Authors reply to Anonymous Referee #2

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 manuscript explores hydrological and riparian controls on DOC and NO3- stream chemistry in an oligotrophic Mediterranean stream. Their rationale was to understand how these dynamics may impact in-stream heterotrophic activity. To do this, the authors use data collected at three sites within the catchment collected over a period 2 years (2010-2012). Their results suggest that spatial (upstream to downstream) patterns are explained by riparian geomorphology and forest coverage and that temporal variability results from hydrological variability. The manuscript is well written, figures are clear, and conclusions are straightforward and supported by data. I particularly enjoyed the use of the hydrological metrics to understand controls on lateral exchanges of DOC and NO3- between the riparian zone. That said, the contributions to the literature are modest. I wonder if the authors could strengthen the manuscript by adding data beyond 2010-2012 and include more (smaller) storm events in their PLSR analysis. Large storms are relatively rare and DOC and NO3- transport during smaller, and likely more frequent, storms may influence stream metabolism more than the larger events. Also, I suspect antecedent conditions are even more important for DOC and NO3- transport dynamics during these smaller events. I also wonder if the authors have NH4+ and/or DON concentrations and might consider looking at the DOC:DIN or DOC:TDN ratios. Perhaps NO3- is the dominant form of N and these other N forms won't impact the story much?

[Reply]: We are happy that you found the manuscript "well written" and the conclusions "straightforward and supported by data" and we acknowledge the appreciation of the use of hydrological metrics. Please, see below our detail responses to the three main points we identified in this general comment.

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

Following your suggestion, we have explored the role of not only the large storm events, but also of smaller events on DOC and NO_3^- mobilization in the context of the present study. For that, we 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).

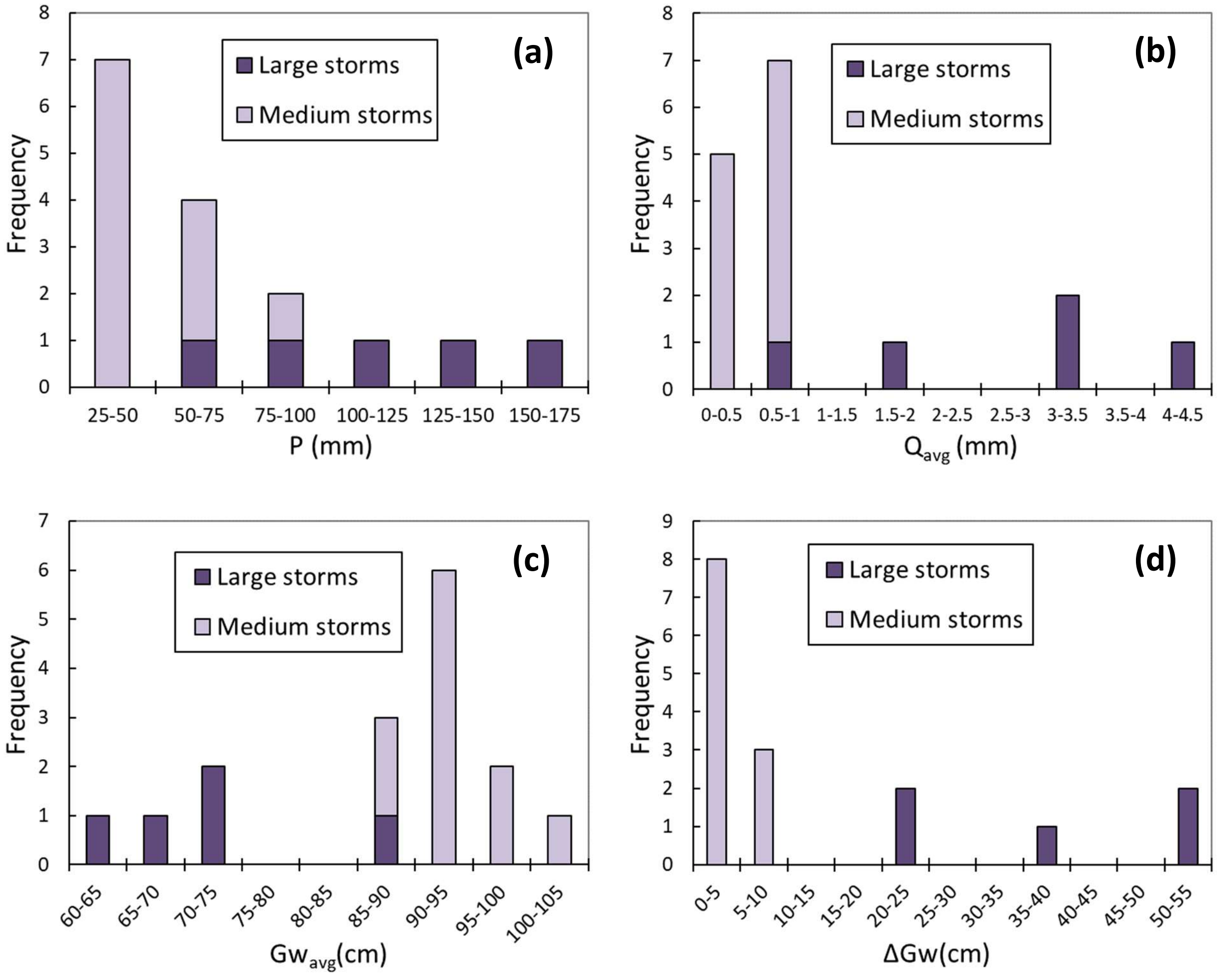

Figure R1. Histograms of the hydroclimatic descriptors (a) precipitation amount (P), (b) average stream flow (Q_{avg}), (c) average groundwater table (Gw_{avg}), and (d) groundwater table range (ΔGw) for the 5 large storm events included in the former version of the manuscript ("large storms") and the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

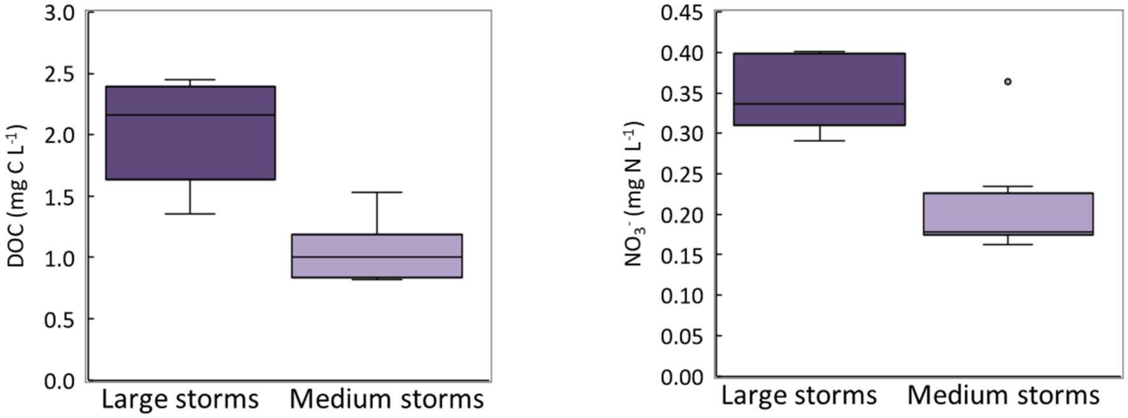

Figure R2. Box plots of dissolved organic carbon (DOC) concentrations and nitrate (NO_3^-) concentrations in stream waer for the 5 large storm events included in the former version of the manuscript ("large storms") and the 11 new medium-size events ("medium storms").

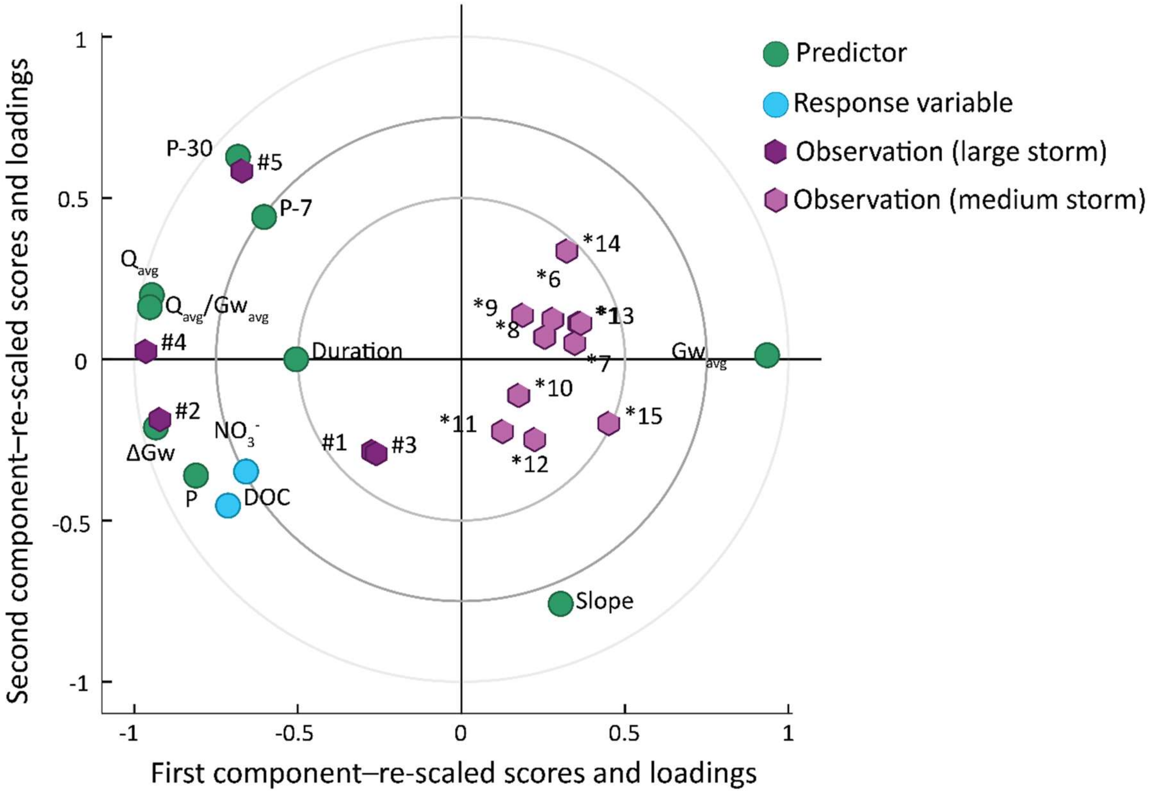

Figure R3. Biplot of a two-component partial least square (PLS) regression model with score and loading vectors re-scaled into the -1 to +1 numerical range in order to display the relative relationships between observations (i.e. 5 large storm events versus 11 medium storm events), predictors, and response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

Adding data beyond 2010-2012

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 (i.e. 2010-2012). Therefore, there is no other data outside this period that can be sensibly integrated into our analyses and we only use other studies on the catchment to support our discussion.

Ammonium and DON concentrations and other carbon to nitrogen ratios

You have made the right point, NO_3^- is the overwhelmingly dominant form of both inorganic and total nitrogen in our system and thus the other forms lack relevance in the context of the story. For the study period (2010-2012), we 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. We are therefore confident that NO_3^- is the major source of nitrogen for stream heterotrophic microorganisms in our system and decided to only analyze spatiotemporal patterns in NO_3^- concentrations. To support this choice, we will now make clear that NO_3^- is the dominant form of nitrogen in Font del Regàs in the revised manuscript.

Minor concerns

Lines 25-26: The authors state "These results suggest that (i) increased supply of limited resources during storms can promote in-stream heterotrophic activity during high flows . . ." They don't actually measure this process, instead they infer it from DOC:NO3- ratios. Perhaps reword this result to be more accurate.

[Reply]: It is true that we do not explicitly measure heterotrophic activity, but we do provide data and analyses that support an "increased supply of limited resources during storms", which is where most of the weight of this statement lies on. The subsequent implication that this increased supply can promote in-stream heterotrophic activity is indeed inferred from the analyses on DOC:NO_3^- molar ratios and, more importantly, from the increase in DOC and NO_3^- concentrations during storm flow compared to base flow. We will tone down the statement by using "could sustain" instead of "can promote", which is more accurate.

Lines 108-110: "All data and analyses were integrated and carried out for daily resolutions, which were determined by the availability of the stream chemical data." This line is confusing, please reword.

[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".

Line 114: Please avoid use of "fortnightly" as most readers will not know what this means. Instead state the actual frequency (i.e., every two weeks).

[Reply]: This is most likely true and so we will remove the word "fortnightly" and use "every two weeks" instead. Thanks.