Authors reply to Anonymous Referee #1

Manuscript title: Hydrology and riparian forests drive carbon and nitrogen supply and DOC:NO_3^- stoichiometry along a headwater Mediterranean stream; by Ledesma, Lupon, Martí, and Bernal

General comments

This study analyses DOC, NO3- and the DOC:NO3- ratio along three stations in a Mediterranean headwater, and across flow conditions, during a 2-year period. Spatial variability is controlled by the presence of riparian forest and topography, while temporal variability is controlled by hydroclimatic conditions. The authors conclude that this spatiotemporal variability influences stream metabolic processes.

The manuscript is well written and the conclusions are clear. This work is within the scope of HESS but I find this is a modest contribution to the literature.

Here are three options to make a stronger paper:

- *Relax the selection criteria for the storm events to include in the PLS regression (currently only 5 observations)*
- *Include data on DOC and N composition, not only in the discussion. The discussion suggests that such data is available. Is nitrate the only N form in this stream?*
- *Include more recent data to make a more complete synthesis of research in this catchment. The references indicate that other studies have taken place in this catchment since the monitoring period 2010-2012 considered here.*

[Reply]: We are happy that you found the manuscript "well written" and the conclusions "clear" and thank you for the three suggestions to make the paper stronger. They are sensible and, in fact, we had already considered these options during the preparation of the manuscript. Below we disclose relevant information and data related to each of these points and argue about the actions that we will take in each case.

<u>Including more storm events in the PLS regression</u>

Following your suggestion, we have relaxed the selection criteria for including storm events in our analyses. For this exercise, new events were included if they fulfilled two requirements: (i) a precipitation amount during the days included in the event of at least 25 mm (events below these threshold showed no groundwater table response at the temporal resolution of the study and marginal stream flow responses), and (ii) stream flow was classified as storm flow during the days of the event (not all events with precipitation amounts higher than 25 mm generated storm flow at the temporal resolution of the study).

Using these more relaxed criteria, a total of 15 new events were identified. The same nine hydroclimatic descriptors used in Table 2 of the manuscript for the former events, together with the average DOC and

NO_3^- concentrations during each event and the relative increase in DOC and NO_3^- concentrations with respect to base flow conditions (within brackets), are shown in Table R1 below for these new events.

Table R1. Hydroclimatic descriptors of the new 11 medium storm events identified during the study period

Event	Period	Duration (days)	P (mm)	P-7 (mm)	P-30 (mm)	Q_{avg} (mm)	Gw_{avg} (m)	ΔGw (m)	Slope (m mm^{-1})	Q_{avg}/Gw_{avg} (mm m^{-1})	DOC (mg C L^{-1})	NO_3^- (mg N L^{-1})
*1	16-19/09/2010	4	52	9	36	0.4	0.97	0.04	0.10	0.4	1.01 (15%)	0.23 (36%)
*2	09-17/10/2010	9	147	6	88	1.3	0.87	0.27	0.10	1.5	na	na
*3	21-29/12/2010	9	67	1	44	0.6	0.93	0.09	0.19	0.7	na	na
*4	27/01-02/02/2011	7	57	0	20	0.5	0.96	0.03	0.05	0.6	na	na
*5	24-27/04/2011	4	53	15	44	1.0	0.88	0.02	-0.02	1.1	na	na
*6	14-17/05/2011	4	30	22	122	0.7	0.90	0.02	0.15	0.8	0.99 (13%)	0.17 (5%)
*7	30/05-02/06/2011	4	36	0	69	0.6	0.92	0.02	0.15	0.6	1.19 (36%)	0.18 (7%)
*8	03-06/06/2011	4	50	36	96	0.6	0.92	0.03	0.14	0.7	0.83 (-5%)	0.17 (-1%)
*9	09-15/06/2011	7	34	66	148	0.6	0.91	0.03	0.15	0.7	1.1 (26%)	0.18 (7%)
*10	25/07-03/08/2011	10	74	4	48	0.5	0.94	0.04	0.16	0.5	0.82 (-7%)	0.18 (5%)
*11	23-30/10/2011	8	98	17	39	0.3	0.95	0.09	0.17	0.3	1.53 (75%)	0.23 (41%)
*12	21-23/03/2012	3	57	6	8	0.8	0.88	0.06	0.22	0.9	0.95 (8%)	0.36 (118%)
*13	03-05/04/2012	3	29	0	63	0.7	0.95	0.06	0.13	0.7	1.23 (40%)	0.16 (-2%)
*14	18-24/05/2012	7	49	10	62	0.5	0.97	0.04	0.02	0.5	0.82 (-7%)	0.19 (12%)
*15	04-09/08/2012	6	33	0	4	0.2	1.05	0.03	0.23	0.2	1.08 (23%)	0.18 (5%)

na: not available or incomplete

Unfortunately, four of the events (*2, *3, *4, and *5) had no available chemical data associated with them or these data were incomplete. The ultimate goal of our PLS regression analysis was to relate hydroclimatic characteristics of storm events with resulting DOC and NO_3^- concentrations in the stream and thus these four events cannot be integrated into this analysis because they lack relevant information. This is the reason why we introduced the requirement "a complete stream chemistry data series associated with the event for the downstream site (i.e. no gaps in chemical data for the event dates)" (L. 185-186) for including events in the analysis in the original manuscript. These four events will not be further discussed.

From the remaining new 11 events, hereafter referred as "medium storm events", (i) 82% (9 out of 11) accumulated lower precipitation amounts than the lowest of the precipitation amounts of the former large storm events (Figure R1a below), (ii) 91% (10 out of 11) sowed lower average stream flow than the lowest of the average stream flows of the former large storm events (Figure R1b), (iii) 100% displayed deeper groundwater tables than the deepest of all groundwater tables of the former large storm events (Figure R1c), and (iv) 100% exhibited smaller groundwater table ranges (i.e. the thickness of the riparian layer that becomes hydrologically activated during the event) than the smallest of the groundwater table ranges of the former large storm events (Figure R1d).

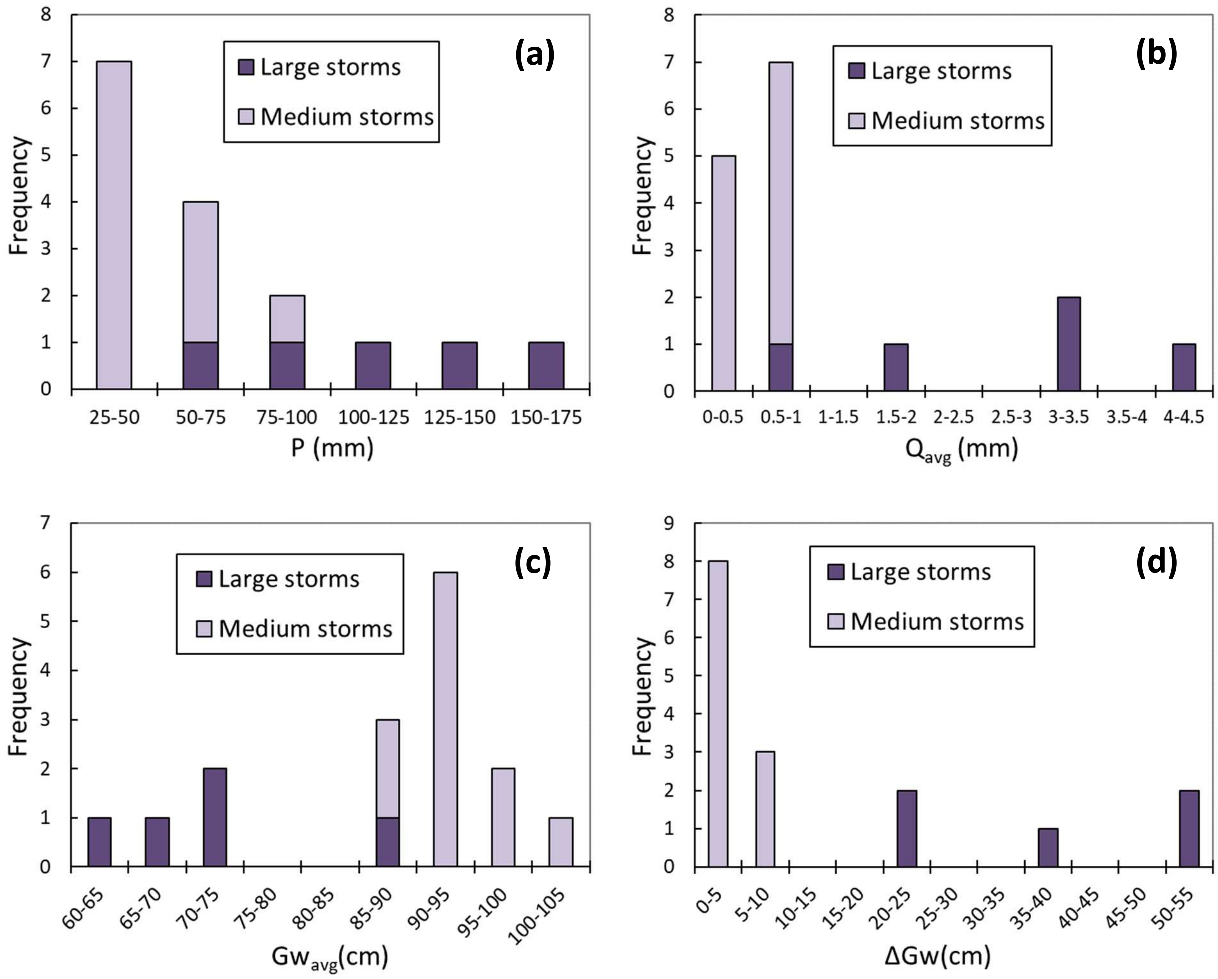


Figure R1. Histograms of the hydroclimatic descriptors (a) precipitation amount (P), (b) average stream flow (Q_{avg}), (c) average groundwater table (Gw_{avg}), and (d) groundwater table range (ΔGw) for the 5 large storm events included in the former version of the manuscript ("large storms") and the 11 new medium-size events ("medium storms").

Importantly, these differences in climatic and, especially, hydrological chatacteristics between the large and medium storm events led to marked differences in the resulting stream chemistry: both DOC and NO_3^- concentrations were substantially lower during the medium storm events than during the large storm events (Figure R2 below). This observed pattern is likely caused by the hydrological activation of a thicker and shallower riparian layer during large storm events that leads to the mobilization of relatively larger amounts of DOC and NO_3^- stored in the riparian soil compared to the amounts mobilized from the deeper and narrower layers that are hydrologically activated during medium storm events. These results suggest that large and medium storm events display a distinct mechanism of DOC and NO_3^- mobilization from the riparian zone that precludes a direct integration of the samples from the two type of events into the PLS regression analysis and into the conceptual model we propose in Figure 7 of the manuscript.

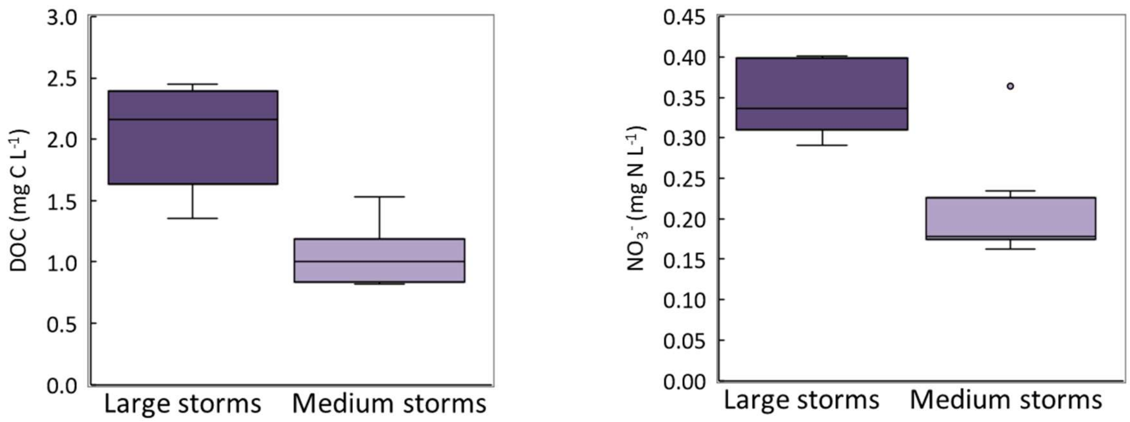


Figure R2. Box plots of dissolved organic carbon (DOC) concentrations and nitrate (NO_3^-) concentrations in stream waer for the 5 large storm events included in the former version of the manuscript ("large storms") and the 11 new medium-size events ("medium storms").

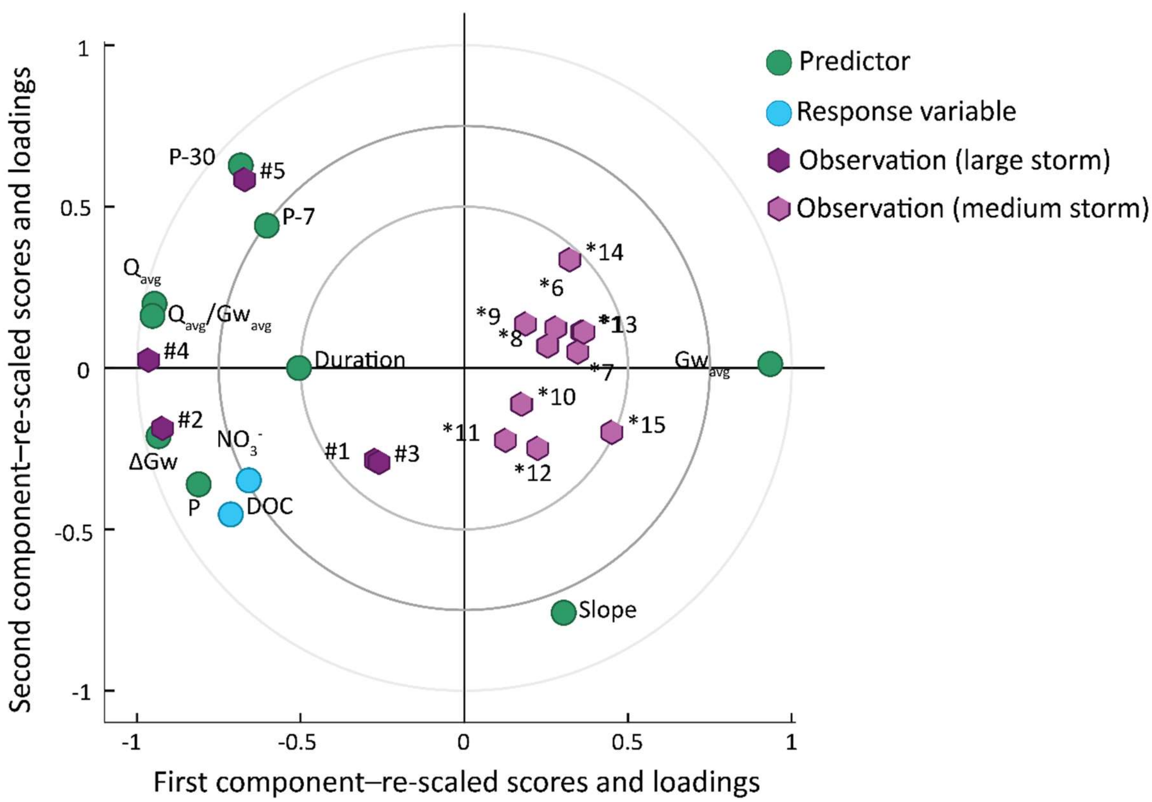


Figure R3. Biplot of a two-component partial least square (PLS) regression model with score and loading vectors re-scaled into the -1 to +1 numerical range in order to display the relative relationships between observations (i.e. 5 large storm events versus 11 medium storm events), predictors, and response variables.

To illustrate our point further, we have also reanalyzed the PLS regression model including both the former large (N = 5, denoted with #) and the new medium (N = 11, denoted with *) storm events (Figure R3 above). The relative ordination of the predictors is similar to the original PLS regression model, with *P-30*, Q_{avg}, and Q_{avg}/Gw_{avg} located relatively contigous in one side of the ordination and *slope* located in the opposite side. However, compared to the original analysis, the model goodness of fit is reduced (R^2Y decreased from 0.96 to 0.72 for DOC and from 0.94 to 0.55 for NO_3^-) and the model predictive ability is compromised (Q^2Y decreased from 0.88 to 0.49 for DOC and from 0.82 to 0.25 for NO_3^-). Remarkably, the two type of storm events fall in two opposite regions of the biplot: medium storm events cluster in the right side of the ordination, while large storm events make a broader cluster in the left side of the ordination. This result further demonstrate the different nature of the two type of events and the different implications for DOC and NO_3^- mobilization.

All in all, in the revised manuscript we will keep the five large storm events in our PLS regression model, which is the basis for our suggested conceptual model that only applies to such large events. Nevertheless, we will also underscore the hydrological differences between large and medium storm events and discuss the biogeochemical implications of such differences in terms of DOC and NO_3^- mobilization and concentrations in the stream. For that, we will integrate in the main body and the supplementary material of the revised manuscript a condensed version of the information we have presented in this document in this regard.

<u>DOM composition and other forms of nitrogen</u>

Unfortunately, we do not have data on DOM composition at daily resolution or during storm flow conditions, which prevents us for directly integrating DOM composition data into the analyses of the present study. Instead, during the same study period (2010-2012), we performed longitudinal surveys of DOM composition on 11 occasions only during base flow conditions. The data from these surveys showed that DOM in Font del Regàs has a prominent protein-like character in both riparian groundwater and stream water and we published these results in a previous study (Bernal et al. 2018). Here we use this information by referring to the published study to support our suggestion that in-stream heterotrophic activity could be partially sustained during storm flow conditions. We will reword the sentence in the discussion where we included this information to make clear that the data we have on DOM composition is from base flow conditions and that we assume the character is maintained across flow conditions as "Further, another study from Font del Regàs showed that DOM has a prominent protein-like character in both riparian groundwater and stream water during base flow conditions (Bernal et al., 2018), which could lead to rapid assimilation even during periods of short water residence times associated with storm flow conditions, assuming DOM molecular composition is maintained across flow conditions".

Regarding the different forms of nitrogen, NO_3^- is not the only form found at Font del Regàs but it makes up the overwhelming majority of both inorganic and total nitrogen. For the study period (2010-2012), we do have data on daily concentrations of other forms of dissolved inorganic nitrogen (DIN), including NH_4^+ and NO_2^- (partially published in Lupon et al., 2016b). Overall average stream concentrations of NH_4^+

(0.01 ± 0.006 mg N L^{-1}) and NO_2^- (0.006 ± 0.005 mg N L^{-1}) are significantly lower than overall average stream NO_3^- concentrations (0.20 ± 0.09 mg N L^{-1}). Moreover, NH_4^+ and NO_2^- concentrations are in all cases lower than 0.02 mg N L^{-1} and show no differences between baseflow and storm flow conditions. Hence, NO_3^- accounted for more than 90% of DIN during both base flow and storm flow during the study period. It its turn, dissolved organic nitrogen (DON) was always below 0.05 mg mg N L^{-1}, which implies that NO_3^- makes up more than 80% of the total dissolved nitrogen under all circumstances. Given that (i) NO_3^- is the major source of nitrogen for stream heterotrophic microorganisms in our system, and (ii) no significant differences were observed in other DIN forms between base and storm flow conditions, we decided to only analyze spatiotemporal patterns in NO_3^- concentrations. We will add this information in the revised manuscript.

Including more recent data

Unfortunately, we do not have chemistry data at the temporal (i.e. daily) or spatial (i.e. three sites along the stream) resolutions that we used in the present study beyond the period that we have analysed here. Some of the studies from the catchment that we cite and that you refer to did in fact use parts of the data that we present here in order to answer different questions, and thus do not include data beyond 2012. Others use data from specific experiments or campaigns that cannot be directly incorporated into our analyses, e.g. the longitudinal surveys of DOM composition presented in Bernal et al. (2018) that we discuss above. In autumn 2018, we started a mostly hydrological monitoring effort at the downstream site and DOC and NO_3^- concentrations (and consequently DOC:NO_3^- molar ratios), which are the main focus of the present study, have only been measured sporadically in the stream water in the downstream site or during focused short experiments along the streams. Therefore, there is no other data outside the study period that can be integrated into our analyses and we only use other studies on Font del Regàs to support our discussion.

Specific comments

Figure 1: add location of the weather station

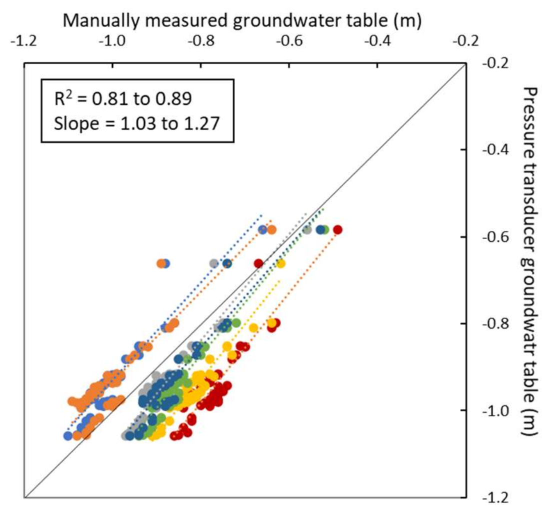
[Reply]: Thanks for pointing this out. We will add the location of the automatic weather station in a revised Figure 1.

L115 "Rating curves obtained from the relationships between stream flow and stream water level measurements were used to construct daily time series of stream flow data at each site" can you provide the rating curves in SI?

[Reply]: The rating curves were presented in the supplementary material of Lupon et al. (2016b), published also in *HESS*, and we can provide them as a new figure in the Appendix of the present study if the Editor thinks this is adequate.

L122 "the dynamics of this dataset capture well the dynamics of the groundwater table variation in the surrounding riparian area and therefore we are confident that the recorded pattern at the monitoring *location was representative of the groundwater table variations in the riparian zone" please provide stronger evidence that this piezometer is representative of the whole downstream area.*

[Reply]: The evidence for this statement was presented in Ledesma et al. (2021). In addition, to the groundwater tables presented in this study (recorded at 15 min intervals using a water pressure transducer installed in a piezometer placed 2.5 m away from the stream channel), we also measured groundwater tables manually every two weeks during the same period at seven equidistant (ca. 3 m) piezometers placed ca. 2 m from the stream channel along the same area where the pressure transducer was located. We compared a total of 45 supplementary groundwater table measurements available at each of the seven piezometers with the data from the pressure transducer, and showed that groundwater table dynamics were notably similar in all cases, which supports the use of the pressure transducer data as representative of the downstream riparian areas in our study. The figure below shows the comparison of pressure transducer versus manually measured groundwater tables (measurements from each piezometer are presented in a different colour and dotted lines are linear regressions between the two variables for each corresponding colour code case ($p<0.0001$ in all cases)):



In the revised manuscript, we will rephrase the sentence to make the evidence more explicit as "In a previous study, we showed that the dynamics of the pressure transducer dataset captured well the dynamics of manually-measured groundwater table variations in seven other piezometers located in the surrounding riparian area (Ledesma et al., 2021). Therefore, we are confident that the recorded pattern at the pressure transducer location was representative of the groundwater table variations in the riparian zone soils in the lower parts of the catchment".

L174 "hydroclimatic analysis of large storm events" I understand that the authors chose to analyze the largest storm events because they probably exhibit the clearest signal, but the selection criteria here are very strict and only 5 storm events were kept for analysis. This is a very small number, even though PLS regression can handle datasets with few observations and many variables. Wouldn't it be more interesting to relax the selection criteria and include more storm events?

[Reply]: Please, see our detailed response to this issue above.

L322 "given that this is a predominantly heterotrophic system (Lupon et al., 2016c)." please explain how this was determined (most readers won't read the reference)

[Reply]: The stream is predominantly heterotrophic because daily rates of ecosystem respiration (5.0 – 10.0 g O_2 m^{-2} day^{-1}) are between 10- and 100-fold higher than daily rates of gross primary production (0.1 – 0.7 g O_2 m^{-2} day^{-1}), as we measured and reported in Lupon et al. (2016c). Following your suggestion, we will include this information in the revised manuscript as "[…] given that this is a predominantly heterotrophic system, i.e. rates of ecosystem respiration are between 10- and 100-fold higher than rates of gross primary production (Lupon et al., 2016c)".

L340 "This result is in line with the idea that headwater streams can remove substantial amounts of NO3 - within relatively short distances (Peterson et al., 2001) […] providing groundwater inputs with low NO3 - concentrations driven by denitrification, as observed in temperate forest catchments (Cirmo and McDonnell, 1997)." Both instream removal and dilution from the middle part of the catchment can explain this decrease. Is it possible to estimate the share of each process?

[Reply]: In general, the data presented in this study cannot be analyzed and evaluated in a way that would allow estimating the relative contribution of *in-stream removal* vs. *dilution from riparian denitrification* to the NO_3^- decrease between the upper and midstream subcatchments. Nevertheless, in this case we are confident than *in-stream removal* overwhelmingly dominates over *dilution from riparian denitrification* because the riparian soils at Font del Regàs do not support high denitrification rates (< 3 µg N kg^{-1} day^{-1}; Poblador et al., 2017), but rather sustain large net nitrification rates (1 – 2 mg N kg^{-1} day^{-1}; Lupon et al., 2016a). Yet, even if *in-stream removal* likely accounts for most of the NO_3^- decrease, we consider that this process alone cannot explain the entire NO_3^- reduction and we propose that "the steep topography of the upstream subcatchment led to rapid drainage of aerated [hillslope] soils and relatively larger NO_3^- mobilization compared to the flatter near-stream zones of the midstream subcatchment" (L. 343-345). Therefore, the second mechanism playing a role on the NO_3^- decrease would be a comparatively lower NO_3^- input in the middle part of the catchment relative to the upper part of the catchment driven by topography, rather than a dilution driven by riparian denitrification.

L375 "The magnitude of change between flow conditions was different for DOC and NO3 - at the upstream site…" please specify which of DOC or NO3- increases more.

[Reply]: We will specify this information in the revised manuscript as "The magnitude of change between flow conditions was different for DOC (which on average increased by 66%) and NO_3^- (which on average increased by 41%) at the upstream site, leading to an increase in the frequency of optimal DOC:NO_3^- stoichiometric conditions for heterotrophic activity during storm flow".

L395 "another study from Font del Regàs showed that DOM has a prominent protein-like character in both riparian groundwater and stream water (Bernal et al., 2018)" suggest to include this data in the analysis (not only just in the discussion) to make a more complete paper. The speciation of DOC and the N species other than nitrate should be analyzed further.

[Reply]: Please, see our detailed responses to these issues above.

Technical corrections

L110 "All data and analyses were integrated and carried out for daily resolutions, which were determined by the availability of the stream chemical data." This sentence is unclear

[Reply]: We agree, and in the revised manuscript we will change this sentence to "All data and analyses were integrated and carried out for daily resolutions, which was the resolution of the stream chemical data".

References used in this authors reply

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