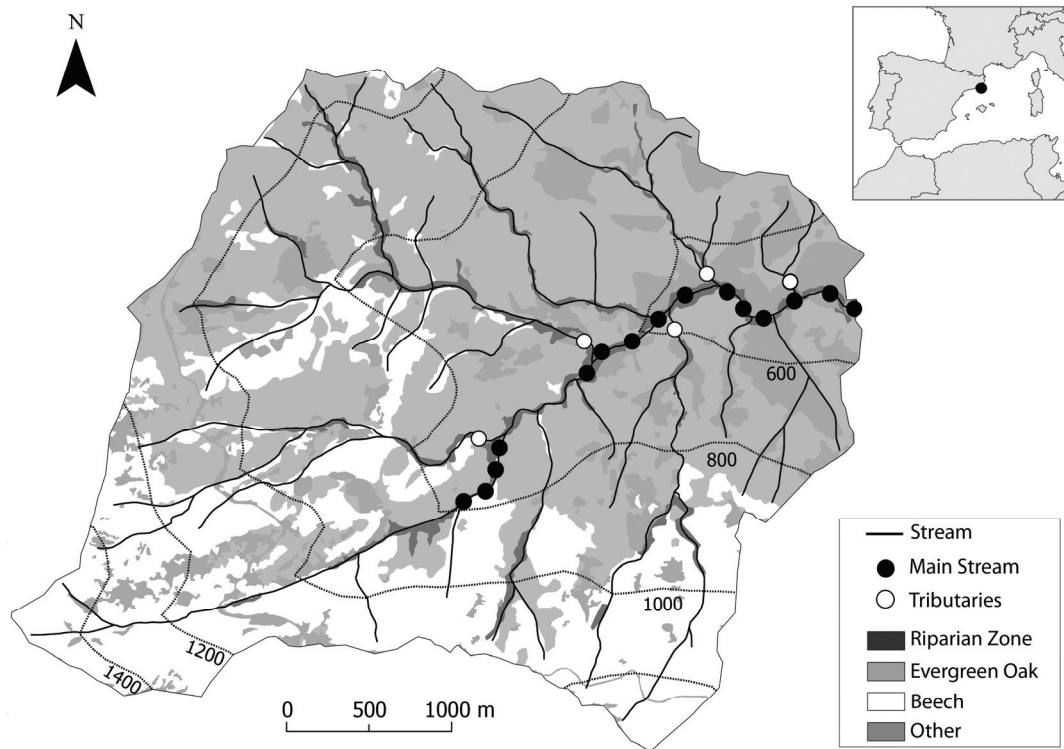
850 **Table 4.** Median and interquartile range [25<sup>th</sup>, 75<sup>th</sup> percentile] of the relative  
 851 contribution of inputs from upstream ( $F_{top}/F_{in}$ ), net riparian groundwater ( $F_{gw}>0/F_{in}$ ),  
 852 tributaries ( $F_{eff}/F_{in}$ ), and in-stream release ( $F_{sw}<0/F_{in}$ ) to stream solute fluxes. For each  
 853 solute, different letters indicate statistically significant differences between solute  
 854 sources (Mann Whitney test with post-hoc Tukey test,  $p > 0.01$ ).  $n = 10$  for the 4  
 855 solutes.

<i>Relative contribution (%)</i>	Cl <sup>-</sup>	N-NO <sub>3</sub> <sup>-</sup>	N-NH <sub>4</sub> <sup>+</sup>	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>

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858

859 **Figures**

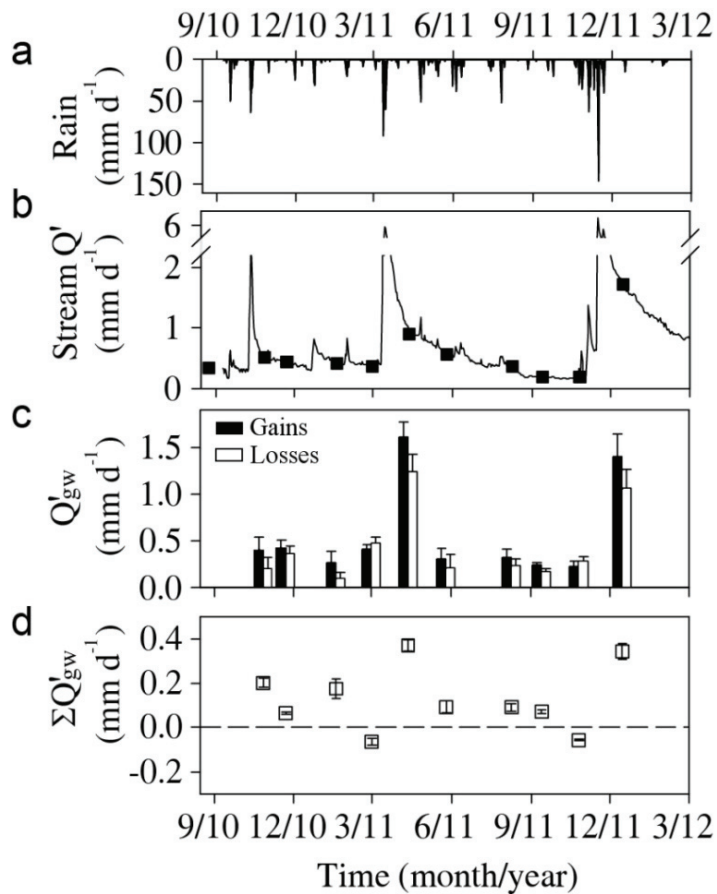


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861

862 **Figure 1.** Map of the Font del Regàs catchment within the Montseny Natural Park (NE,  
863 Spain). The vegetation cover and the main stream sampling stations along the 3.7-km  
864 reach are indicated. There were 5 and 10 sampling stations along the 2<sup>nd</sup> and 3<sup>rd</sup> order  
865 sections, respectively. Four permanent tributaries discharged to the main stream from  
866 the upstream- to the downstream-most site (white circles). Additional water samples  
867 were collected from a small tributary draining through the inhabited area at the lowest  
868 part of the reach. The remaining tributaries were dry during the study period.

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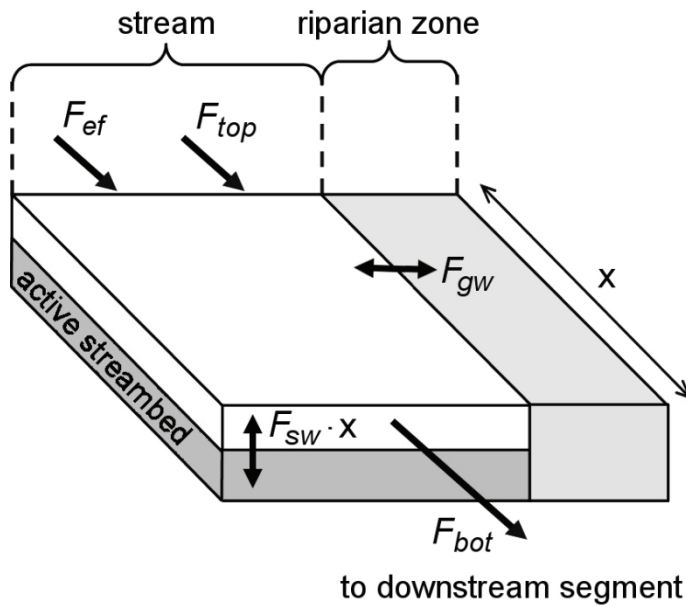


870

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

876

877



878

879 **Figure 3.** Conceptual representation of nutrient fluxes considered to estimate in-stream

880 net nutrient uptake for each stream segment ( $F_{sw} \cdot x$ , Equation 2). For each segment of

881 length  $x$ , the considered nutrient input fluxes were upstream ( $F_{top}$ ) and tributaries ( $F_{ef}$ ).

882 Nutrient fluxes exiting the stream segment ( $F_{bot}$ ) were  $F_{top}$  for the contiguous

883 downstream segment. Riparian groundwater nutrient fluxes could either enter ( $F_{gw} > 0$ )

884 or exit ( $F_{gw} < 0$ ) the stream. Nutrient fluxes for each component were estimated by

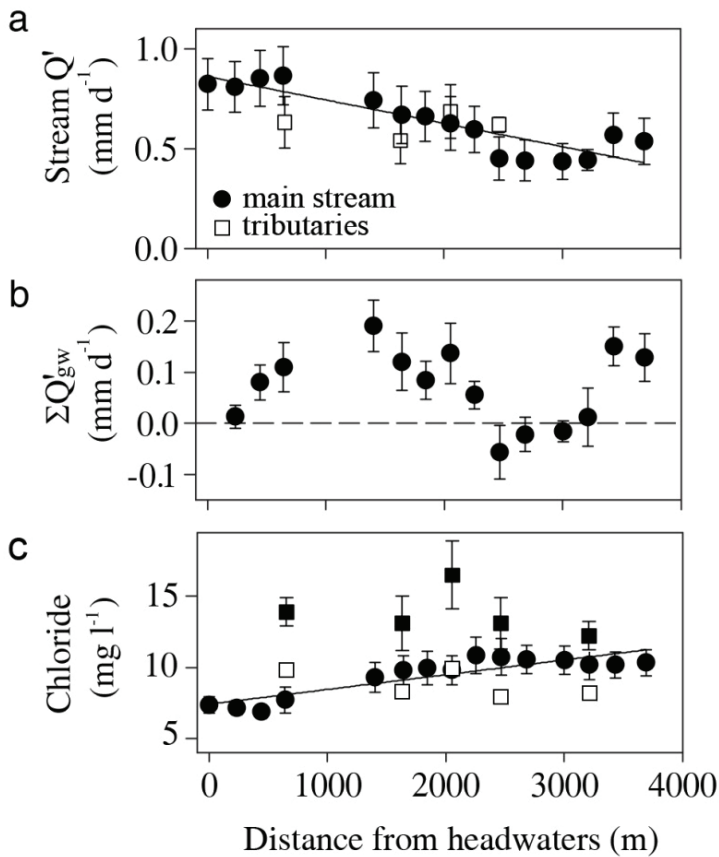
885 multiplying its water flux ( $Q$ ) by its nutrient concentration ( $C$ ). In-stream net nutrient

886 uptake ( $F_{sw} \cdot x$ ) is the result of gross nutrient uptake and release by the active streambed.

887  $F_{sw} \cdot x$  can be positive (gross uptake  $>$  release), negative (gross uptake  $<$  release), or nil

888 (gross uptake  $\sim$  release). See text for details.

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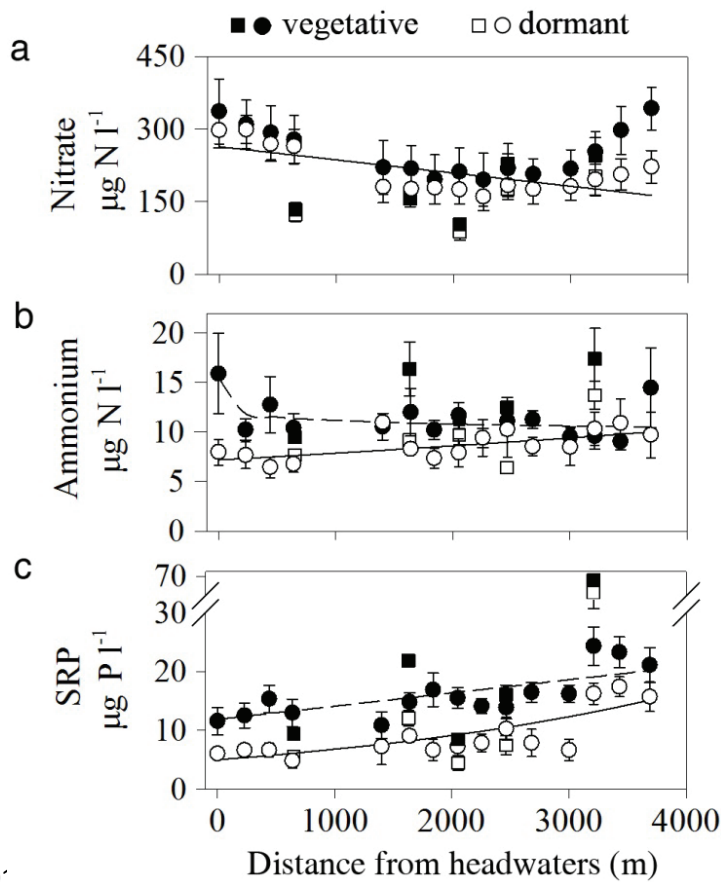


891

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

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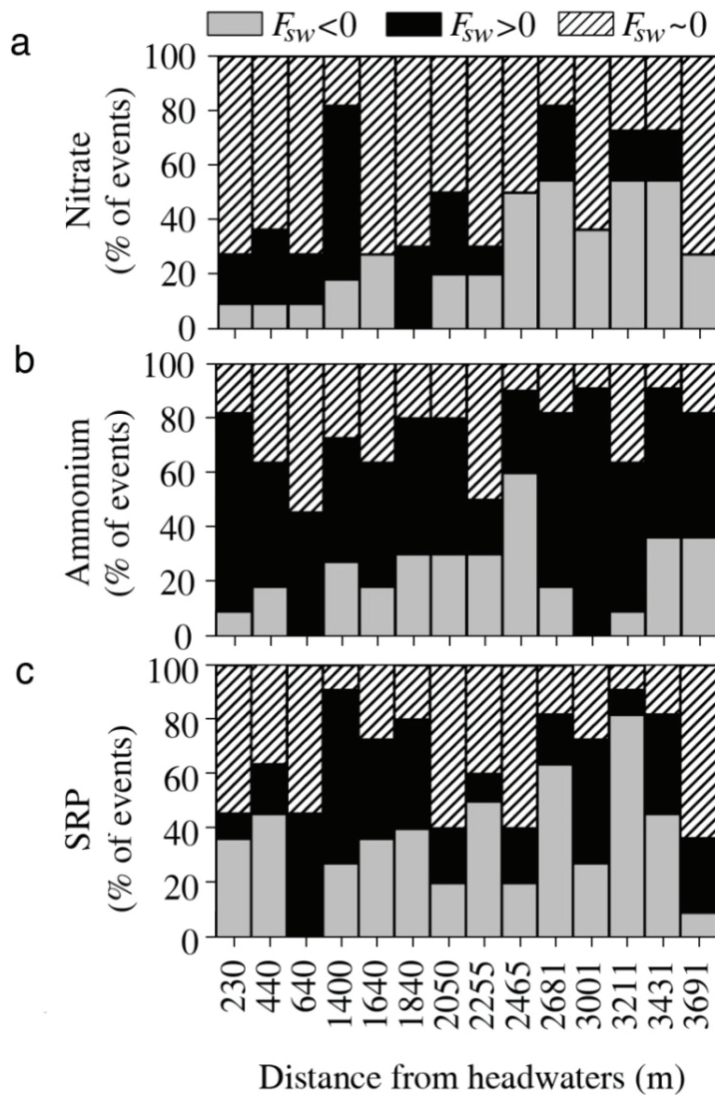
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903 **Figure 5.** Longitudinal pattern of stream nutrient concentrations for (a) nitrate, (b)  
904 ammonium, and (c) solute reactive phosphorus at Font del Regàs. Symbols are average  
905 and standard error (whiskers) for the main stream (circles)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

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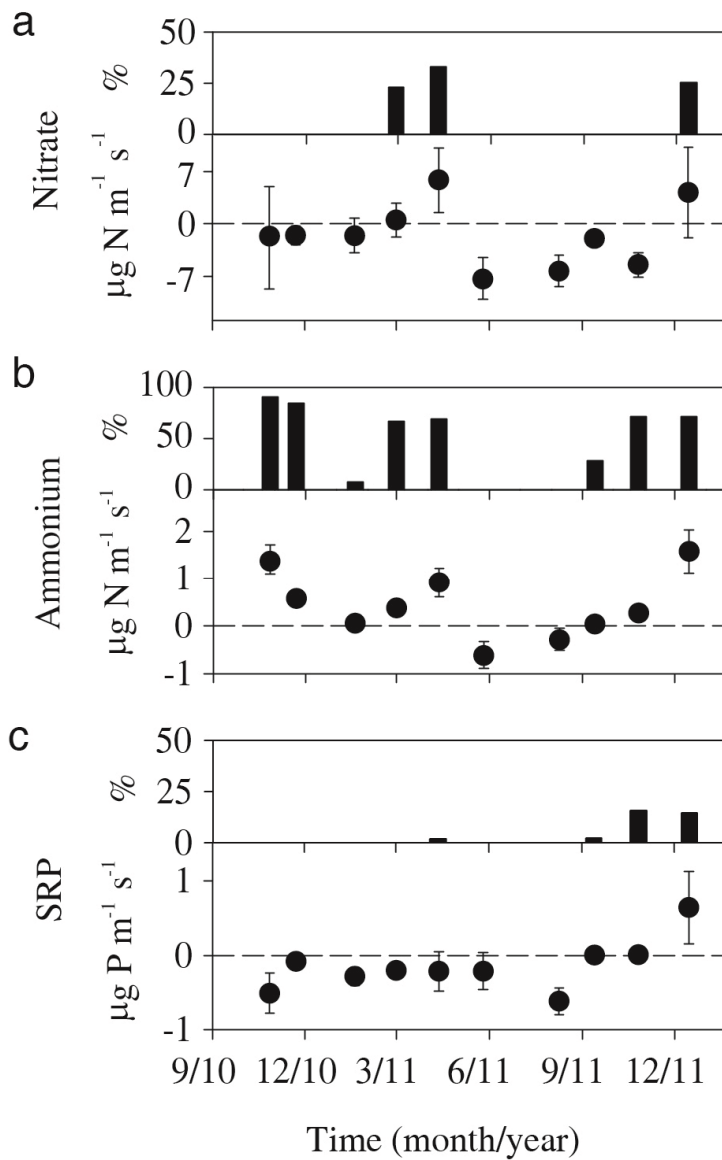




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911 **Figure 6.** Frequency of dates for which  $F_{sw} < 0$  (gross uptake < release),  $F_{sw} > 0$  (gross  
 912 uptake > release), and  $F_{sw} \sim 0$  (gross uptake ~ release) for (a) nitrate, (b) ammonium,  
 913 and (c) soluble reactive phosphorus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

916



918

919 **Figure 7.** Temporal pattern of in-stream net nutrient uptake ( $F_{sw}$ , in  $\mu\text{g m}^{-1} \text{s}^{-1}$ ) for (a)

920 nitrate, (b) ammonium, and (c) soluble reactive phosphorus at the whole-reach scale.

921 Whiskers are the uncertainty associated with the estimation of stream discharge from

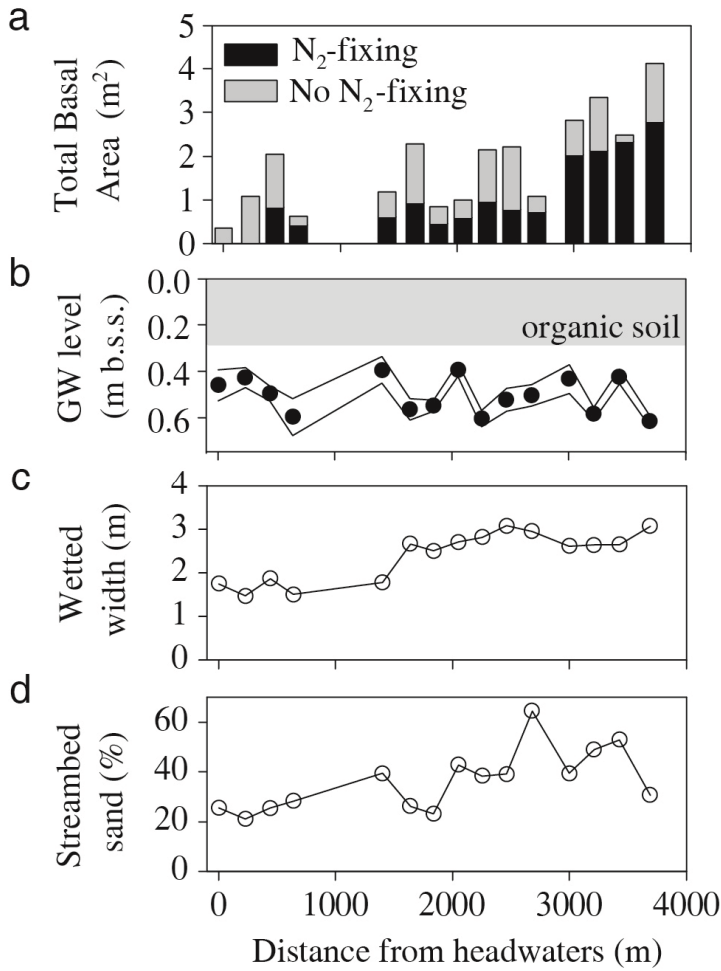
922 slug tracer additions.  $F_{sw} > 0$  indicates that gross uptake prevailed over release, while923  $F_{sw} < 0$  indicates the opposite. For those cases for which  $F_{sw} > 0$ , the contribution of in-924 stream net nutrient uptake to reduce stream nutrient fluxes ( $F_{sw} \cdot x/F_{in}$ , in %) is shown

925 (black bars).

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927

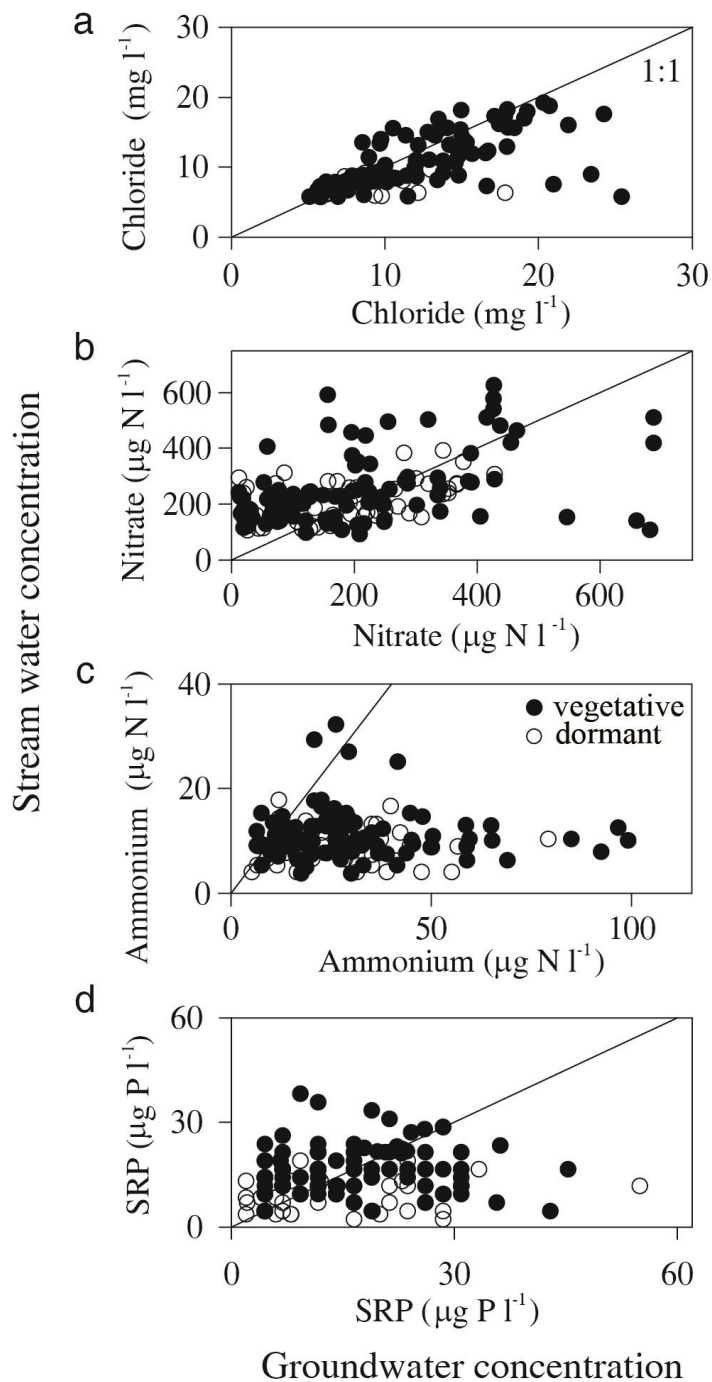
928 **Supplementary Figures**



929

930 **Figure S1.** (a) Total basal area of riparian trees, (b) mean riparian groundwater level (in  
931 m below the soil surface), (c) stream wetted width, and (d) percentage of sands in  
932 streambed for each sampling site along the study reach. Different colors in (a) indicate  
933 the basal area of N<sub>2</sub>- and no N<sub>2</sub>-fixing trees. The solid lines in (b) are the 95% lower and  
934 upper values of the riparian groundwater level.

935



936

937 **Figure S2.** Relationship between riparian groundwater and stream water concentrations  
 938 for (a) chloride, (b) nitrate, (c) ammonium, and (d) soluble reactive phosphorus at each  
 939 sampling site and for each sampling date at Font del Regàs. The 1:1 line is indicated in  
 940 black.