Author responses to reviewer comments on the manuscript entitled
“Temporal and spatial decoupling of CO_2 and N_2O soil emissions in a Mediterranean riparian forest”
by Sílvia Poblador, Anna Lupon, Santiago Sabaté and Francesc Sabater

Dear reviewers and Prof. Akihiko Ito,

Many thanks for your review and your positive and constructive comments on the manuscript "Temporal and spatial decoupling of CO_2 and N_2O soil emissions in a Mediterranean riparian forest". We have considered all the reviewers’ comments and we have worked thoroughly on a new version of the manuscript to tackle them all. As suggested by the reviewers, we have rewritten the introduction in order to clarify our objectives and improve the justification of our study design. In the new version, we have improved the methodology description, included the suggested new figures, and provided estimates of the N_2:N_2O ratios. Finally, we have shortened the discussion and toned down our conclusions. We feel that the reviewers’ suggestions helped to improve the quality and clarity of our manuscript and we believe that you will find our revised version suitable for publication in *Biogeosciences*. Please note that we have changed the manuscript title to “Soil water content drives spatio-temporal patterns of CO_2 and N_2O emissions from a Mediterranean riparian forest soil”.

Below we provide the specific answers to all the questions raised by the three reviewers. Please, do not hesitate to contact us if you considered that further clarifications are needed.

Looking forward hearing from you soon,

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cc: Anna Lupon, Santiago Sabaté, Francesc Sabater

Reviewer #1

General comments:

The manuscript title is not clear for the air emission or soil emission. The paper purpose needs to be sharpened. The details of CO_2 and N_2O measurement, emission calculation formulas, quality assurance, and statistical models should be given in method section.

Answer: We are very grateful for the many insightful comments and suggestions. We agree that the title and the objectives of the older version of the manuscript were confusing. In the new version of the manuscript, we have changed the title to clarify that we measure soil GHG emissions along a riparian zone. In addition, we have rewritten the last paragraph of the introduction by including the constructive comments from the reviewer and Reviewer 2, who also highlighted the need to improve the purpose and hypotheses of the study. We do now clarify our 3 specific objectives as well as develop a competing set of hypotheses regarding the spatio-temporal pattern in GHG emissions that we expected to find based on changes in riparian hydrology. Please, see your and Reviewer 2 comments for more details on this regard.

Moreover, and following the reviewer suggestion, we include now detailed information of the methods and calculations used to estimate carbon dioxide (CO_2) emissions. Among other things, we have carefully checked all the calculations to ensure that there were no mistakes. By doing so, we realized that Figure 4a (Figure 5a in the new version of the manuscript) was wrong: the bars represent mg CO_2 m^{-2} h^{-1} (not mg C m^{-2} h^{-1}). We apologize for the mistake and we are thankful to the reviewer to pointing it out. For more detail on the changes we have made to the manuscript, please see our responses to your specific comments below.

Detailed comments:

1. In title: What is the CO_2 and N_2O soil emissions? It should be the CO_2 and N_2O emissions from soil. And in the context, "decoupling" is not discussed. **Answer:** We agree that we did not use "*decoupling*" in a proper way; we meant that CO_2 and N_2O had opposite spatial patterns. To avoid further confusions, and following the reviewer advice, we have changed the title to: "Soil water content drives spatio-temporal patterns of CO_2 and N_2O emissions from a Mediterranean riparian forest soil".

2. Row 69-80: one sentence is needed to clearly express the paper purpose. **Answer:** The reviewer is right; we admit that our objectives and hypotheses were not clear enough. The main objectives of our study were '(i) to evaluate the spatio-temporal patterns of CO_2 and N_2O emissions in Mediterranean riparian soils, (ii) to analyze under which conditions soil water content rules microbial processes and GHG over other physicochemical variables, and (iii) to provide some reliable estimates of GHG emissions from Mediterranean riparian soils'. These objectives are specified in the new version of the manuscript (page 3, lines 79-82).

3. Sections 2 and 3 may merge into one section as methodology section. **Answer:** OK, we have included the study site in the "Materials and methods" section.

4. Can you give a schematic graph to show CO_2 and N_2O sampling locations in three zones and sampling schedules? **Answer:** We agree with the reviewer that a plot layout of the studied riparian forest would improve the readability and justification of the study design. Accordingly, we have included a new figure showing the sampling locations along the studied riparian forest (Figure R.1). This figure is now included in the manuscript together with a conceptual model of the influence of hydrology on soil microbial processes across a Mediterranean riparian zone (new Figure 1). We strongly believe that this new figure will improve the readability of our study design as well as our main hypotheses and objectives.

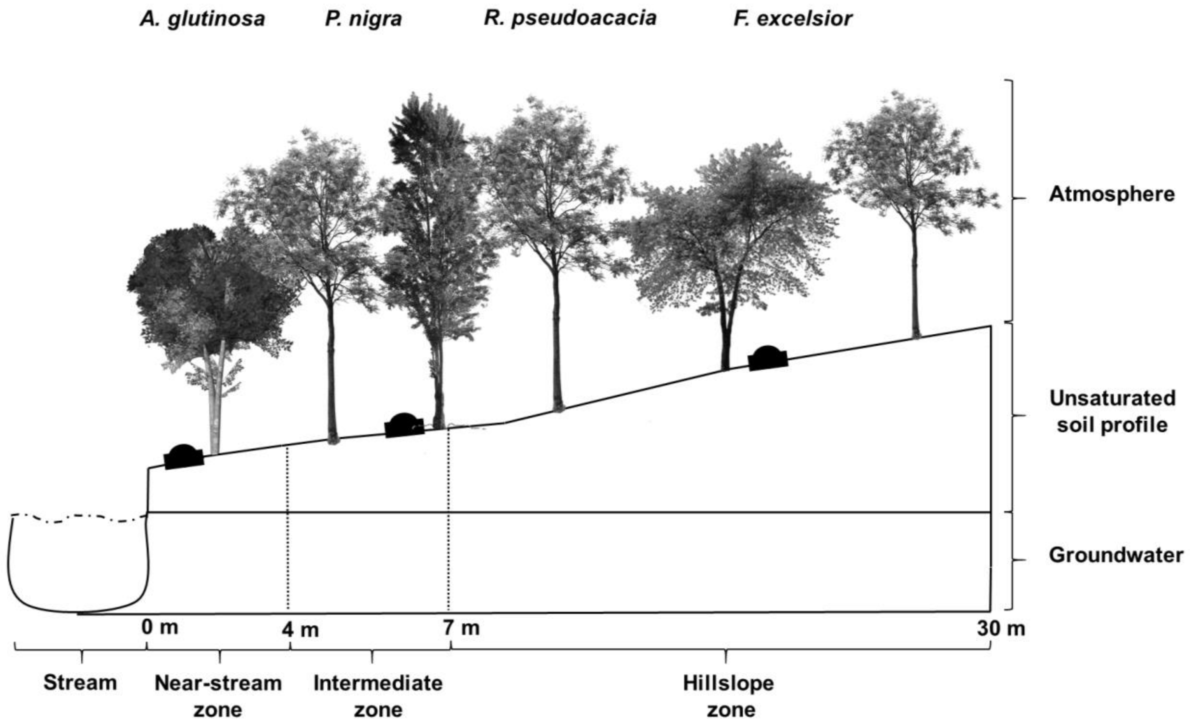

Fig. R1: Plot layout for the studied Mediterranean riparian forest showing the three riparian zones and the location of the chambers (n=5 for each riparian zone).

5. How did you measure CO_2 in three zones at the same time with one sampling system? **Answer:** We acknowledge that the explanation regarding CO_2 measurements was confusing. As the reviewer pointed, soil CO_2 effluxes were not measured simultaneously at all sites. Instead, we used one unique sensor to perform all the measurements. Every day, at 12 pm, we started measuring soil CO_2 effluxes in the first site of the near-stream zone. When finished, we moved to the second site and so on until finished with all sites (15 in total). The incubation time needed for CO_2 efflux measurements was generally short (< 2 min), and thus, we were able to perform all the measurements in a relatively short time (~ 30-45 min). This field protocol ensured that changes in CO_2 emissions were because of the spatial variability rather than diurnal changes in environmental conditions. We have clarified this in the new version of the manuscript as 'Every field day, CO_2 measurements started at 12 p.m. and were conducted consecutively at the 15 plots starting for the near stream zone' (page 5, lines 125-126).

6. Row 111: did you directly place the SRC-1 soil chamber on top of the soil surface? **Answer:** No, the SRC-1 chamber was gently placed on the top soil surface after (i) carefully removing the litter layer and (ii) checking that there was no air exchange with the atmosphere. Furthermore, prior to each measurement, we aerated the sensor (1 min) to minimize the contamination between samples. This procedure is detailed in the new version of the manuscript to avoid confusions: 'Before each measurement, we carefully removed the litter layer to ensure no leaks. Furthermore, we aerated the SCR-1 between samples to ensure the accuracy of the instrument as well as to avoid contamination between samples' (page 5, lines 126-128).

7. What standard operation procedures did you follow for CO_2 and N_2O measurement? How did you conduct the QA/QC? Or are you sure that your measurements were accurate? **Answer:** For CO_2 measurements, we assumed an accuracy of 1% (PP Systems, USA). Moreover, the EGM-4 performed an "auto-zero" at regular intervals during the sampling to ensure the stability of CO_2 signals, minimize contamination, and reduce changes on sensitivity.

For N_2O measurements, we ensure the accuracy of the gas chromatograph measurements (Agilent Technologies, 7820A GC System) by measuring several certified standards (4.66 ppm N_2O; AirLiquide) prior, during, and post the analysis of our samples. Following your advice, we provide a detail description of these procedures in the new methods section (page 5, lines 124-125 for CO_2; page 6, lines 162-163, for N_2O).

8. What is your CO_2 and N_2O emission calculation formulae? **Answer:** The rates of CO_2 and N_2O emission were calculated from the best fit linear or exponential regression of gas concentration with time. GHG fluxes on an areal basis (*Fg*, in µmol m^{-2} h^{-1}) were then calculated following Healy et al. (1996):

$$F_g = \frac{dg}{dt} \times \frac{V\,P_0}{S\,RT_0} \qquad \text{(Eq.1)}$$

where dg/dt is the rate of change in gas concentration (in µmol mol^{-1} h^{-1}) in the chamber, *V* is chamber volume (in m^3), P_0 is initial pressure (in Pa), S is the soil surface area (in m^2), R is the gas constant (8.314 Pa m^3 K^{-1} mol^{-1}), and T_0 is the initial chamber temperature (in K). For budgeting, moles of CO_2 and N_2O were converted to grams of C and N, respectively. Following the reviewer suggestion, we have included the formula in the new of the manuscript (pages 5, lines 130-134).

9. Rows 113 to 114: "CO_2 emissions rates were calculated as the amount of CO_2 accumulated in the head-space of the EGM-4 chamber after an incubation time of c.a. 120 s." Please make sure that is EGM-4 chamber or SRC-1 soil chamber? **Answer:** The reviewer had a point; this sentence was confusing. We have clarified in the text that it was a SRC-1 soil chamber connected to an EGM-4 (page 5, lines 126-127). Sorry for the confusion.

10. In 3.3 section: can you give statistical model for each analysis **Answer:** We are not sure which statistical model the reviewer is referring to. However, in the new manuscript have clarified that (i) we used the best linear or exponential fit model to estimate gas emission rates (page 5, lines 128-129), (ii) we performed linear mixed-model analysis of variance (ANOVA) to test differences in soil properties, microbial N processes, and gas emissions across riparian zones and seasons (pages 6-7, Section 2.4), and (iii) we used partial least squares regression (PLS) to explore how soil properties and soil N processes predict variation in CO_2 and N_2O emissions (pages 6-7, Section 2.4). Moreover, Table 2 and Table 4 show the main statistical results of the linear mixed-model ANOVA and the PLS-models, respectively. We have also included a new figure in the Supplementary Information (Figure S1) showing the models used to estimated gas emissions rates (please, see comment 11 for more information).

11. Can you give a graph to show CO_2 and N_2O concentrations or emission during June measurement with time to show real measurement? **Answer:** Following the reviewer suggestion, Figure R.2 shows the increments of CO_2 and N_2O concentrations during the incubation time for the field campaign of June 2013. The best fit linear regression used to estimate gas effluxes is shown in each case. Based on these regressions, rates of CO_2 and N_2O emissions were 99.11 – 160.98 mg C m^{-2} h^{-1} and 0.0007 – 0.002 mg N m^{-2} h^{-1}. To further clarify the calculations of GHG emissions, we include Figure R2 in the Supplementary Information (Figure S1).

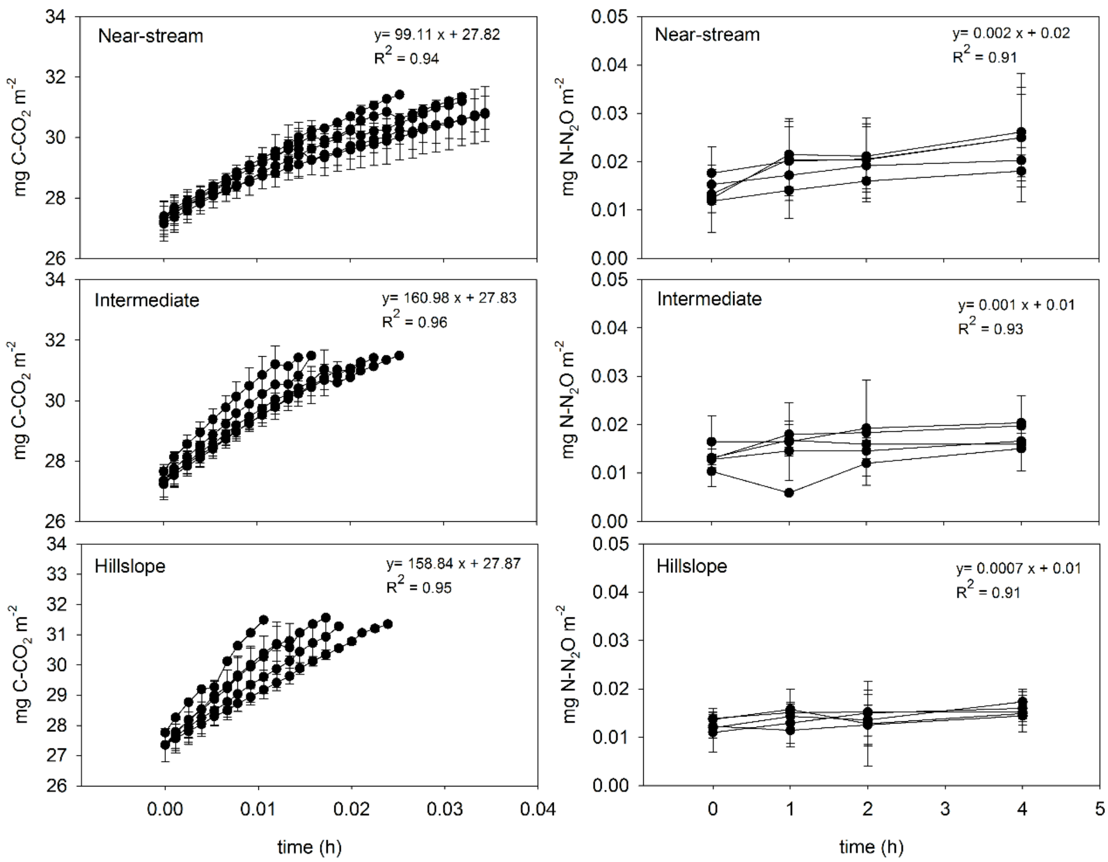

Fig. R2: Concentrations of carbon dioxide (left column) and nitrous oxide (right column) during the incubation time for the sampling campaign of June 2013. Data is shown for the near-stream, intermediate and hillslope zones separately. For each plot, data is shown as mean ± SD (n = 5) for all sampling days of June. The best fit linear model used to calculate gas emissions is shown for each plot.

12. How do you conclude the decoupling of CO_2 and N_2O emission from soils in time and location? **Answer:** As we acknowledged in your earlier comment, we did not used properly the term **"***decoupling". By decoupling we meant that CO_2 and N_2O fluxes were negatively correlated in space: CO_2 fluxes were higher at the hillslope zone, while higher N_2O emissions were observed at the near-stream zone. Similarly, the temporal pattern of CO_2 and N_2O emissions also differed: CO_2 emissions were maxima in June, while highest N_2O emissions occurred in April. In the new conclusions, we do not use the term "*decoupling*" anymore but focus on highlighting the main findings of our experiment (see you comment below).

13. In conclusion, you may report your own conclusions. Why did you cite the references in your conclusion? What is your meaning about the large emission of GHG? **Answer:** Following the reviewer suggestion, we have removed all citations from the conclusion section to avoid confusions and improve the readability of our findings and their meaningful. For instance, we do now explain that our results clearly illustrate the close linkage between riparian soil water content and the microbial processes that produce GHG at both temporal and spatial scales (page 12, lines 344-351). Overall, these findings highlight that future variations in soil water availability due to climate change can potentially affect the riparian functionality in Mediterranean zones, as well as their contribution to regional and global C and N cycles (page 12, lines 351-352).

Reviewer #2

In this paper entitled "Temporal and spatial decoupling of CO_2 and N_2O soil emissions in a Mediterranean riparian forest" Poblador et al report annual and seasonal greenhouse gas (GHG) emissions from a Spanish riparian zone. The authors found that N_2O fluxes from denitrification were lower than in previous studies but that CO_2 flux was quite high. As expected, these fluxes were negatively correlated in space and time. The authors conduct a sound observational study with interesting results, but I was left wondering what the key findings were and how they advanced our understanding of the role of riparian zones as a terrestrial-atmospheric-aquatic interface. I outline a few general concerns below before providing line edits. If the paper can be restructured around a compelling question, it could be a valuable contribution to our understanding of riparian zones in the larger landscape context, but the current lack of focus limits the paper in its current state.

Answer: Thank you for your positive and constructive comments, which have helped us to improve the manuscript significantly. Following your advice, we have carefully rewritten both the introduction and discussion sections in order to (i) develop a more meaningful objectives and hypotheses and (ii) highlight the novelties of our study. For more detail of the changes we have made to the manuscript, please see our responses below.

Major concerns:

1. The manuscript focuses on GHG emissions, but the temporal and spatial scale and the methods of the study do not seem suited to answer this question. High CO_2 fluxes in themselves do not indicate whether an ecosystem is a carbon source or sink, since net ecosystem carbon balance is the relevant parameter. Furthermore, there is large inter-annual variability in both CO_2 and N_2O fluxes in Mediterranean ecosystems, and the magnitude of difference observed here does not seem strong enough to infer landscape functions. I think the study has many other interesting implications about the link between microbial, physicochemical, and hydrological variation, but I feel it does not shine when framed as an assessment of GHG budgets.

Answer: Thank you for your remarks. As we designed this study, we initially set out to address the relationship between soil water availability, microbial processes, and GHG emissions in a Mediterranean riparian forest. Later on, the editor pinpoint that our results could be used to understand the role of riparian zones as active components of the regional and global carbon (C) and nitrogen (N) cycle. We agreed with him because our findings suggest that, on annual terms, Mediterranean riparian zones can transform and outgas large amounts of C and N. However, finding a balance between explaining the rationale of the study (which clearly involves the link between water availability, microbial transformations, and gas emissions) and focusing on more interesting implications of our results has proven challenging. We agree that the previous version of the manuscript may have put too much emphasis on the magnitude of greenhouse gas emissions. Therefore, we have made changes according to the reviewer suggestions (which we believe that have improved the logical structure of the introduction) (page 2-3), while maintaining a mention of the C and N annual emissions in the objectives and discussion (page 2, lines 72-75; pages 11-12, section 4.4). Moreover, we also acknowledge that we cannot state if riparian zones are sink or source of C because we did not take into account C uptake rates. We have omitted that concept in the new version of the manuscript.

2. The introduction reports many interesting observations but the lack of a focused research question, hypothesis, and broader conceptual framework make it hard to identify the salient points. Revising the intro to focus on clear question (rather than just stating multiple times that little is known about GHG flux from Mediterranean riparian zones) would strengthen the paper substantially.

Answer: Thanks for the suggestion; we have rewritten the introduction to improve the readability of our objectives and the justification of our study design. First, we do now specify that our goals were: '(i) to evaluate the spatiotemporal patterns of CO_2 and N_2O emissions in Mediterranean riparian soils, (ii) to analyze under which conditions soil water content rules microbial processes and GHG over other physicochemical variables, and (iii) to provide some reliable estimates of GHG emissions from Mediterranean riparian soils' (page 3, lines 79-82).

Further, we have improved our hypotheses by focusing on the role of soil water availability on microbial processes. We hypothesized that the magnitude and the relative contribution of N_2O and CO_2 to total GHG emission would strongly depend on soil water content. In the near-stream zone, we expected that saturated soils would enhance denitrification and methanogenesis, but inhibited both respiration and nitrification. Thus, we predicted higher N_2O than CO_2 emissions in this zone. In the intermediate zone, we expected that wet (but not saturated) soils would enhance aerobic processes, and thus, we predicted high CO_2 emissions compared to N_2O. Finally, we expected that dry soils would depleted (or even inhibited) microbial activity near the hillslope edge, and therefore, we predicted low GHG emissions in this zone. Because Mediterranean regions are subjected to strong intra-annual variations in soil water content and temperature, we predicted that this general behavior may be maximized in summer, when only near-stream soils would keep wet. Conversely, differences would be minimized during wet periods, where all zones would have high rates of GHG emissions (page 3; lines 82-92).

Finally, we have rewritten the introduction to focus more on the relationship between soil water availability, microbial processes, and GHG emission rather than in the source/sink behavior of riparian forests in the global C and N cycle. We do now highlight that riparian hydrology may play a fundamental role in regulating soil C and N cycling and emissions. However, there are still relatively few studies that analyze the direct influence of soil water content on several GHG effluxes simultaneously (but see Harms and Grimm, 2003; Jacinthe et al., 2015), and even less that combine such analyses with soil microbial processes (especially mineralization and nitrification). This gap of knowledge makes extremely difficult to assess under which circumstances soil water content (rather than temperature or substrate availability) is the primary control factor of the riparian functionality. Moreover, we also highlight that Mediterranean systems are a unique natural laboratory to understand the close link between the spatio-temporal variations in hydrology and riparian functionality because (i) they are characterized by a marked spatial gradient in soil water content and (ii) they are subjected to seasonal alterations of precipitation and temperature regimes (pages 2-3, lines 62-67). With these improvements, we strongly believe that the introduction is now better framed within the context of riparian functionality and explicitly addresses the hypotheses that were implicit in our study design.

3. The discussion currently feels like a continuation or repetition of the results sections. Clearly summarizing the key findings and their implications at the beginning of the discussion would orient the reader to better appreciate the value of this study. Subheadings could be effective at organizing the content and an overall shortening is probably in order since the discussion is quite long for the amount of new material it presents.

Answer: We agreed that the discussion was quite long and repetitive. We have reduce one page of text in that section. Following the reviewer advice, we have opened the discussion with a short summary highlighting the main objectives and results of the study (i.e. that soil water content has a major role in driving spatio-temporal variations in riparian C and N cycling as well as in regulating the overall role of Mediterranean riparian soils in the global C and N cycles and emissions).

Moreover, we do now include 4 different subsections to reduce the redundancy throughout the discussion. The first subsection (section 4.1) focuses on the microbial processes that regulated GHG emissions. Briefly, our results suggest that CO_2 emissions are close link to heterotrophic respiration, while N_2O emissions depend on denitrification. Ultimately, the absence of a relationship between N_2O and CO_2 emissions suggests that soil C and N cycles are decoupled in Mediterranean riparian forests (page 9, lines 263-267).In the second and third sections (section 4.2 and 4.3), we evaluate in detail the effect of soil water content on the spatio-temporal patterns of CO_2 and N_2O emissions by directly relating our findings with our initial hypotheses. We do now discuss how riparian hydrology overrule other environmental parameters on controlling riparian functionality in terms of microbial activity, GHG production and denitrification efficiency (page 10, lines 280-284; page 10, lines 302-304; page 11, lines 309-314). Finally, the last section (section 4.4) discuss put our results in a broader scale by quantifying the potential role of riparian soils as a hot spot of GHG emissions within Mediterranean regions (pages 11-12, lines 330-341).

We believe that these changes, together with those proposed for the other reviewers, have reduced significantly the length of the discussion as well as have better highlighted the implications of our findings.

4. A conceptual figure laying out the expected or observed functioning of the riparian zone in regards to respiration and denitrification would be useful and could help focus the paper.

Answer: As suggested, we performed a conceptual figure showing the expected link between water availability and microbial processes along our riparian forest (Figure R.3). Denitrification and methanogenesis are anaerobic processes that only occur under anoxic conditions (water content > 60%), while respiration, mineralization and nitrification are aerobic process that mostly occur under moist soils (water content = 30-60%) (Pinay et al., 2007). Based on these premises, we hypothesized that soil water content would control the microbial processes and GHG emissions in our Mediterranean riparian soils. We expected high denitrification rates in the near-stream zone, where high groundwater levels sustain saturated soil. Conversely, we expected high respiration and mineralization rates in the intermediate zone, where soils are wet but not saturated. Finally, we expected low (or nil) microbial activity in the hillslope zone, where deep groundwater tables are not sufficient to keep the top soils moist.

This figure is now included in the main manuscript together with a plot layout of the riparian plot (Figure 1). We believe that the new figure helps to the reader to understand our sampling design as well as our main hypotheses.

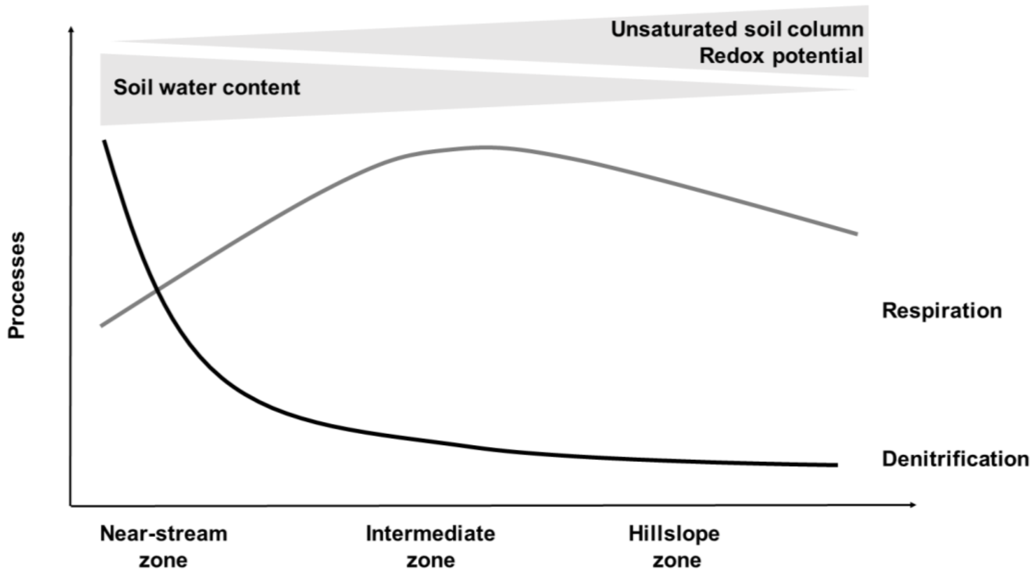

Fig. R3. Conceptual model of the influence of hydrology on soil microbial processes across a Mediterranean riparian zone. Soil water content decreases from the near-stream to the hillslope zones due to changes in groundwater table, increasing unsaturated soil column and oxic conditions. Anaerobic processes such as denitrification and methanogenesis occur under anoxic conditions, and thus, high rates of these processes are expected in the near-stream zone. Conversely, aerobic processes such as respiration and mineralization are optimized under a moderate range of soil water content, and therefore, they would increase in the intermediate zone. Finally, we expected low (or nil) microbial activity in the hillslope zone because dry soils are unfavorable for both aerobic and anaerobic processes. Accordingly, we predicted high rates of soil CO_2 effluxes in the intermediate zone, while high rates of N_2O emissions are predicted in the near-stream zone.

5. There are many unnecessary acronyms that make the text unwieldy. Avoiding uncommon acronyms (e.g. GWL, SWC, NNM, NN, DNT, PLS, DEA, TCD) would make the paper more accessible.

Answer: OK; we have avoided the use of acronyms throughout the manuscript. However, we have decided to keep some of them in the tables and figures to improve their readability.

6. The paper is generally well written but it has quite a few non-standard phrasings and English formulations. Asking for a proofread from a native speaker would be worthwhile.

Answer: OK, a native English speaker have carefully read the new version of the manuscript before submission.

<u>Detailed comments:</u>

Line 21: powerless is a strange word choice **Answer:** OK, “low amounts of N_2O emissions”.

Line 38: Not clear what 70% of total emissions means. Also no need to put greenhouse in quotes **Answer:** We meant that riparian zones can emit up to 70% of global GHG emissions, which take into account both natural processes and human activities. We have clarified it in the text (page 2, lines 36-39).

Line 40: Not clear what this means? **Answer:** We meant that soil water saturation in riparian soils can support large methane (CH_4) effluxes to the atmosphere that account for the 15 – 40 % of the global CH_4 emissions. This sentence has been clarified in the text (page 2, lines 42-43).

Line 44: contribute to increasing **Answer:** Not needed now. Thank you anyway.

Line 59: may complicate upscaling (instead of may difficult to upscale) **Answer:** Not needed now. Thank you anyway.

Line 72: alter instead of vary and measure instead of measured **Answer:** Not needed now. Thank you anyway.

Line 88: compositions **Answer:** OK (page 4, line 101).

Line 111: with instead of by using. **Answer:** OK (page 5, line 123).

Line 262: There is a large body of research on scaling riparian soil measurements. **Answer:** That's right; several studies have attempted to upscale soil processes and how the mechanisms underlying such GHG emissions can ultimately modify catchment GHG fluxes (e.g. Hagedorn, 2010; Pinay et al., 2015; Vidon and Hill, 2006). However, there are still fundamental uncertainties regarding the magnitude, spatiotemporal variation, and sources of GHG emissions from riparian zones (see Pinay et al., 2015). We have clarified it in the new version of the manuscript (page 11, lines 330-332).

References: There are several inconsistently formatted references. **Answer:** OK, references have been carefully checked before submitting the new manuscript.

Line 620: Figure 5 has a pretty low information content. I wonder if it could be included in the supplementary information. **Answer:** OK, Figure 5 has been moved to supplementary information (new Figure S.2.).

Reviewer #3

The paper is interesting, methods reliable and results almost as expected. The main concern is missing of data on potential N_2 emission which is the main product of denitrification. Therefore, I cannot agree with the statement in paper that low N_2O emission is a result of low intensity denitrification. In opposite, it could be that the denitrification process is complete and most of N_2O produced will be transformed to N_2. However, without evidences on (potential) N_2 emission (either based on 15N or He-O_2 analysis or even the acetylene method which gives underestimated but at least some values) and denitrification control genes (nirS+nirK and nosZi+II) it is hard to say about the intensity of denitrification. It can also be that a part of N_2O is coming from nitrification. This kind of discussion is missing and may be it is too much to require analysis of all those components. However, authors should avoid to declare that denitrification intensity is low because the N_2O flux is low. Also, it is recommended to include some relevant references on denitrification intensity (N_2:N_2O ratio) in riparian zones and develop a short discussion based on this knowledge.

Answer: Certainty, measures of denitrification intensity (i.e. N_2:N_2O ratio) can be an added value to our discussion. Unfortunately, we did not measure N_2 nor denitrification control genes to identify the source of N_2O emissions (Butterbach-bahl et al., 2013; Mander et al., 2014; Saarenheimo et al., 2015). However, we indeed measured denitrification rates by the acetylene method. Therefore, as the reviewer pinpointed, we can calculate some rough estimates of N_2:N_2O ratio. As shown in Table R.1, N_2:N_2O ratios ranged from 4 (hillslope zone) to 22 (near-stream zone). Although these values are within the literature range, most of studies in temperate riparian forests have reported N_2:N_2O ratios at least one order of magnitude higher than in Font del Regàs (i.e., 184 – 844; Mander et al., 2014). These results support the idea that low N_2O emissions in our riparian site can be attributed to both low denitrification rates (0.72 - 2.69 mg N kg^{-1} d^{-1}) and high denitrification efficiency. The highest denitrification efficiency was observed in the near-stream zone, suggesting that saturated soils favored the complete denitrification process and can potentially emit less N_2O compared to less saturated soils. Following the reviewer suggestion, we have included this rationale in the new discussion (pages 11, lines 309-314); thanks for pointing it out.

Table R.1: Mean values (± standard deviation) of N_2 emissions, N_2O emissions and molar N_2:N_2O ratios for each riparian zone during the study period.

	N_2 Emission (mg m^{-2} d^{-1})	**N_2O Emission (mg m^{-2} d^{-1})**	**N_2:N_2O ratio**
Near-stream	4.93 ± 10.17	0.80 ± 0.79	21.50 ± 40.32
Intermediate	0.41 ± 1.41	0.79 ± 0.81	5.90 ± 16.02
Hillslope	0.42 ± 1.05	0.52 ± 0.52	4.23 ± 8.31

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Soil water content drives spatio-temporal patterns of CO_2 and N_2O emissions from a Mediterranean riparian forest soil

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Abstract.

Riparian zones play a fundamental role in regulating the amount of carbon (C) and nitrogen (N) that is exported from catchments. However, C and N removal via soil gaseous pathways can influence local budgets of greenhouse gases (GHG) emissions and contribute to climate change. Over a year, we quantified soil effluxes of carbon dioxide (CO_2) and nitrous oxide (N_2O) from a Mediterranean riparian forest in order to understand the role of these ecosystems on catchment GHG emissions. In addition, we evaluated the main soil microbial processes that produce GHG (mineralization, nitrification, and denitrification) and how changes in soil properties can modify the GHG production over time and space. Mediterranean riparian soils emitted larger amounts of CO_2 (1.2 – 10 g C m^{-2} d^{-1}) than N_2O (0.001 – 0.2 mg N m^{-2} d^{-1}) to the atmosphere attributed to high respiration and low denitrification rates. Both CO_2 and N_2O emissions showed a marked (but antagonistic) spatial gradient as a result of variations in soil water content across the riparian zone. Deep groundwater tables fueled large soil CO_2 effluxes near the hillslope, while N_2O emissions were higher in the wet zones adjacent to the stream channel. However, both CO_2 and N_2O emissions peaked after spring rewetting events, when optimal conditions of soil water content, temperature, and N availability favor microbial respiration, nitrification, and denitrification. Overall, our results highlight the role of water availability on riparian soil biogeochemistry and GHG emissions and suggest that climate change alterations in hydrologic regimes can affect the microbial processes that produce GHG as well as the contribution of these systems to regional and global biogeochemical cycles.

Keywords.

greenhouse gas emissions, riparian soils, denitrification, microbial respiration, soil water content.

1 Introduction

Riparian zones are hotspots of nitrogen (N) transformations across the landscape, providing a natural filter for nitrate (NO_3^-) transported from surrounding lands via runoff and subsurface flow paths (Hill, 1996; Vidon et al., 2010). Although interest in riparian zones has primarily been motivated by the benefits of these ecotones as effective N sinks, enhanced microbial activity in riparian landscapes can play a key role on atmospheric pollution. For instance, riparian zones can account by 70% of global (natural processes and human activities) terrestrial emissions of nitrous oxide (N_2O) to the atmosphere, a powerful greenhouse gas (GHG) with 298 times the global warning potential of carbon dioxide (CO_2) (Audet et al., 2014; Groffman et al., 2000; Hefting et al., 2003). Moreover, riparian soils can significantly contribute to global CO_2 emissions because they can hold high rates of heterotrophic and autotrophic respiration (Chang et al., 2014). Soil respiration is the main natural carbon (C) efflux to the atmosphere, contributing to 20% of the global emission of CO_2 (Kim and Verma, 1990; Raich et al., 2002; Rastogi et al., 2002). Finally, riparian zones can support large methane (CH_4) fluxes that account for the 15 – 40 % of global emissions (Audet et al., 2014; Segers, 1998). However, there are still many uncertainties regarding the magnitude and spatio-temporal variability of soils GHG emissions in riparian zones, reaching contradictory results concerning the potential role of riparian zones as sinks or sources of C and N (Bruland et al., 2006; Groffman et al., 1992; Harms et al., 2009; Walker et al., 2002).

Understanding the processes regulating GHG emissions from riparian soils is essential to quantify the role of riparian zones in the global C and N cycles. Multiple environmental variables, such as soil temperature, soil water content, and both C and N availability have been identified as key factors influencing the rate and variability of soil microbial activities that produce GHG (Chang et al., 2014; Hefting et al., 2003; Mander et al., 2008; McGlynn and Seibert, 2003). Among them, riparian hydrology seems to play a fundamental role on GHG production because it controls the substrate subsides and, most importantly, the redox conditions of riparian soils (Jacinthe et al., 2015; Vidon, 2017). Under saturated conditions, anaerobic processes such as methanogenesis (i.e. the transformation of CO_2 to CH_4) and denitrification (i.e. the transformation of NO_3^- to N gas (N_2) or N_2O) are the primary processes involved in the C and N cycles (Clément et al., 2002). Conversely, in dry soils, aerobic transformations involved in the oxidation of the organic matter (i.e. respiration, mineralization, nitrification, methane oxidation) dominate the riparian biogeochemistry (Harms and Grimm, 2008). From such observations, some one would expect that there is a strong correlation between soil wetness and the relative importance of CO_2, N_2O and CH_4 riparian soil emissions to the total GHG fluxes. However, there are still relatively few studies that analyze the direct influence of soil water content on several GHG effluxes simultaneously (but see Harms and Grimm, 2008; Jacinthe et al., 2015), and even less that combine such analyses with other environmental factors and soil processes. Thus, it is still unclear under which circumstances soil water content (rather than temperature or substrate availability) is the primary control factor of the riparian functionality.

Mediterranean systems are a unique natural laboratory to understand the close link between spatio-temporal variations in hydrology and riparian biogeochemistry because they are characterized by a marked spatial gradient of soil water content, that can range from <10% in the hillslope edge to > 80% close to the stream (Chang et al., 2014; Lupon et al., 2016). Moreover, Mediterranean regions are subjected to seasonal alterations of precipitation and temperature regimes that might affect riparian hydrology as well as microbial activity in the riparian soils (Bernal et al., 2007; Bruland et al., 2006; Harms and Grimm, 2008; Harms et al., 2009). Increments in GHG emissions in riparian zones might occur following storms or flood events because sharp increments in soil water content enhance nitrification, denitrification, respiration, and methanogenesis rates (Casals et al., 2011; Jacinthe et al., 2015; Werner et al., 2014). However, because recent studies have shown that high temperatures and relatively moist soils can sustain large rates of C respiration and N mineralization in summer in the near-stream zone (Chang et al., 2014; Lupon et al., 2016), the contribution of such microbial pulses to annual CO_2 and N_2O production in Mediterranean riparian soils is still under debate. Moreover, improved understanding of interactions among hydrology, microbial processes, and gas emissions within Mediterranean riparian zones is not only fundamental to understand the temporal pattern of riparian biogeochemistry, buy also necessary to estimate the contribution of these ecosystems to atmospheric GHG budgets at local and global scale.

In this study, soil properties, soil N processes, and CO_2 and N_2O soil emissions were measured over a year across a Mediterranean riparian forest that exhibited a strong gradient in soil water content (Fig. 1a). We did not measure CH_4 emissions because previous studies reported extremely low values in dry systems (-0.06 – 0.42 mg C m^{-2} d^{-1}; Batson et al., 2015; Gómez-Gener et al., 2015). Specifically, we aimed (i) to evaluate the spatio-temporal patterns of CO_2 and N_2O emissions in Mediterranean riparian soils, (ii) to analyze under which conditions soil water content rules microbial processes and GHG over other physicochemical variables, and (iii) to provide some reliable estimates of GHG emissions from Mediterranean riparian soils. We hypothesized that the magnitude and the relative contribution of N_2O and CO_2 to total GHG emission strongly depend on soil water content and redox conditions rather than other variables during all year long (see conceptual approach in Fig. 1b). In the near-stream zone, we expected that saturated anoxic soils would enhance denitrification but constrain both respiration and nitrification. Thus, we predicted higher N_2O than CO_2 emissions in this zone. In the intermediate zone, we expected that wet (but not saturated) soils would enhance aerobic processes such as respiration, N mineralization or nitrification, and thus, we predicted high CO_2 emissions compared to N_2O. Finally, we expect that dry soils would deplete (or even inhibit) the soil microbial activity near the hillslope edge, and therefore, we predicted low GHG emissions in this zone. Because Mediterranean regions are subjected to strong intra-annual variations in soil water content, we expected that this general behavior would be maximized in summer, when only near-stream soils would keep wet. Conversely, we expected that all microbial processes would be enhanced short-after rainfall events, and thus, simultaneous pulses of CO_2 and N_2O emissions would occur in spring and fall.

2 Materials and methods

2.1 Study site

The research was conducted in a riparian forest of Font del Regàs, a forested headwater catchment (14.2 km^2, 500 – 1500 m above the sea level (a.s.l.)) located in the Montseny Natural Park, NE Spain (41º50'N, 2º30'E) (Fig. 1a). The climate is sub-humid Mediterranean; with mean temperature ranging from 5ºC in February to 25ºC in August. In 2013, annual precipitation (1020 mm) was higher than long-term average (925 ± 151 mm), with most of rain falling in spring (500 mm) (Fig. 2a). Total inorganic N deposition oscillates between 15 – 30 kg N ha^{-1} yr^{-1} (period 1983 – 2007; Àvila and Rodà, 2012).

We selected a riparian site (~600 m^2, ~30 m wide) that flanked a 3rd order stream close to the catchment outlet (536 m a.s.l., 5.3 km from headwaters). The riparian site was divided into three zones characterized by different species compositions (Fig. 1a). The near-stream zone was located adjacent to the stream (0 – 4 m from the stream edge) and was composed of *Alnus glutinosa* (45% of basal area) and *Populus nigra* (33% of basal area). The intermediate zone (4 – 7 m from the stream edge) was composed by *P. nigra* and *Robinia pseudoacacia* (29% and 71% of basal area respectively). Finally, the hillslope zone (7 – 30 m from the stream edge) bordered upland forests and was composed by *R. pseudoacacia* (93% of basal area) and *Fraxinus excelsior* (7% of basal area). The three riparian zones had sandy-loam soils (bulk density = 0.9 – 1.1 g cm^{-3}), with a 5-cm deep organic layer followed by a 30-cm deep A-horizon. The top soil layer (0 – 10 cm depth) was mainly composed by sands (~90%) and silts (~7%) at the near-stream zone, whereas gravels (~16%) and sands (~80%) were the dominant particle sizes at the intermediate and hillslope zones. During the study period, groundwater level averaged -54 ± 14 cm below the soil surface (b.s.s.) at the near-stream zone, and decreased to -125 ± 4 and -358 ± 26 cm b.s.s. at the intermediate and hillslope zones, respectively (Fig. 1a and Fig. 2b).

2.2 Field sampling

We delimited five plots (1 x 1 m) within each riparian zone (near-stream, intermediate and hillslope) (Fig. 1a). During the year 2013, soil physicochemical properties, soil N processes, and gas emissions were measured in each plot every 2 – 3 months in order to cover a wide range of soil water content and temperature conditions. On each sampling month, one soil sample (0 – 10 cm depth, including O- and A- horizons) was collected randomly from each plot to analyze soil physicochemical properties. Soil samples were taken with a 5-cm diameter core sampler and placed gently into plastic bags after carefully removing the litter layer. Close to each soil sample, we performed *in situ* soil incubations to measure soil net N mineralization and net nitrification rates (Eno, 1960). For this purpose, a second soil core (0 – 10 cm depth) was taken, placed in a polyethylene bag, and buried at the same depth. Soil incubations were buried 4 days and then removed from the soil.

Gas emissions and denitrification rates were measured simultaneously and during four consecutive days (i.e. during the entire soil incubation period) in order to facilitate the direct comparison between microbial rates and gas fluxes. Soil CO_2 effluxes were measured with a SRC-1 soil chamber attached to an EGM-4 portable infrared gas analyzer (IRGA) (PP Systems, Amesbury, MA). The EGM-4 has a measurement range of 0 – 2000 ppm (µmol mol^{-1}), with an accuracy of 1% and a linearity of 1% throughout the range. Every field day, CO_2 measurements started at 12 p.m. and were conducted consecutively at the 15 plots starting for the near stream zone. At each plot, the SCR-1 soil chamber was placed over the top soil for a 120 s incubation. Before each measurement, we carefully removed the litter layer to ensure no leaks. Furthermore, we aerated the SCR-1 between samples to ensure the accuracy of the instrument as well as to avoid contamination between samples. For each plot, CO_2 emissions rates were calculated from the best fit linear regression of the CO_2 accumulated in the head-space with incubation time (Fig. S1). CO_2 fluxes on an areal basis (F_{CO2}, in µmol m^{-2} h^{-1}) were calculated following Healy et al. (1996):

$$F_g = \frac{dg}{dt} \times \frac{V\,P_0}{S\,RT_0} \qquad \text{(Eq.1)}$$

where dg/dt is the rate of change in gas concentration (in µmol mol^{-1} h^{-1}) in the chamber, *V* is chamber volume (in m^3), P_0 is initial pressure (in Pa), S is the soil surface area (in m^2), R is the gas constant (8.314 Pa m^3 K^{-1} mol^{-1}), and T_0 is the initial chamber temperature (in °K). For budgeting, moles of CO_2 and N_2O were converted to grams of C and N, respectively.

In situ denitrification rates and N_2O emissions were measured using closed cylinder (0.37 L) and open cylinder (0.314 m^2) chambers, respectively. For denitrification analyses, an intact soil core (0 – 10 cm depth) was introduced in the chamber, closed with a rubber serum stopper, amended with acetone-free acetylene to inhibit the transformation of N_2O to N_2 (10% v/v atmosphere), and placed at the same depth. For N_2O analysis, chambers were placed directly on the soil and no special treatment was carried out. Gas samples for both denitrification and N_2O chambers were taken at the same time (0h, 1h, 2h, and 4h of incubation) with a 20-mL syringe and stored in evacuated tubes. All soil and gas samples were kept at < 4ºC until laboratory analysis (< 24 h after collection).

Soil physical properties were measured within each plot simultaneously to gas emissions. Volumetric soil water content (%) (5 replicates per plot) and soil temperature (ºC) (1 replicate per plot) were measured at 10-cm depth by using a time-domain reflectometer sensor (HH2 Delta-T Devices Moisture Meter) and a temperature sensor (CRISON 25), respectively. Soil pH and reduction potential (Eh, mV) (1 replicate per plot) were measured at 0 – 10 cm depth by water extraction (1:2.5 v/v) using a Thermo-Scientific ORION sensor (STAR 9107BNMD). Although Eh measures performed by water extraction may not be as accurate as other field techniques, these values have been previously used as a good proxy of the soil redox potential (Yu and Rinklebe, 2013).

2.3 Laboratory analyses

Pre-incubation soil samples were oven dried at 60ºC, sieved, and the fraction < 2 mm was used for measuring soil chemical properties. The relative soil organic matter content (%) was measured by loss on ignition (450ºC, 4 h). Total soil C and N contents were determined on a gas chromatograph coupled to a TCD detector after combustion at 1000ºC at the Scientific Technical Service of the University of Barcelona.

To estimate microbial N processes, we extracted 5 g of pre- and post- incubation field-moist soil samples with 50 ml of 2 M KCl (1g : 10ml, ww : v; 1 h shacking at 110 r.p.m. and 20ºC). The supernatant was filtered (Whatman GF/F 0.7 µm pore diameter) and analyzed for ammonium (NH_4^+) and nitrate (NO_3^-). NH_4^+ was analyzed by the salicilate-nitropruside method (Baethgen and Alley, 1989) using a spectrophotometer (PharmaSpec UV-1700, SHIMADZU). NO_3^- was analyzed by the cadmium reduction method (Keeney and Nelson, 1982) using a Technicon Autoanalyzer (Technicon, 1987). For each pair of samples, net N mineralization and net nitrification were calculated as the differences between pre- and post-incubations values of inorganic N (NH_4^+ and NO_3^-) and NO_3^-, respectively (Eno, 1960). Pre-incubation NH_4^+ and NO_3^- concentrations were further used to calculate the availability of dissolved inorganic nitrogen in riparian soils.

To estimate denitrification and natural N_2O emissions, we analyzed the N_2O of all gas samples using a gas chromatograph (Agilent Technologies, 7820A GC System) that was calibrated using certified standards (4.66 ppm N_2O; , AirLiquide). Both denitrification and N_2O emissions rates were calculated similarly to CO_2 fluxes (Fig. S1). In addition, we measured the denitrification enzyme activity (DEA) for 3 soil cores of each riparian zone to determine the factors limiting denitrification. For each soil core, four sub-samples (20 g of fresh soil) were placed into 125-ml glass jars containing different treatments. The first jar (DEA_{MQ}) contained Milli-Q water (20 ml) to test anaerobiosis limitation. The second jar (DEA_C) was amended with glucose solution (4 g glucose kg soil^{-1}) to test C limitation. The third jar (DEA_{NO3}) was amended with nitrate solution (72.22mg KNO_3 kg soil^{-1}) to test N limitation. Finally, the fourth jar (DEA_{C+NO3}) was amended with both nitrate and glucose solutions (4 g glucose kg soil^{-1} and 72.22mg KNO_3 kg soil^{-1}) to test simultaneously C and N limitation. All jars were capped with rubber serum stoppers, made anaerobic by flushing N_2, and amended with acetone-free acetylene (10% v/v) (Smith and Tiedje, 1979). Gas samples were collected after 4 h and 8 h of incubation and analyzed following the same procedure of field DNT samples. DEA rates were calculated similarly to denitrification rates.

2.4 Statistical analysis

Statistical analyses were carried out using the package *lmer* and *pls* of R 2.15.1 statistical software (R Core Team, 2012). We performed linear mixed-model analysis of variance (ANOVA) to test differences in soil properties, microbial N processes, and gas emissions across riparian zones and seasons. We used riparian zone and season as fixed effects, and plot (nested within riparian zones) as a random effect. When multiple samples were taken within a plot (soil physical properties, denitrification, and gas emissions), the ANOVA was performed on plot means, with n = 75 (5 plots x 3 zones x 5 dates). For each model, post-hoc Tukey contrasts were used to test which zones or seasons differed from each other. In all cases, residuals were tested for normality using a Shapiro-Wilk test, and homogeneity of variance was examined visually by plotting the predicted and residual values. In those cases that the normality assumption was unmet, data was log transformed. In all analyses, differences were considered significant when $p < 0.05$.

We used partial least squares regression (PLS) to explore how soil properties, C and N availability, groundwater level, and soil N processes predict variation in CO_2 and N_2O emissions. PLS identifies the relationship between independent (X) and dependent (Y) data matrices through a linear, multivariate model; and produces latent variables (PLS components) representing the combination of X variables that best describe the distribution of observations in 'Y space' (Eriksson et al., 2006). We determined the goodness of fit (R^2Y) and the predictive ability (Q^2Y) of the model by comparing modeled and actual Y observations through a cross-validation process. Each model was refined by iteratively removing variables that had non-significant coefficients in order to minimize the model overfitting (i.e. low Q^2Y values) as well as the multicollinearity of the explanatory variables (i.e. variance inflation factor (VIF) < 5). Furthermore, we identified the importance of each X variable by using variable importance on the projection (VIP) scores, calculated as the sum of square of the PLS weights across all components. VIP values > 1 indicate variables that are most important to the overall model (Eriksson et al., 2006). In all PLS models, data was ranked and centered prior analysis.

3 Results

3.1 Spatial pattern of soil properties, microbial rates, and gas emissions

During the study period, all riparian zones had similar mean soil temperature (11 – 12ºC), pH (6 – 7) and redox potential (170 – 185 mV) (Table 1). However, soil water content exhibited strong differences across riparian zones (Table 2), with the near-stream zone holding wetter soils than the intermediate and the hillslope zones (Table 1). There were significant differences in most of soil chemical properties (Table 1, Table 2). Both organic matter and soil C and N content were 2-fold lower in the near-stream zone than in the intermediate and hillslope zones, though all zones exhibited similar C:N ratios (CN = 14). Moreover, inorganic N concentrations (NH_4^+ and NO_3^-) were from 2- to 5-fold lower for the near-stream zone than for the other two zones.

On annual basis, net N mineralization averaged 0.14 ± 0.40, 0.39 ± 1.23, and 0.22 ± 1.03 mg N kg^{-1} d^{-1} at the near-stream, intermediate, and hillslope zones, respectively. Mean annual net nitrification rates were close to net N mineralization, averaging 0.17 ± 0.38, 0.25 ± 0.69, and 0.28 ± 0.73 mg N kg^{-1} d^{-1} at the near-stream, intermediate, and hillslope zones, respectively. There were no significant differences in mean annual net N mineralization and net nitrification rates among riparian zones (in both cases: mixed-model ANOVA test, $F > F_{0.05}$, $p > 0.05$). Mean annual denitrification was higher at the near-stream zone (2.69 ± 5.30 mg N kg^{-1} d^{-1}) than at the intermediate (0.72 ± 1.85 mg N kg^{-1} d^{-1}) and hillslope (0.76 ± 1.59 mg N kg^{-1} d^{-1}) zones (mixed-model ANOVA test, $F = 4.33$, $p = 0.038$). However, potential denitrification rates were lower in the near-stream zone (0.3 – 0.6 mg N kg^{-1} d^{-1}) compared to intermediate (1.0 – 2.4 mg N kg^{-1} d^{-1}) and hillslope (1.3 – 3.8 mg N kg^{-1} d^{-1}) zones (Table 3).

Natural CO_2 and N_2O emissions differed among riparian zones, yet they showed opposite spatial patterns. Near-stream zone exhibited lower CO_2 emissions (318 ± 195 mg C m^{-2} h^{-1}) compared to the intermediate (472 ± 298 mg C m^{-2} h^{-1}) and hillslope

(458 ± 308 mg C m^{-2} h^{-1}) zones (mixed-model ANOVA test, $F = 7.08$, $p = 0.009$). Conversely, near-stream zone showed higher N_2O emissions (0.035 ± 0.022 mg N m^{-2} h^{-1}) than the other two zones (intermediate = 0.032 ± 0.025 mg N m^{-2} h^{-1}; hillslope = 0.022 ± 0.012 mg N m^{-2} h^{-1}) (mixed-model ANOVA test, $F = 7.31$, $p = 0.008$).

3.2 Temporal pattern of soil properties, microbial rates, and gas emissions

During the study period, there was a marked seasonality in most of soil physical properties, except for pH and Eh, which did not show any temporal pattern (Table 2). Soil water content exhibited a marked seasonality, though it differed among riparian zones (Table 2, "zone x season"). In the intermediate and hillslope zones, soil water content was maxima in November and minima in August, while the near-stream soils were wetter during both spring (April-June) and autumn (November) (Fig. 3a). Conversely, soil temperature showed similar seasonality but opposite values in all riparian zones (Table 2), with a maxima in summer (August) and minima in winter (February) (Fig. 3b). Soil chemical properties (soil organic matter and both soil C and N content) did not show any seasonal trend, but all riparian zones exhibited lower C:N ratios in February compared to the other seasons (Fig. 3c). There was no seasonality in soil NH_4^+ concentrations at any riparian zone (Table 2). However, soil NO_3^- concentrations showed a marked temporal pattern, yet it differed among riparian zones (Table 2, "zone x season"). The highest soil NO_3^- concentrations occurred in February at both the near-stream and hillslope zones, but in June-August at the intermediate zone (Fig. 3d).

Soil N processes showed similar seasonal patterns in all riparian zones (in all cases: $F_{date} < F_{0.05}$, $F_{interaction} > F_{0.05}$). Both net N mineralization and net nitrification rates were higher in April than February, June, and November (Fig. 4a and 4b), while denitrification rates were higher in April and June compared to the rest of the year (Fig. 4c). In April, both net N mineralization and net nitrification rates differed across riparian zone, with higher rates in the intermediate zone than in the near-stream one. Net N mineralization rates also differed in August, when the intermediate zone exhibited 2-fold higher rates than the other two zones. Finally, denitrification was higher at the near-stream than at the other two zones in both June and August.

Natural gas emissions showed a clear seasonal pattern (in both cases: mixed-model ANOVA test, $F_{date} < F_{0.05}$, $p < 0.001$), yet it differed between CO_2 and N_2O emissions. In all zones, CO_2 emissions were maxima in June and minima in February (Fig. 5a), while highest N_2O emission rates occurred in April and lowest in both February and August (Fig. 5b). In spring (April and June), CO_2 emissions were higher at the intermediate and hillslope zones compared to the near-stream one (Fig. 5a). Moreover, the near-stream zone showed higher N_2O emissions than the hillslope zone in February, April, and June (Fig. 5b).

3.3 Relationship between soil properties, microbial processes, and gas emissions

PLS models extracted two components that explained the 71% and the 40% of the variance in CO_2 and N_2O emissions, respectively (Table 4). The model predictability was high for CO_2 ($Q^2Y = 0.66$), but weak for N_2O ($Q^2Y = 0.34$). Moreover, PLS models identified few variables as key predictors of GHG emissions (VIF < 2, VIP > 0.8), yet these variables differed between CO_2 and N_2O emissions (Table 4). Soil temperature (PLS coefficient [coef] = +0.60), and soil water content (coef =

-0.24) explained most of the variation in CO_2 emissions (Table 4, Fig. S2a). Conversely, variations in N_2O emissions were primarily related to changes in denitrification rates (coef = +0.45), soil water content (coef = +0.21) and, to less extent, groundwater level (coef = -0.16) (Table 4, Fig. S2b).

4 Discussion

This study emphasized the role of soil water content as a main driver of riparian biogeochemistry and GHG emissions. By analyzing soil microbial processes and GHG emissions over a year in a Mediterranean riparian forest, we clearly demonstrate that soil water content has a major role in driving soil microbial processes, the spatio-temporal patters of CO_2 and N_2O emissions and the overall role of Mediterranean riparian soils in the global C and N cycles.

4.1 Microbial processes regulating GHG emissions

Mean daily emissions of CO_2 found in the present study (1.2 – 10 g C m^{-2} d^{-1}) were generally high, especially during spring and summer months. These soil CO_2 emissions were higher than those reported for temperate riparian regions (0.2 – 4.8 g C m^{-2} d^{-1}; Batson et al., 2015; Bond-Lamberty and Thomson, 2010; Mander et al., 2008), although similar values have been reported in some dry forested wetlands of Europe and North America (Harms and Grimm, 2008; Oertel et al., 2016). These substantially high CO_2 emissions observed in Font del Regàs may be attributed to high microbial respiration rates associated with relatively moist and organic matter enriched soils (Mitsch and Gosselink, 2007; Pacific et al., 2008; Stern, 2006). In agreement, previous studies have reported that microbial heterotrophic respiration can be an important contributor (> 60%) to CO_2 soil effluxes in water-limited riparian zones (Harms and Grimm, 2012; McLain and Martens, 2006). However, the absence of a relationship between soil N processes and CO_2 emissions suggests that soil C and N cycles are decoupled in Mediterranean riparian forests, and thus, soil N mineralization may be not a good descriptor of bulk organic matter mineralization. Moreover, plant roots respiration and methane oxidation can increase the CO_2 emissions in riparian soils with deep groundwater tables such as in Font del Regàs (Chang et al., 2014).

Conversely, N_2O emissions of our riparian site (0.001 – 0.2 mg N m^{-2} d^{-1}) were relatively low during the whole year. Similar N_2O emissions were reported in other water-limited riparian forests that are rarely flooded (-0.9 – 0.39 mg m^{-2} d^{-1}; Bernal et al., 2003; Harms and Grimm, 2012; Vidon et al., 2016), yet these values were, on average, much lower than those found in temperate riparian regions (0 – 54 mg N m^{-2} d^{-1}; Burgin and Groffman, 2012; Hefting et al., 2003; Mander et al., 2008). In Font del Regàs, most N_2O was produced by denitrification, as we found an intimate link between this microbial process and N_2O emissions. Additionally, other processes such as nitrification or nitrate ammonification can contribute to N_2O emissions (Baggs, 2008; Hefting et al., 2003). However, it seems unlikely that nitrification could account for the observed N_2O emissions because no relationship was found between net nitrification rates and N_2O emissions. Likewise relatively oxic conditions (Eh > 100) and low C:N ratios (C:N < 20) in Font del Regàs suggest low nitrate ammonification in riparian soils (Schmidt et al., 2011). Currently, the influence of soil denitrification on N_2O emissions in riparian zones is still under debate (Giles et al., 2012). Nonetheless, our results suggest that performing simultaneous measurements of different soil N can contribute to disentangling the mechanisms underlying net N_2O emissions in riparian areas.

4.2 Effects of soil water content on soil CO_2 effluxes

As expected, we found higher soil CO_2 effluxes at the intermediate and hillslope zones than at the near-stream zone. This spatial pattern was negative and strongly related to soil water content (Table 4), suggesting that, as soils become less moist and more aerated, oxidizing aerobic respiration increases, ultimately stimulating CO_2 production in the top soil layer (Muller et al., 2015). In agreement, other aerobic processes, such as N mineralization were also higher in the intermediate and hillslope zones. Moreover, deep groundwater tables in the hillslope zone can increase the volume of aerated soil, which can increase the area-specific soil CO_2 emissions near the hillslope edge (Chang et al., 2014). Increasing CO_2 emissions from wet to dry zones has been reported in other wetlands and riparian forests (Batson et al., 2015; Morse et al., 2012; Welti et al., 2012), pinpointing a close linkage between riparian hydrology and spatial variations in microbial respiration rates..

Nonetheless, the intra-annual variations of soil CO_2 emissions were strongly dependent on soil temperature (Table 4). Probably, cold temperatures (< 4ºC) limited soil respiration during winter, while warmer conditions (> 15ºC) stimulated this process in June and August (Emmett et al., 2004; Suseela et al., 2012; Teiter and Mander, 2005). However, lower CO_2 emissions than expected for temperature dynamics were reported in summer at the intermediate and hillslope zones, likely because extreme soil dryness (soil water content < 20%) limited respiration rates during such period (Chang et al., 2014; Goulden et al., 2004; Wickland et al., 2010). Although the mechanisms by which soil dryness may affect microbial C demand are still poorly understood, suppressed microbial respiration in summer can be attributed to a disconnection between microbes and resources (Belnap et al., 2005; Davidson et al., 2006), decreases in photosynthetic and exo-enzimatic activities (Stark and Firestone, 1995; Williams et al., 2000), or a relocation of the invested energy on growth (Allison et al., 2010). Altogether, these results suggest that soil water content may be as important as soil temperature to understand soil CO_2 effluxes, and therefore, future warmer conditions may not fuel higher CO_2 emissions, at least in those regions experiencing severe water limitation.

4.3 Effects of soil water content on soil N_2O effluxes

As occurred for CO_2 emissions, N_2O fluxes showed a clear spatial pattern associated with changes in soil water content across the riparian zone. In the near-stream zone, relatively wet conditions (SWC = 30 – 40%) likely promoted denitrification rates, while dry soils (SWC = 10 – 25%) could limit both nitrification and denitrification in the intermediate and hillslope zones (Linn and Doran, 1984; Pinay et al., 2007). Such spatial pattern differed from those found in non-water limited riparian forests, where higher N_2O emissions occurred in the hillslope edge as a result of high resource supply (DeSimone et al., 2010; Dhondt et al., 2004; Hedin et al., 1998). These results suggest that riparian hydrology is the primary mechanisms controlling denitrification but, once water is unlimited, substrate availability controls the magnitude of denitrification rates. This former idea is supported by our potential denitrification results, which showed that, after adding water, denitrification rates were similar to those observed in the field for the near-stream zone, but increase by 3-4 fold in the other two zones. Moreover, N_2:N_2O ratios estimated from acetylene method suggest that there was a spatial pattern in denitrification efficiency as well. During the study period, N_2:N_2O ratios were always higher at the near-stream (21.50 ± 40.32) than at the intermediate and hillslope zones (5.90 ± 16.02 and 4.23 ± 8.31, respectively), yet all values were much lower than those reported for temperate riparian forests (184 – 844; Mander et al., 2014). All together, these results support the idea that saturated soils favored the complete denitrification process to N_2 and can potentially emit less N_2O compared to less saturated soils (Giles et al., 2012).

Intra-annual variation in N_2O emission was also related to riparian hydrology because high rates of N_2O effluxes occurred in April, when large precipitation events (400 mm) raised the groundwater level and increased soil water content at the whole riparian plot. Such pulses of N_2O emissions short-after rewetting events can reflect the microbial use of the NO_3^- that has been accumulated during dry antecedent periods (Chang et al., 2014; Hefting et al., 2004; Pinay et al., 2007). In agreement, the PLS model showed a negative relation between soil water content and NO_3^- concentrations. Moreover, our results further suggest that rewetting events promote a fast N cycle because all microbial N processes were maxima in April. Nevertheless, we also expected a fast N cycle as well as large N_2O emissions following rains in November because, similarly to spring, environmental conditions (i.e. high soil water content and increments in soil NO_3^- concentrations during the antecedent dry summer) should enhance microbial activity. Likely, low rates of N transformations during fall may be attributed to an increase in microbial N demand following large C inputs from litterfall (Guckland et al., 2010). Moreover, leaf litter from *R. pseudoacacia*, the main tree species in our study site, holds a high lignin content (Castro-Díez et al., 2009; Yavitt et al., 1997), which might enrich the riparian soil with phenolic compounds and ultimately limit the use of N by microbes (Bardon et al., 2014). These results suggest that the response of N cycling to changes in water availability is more complex and less predictable than C cycling, likely because N processes depend on the interplay of additional ecosystem factors not included in this study.

4.4 Riparian soils as hot spots of GHG effluxes

There are several studies that attempt to upscale riparian GHG emissions at catchment scale, yet there are still fundamental uncertainties regarding the magnitude and sources of GHG emissions (Hagedorn, 2010; Pinay et al., 2015; Vidon and Hill, 2006). When accounting for all GHG (CO_2 + N_2O), our study suggest that our riparian soils can emit between 438 – 3650 g C m^{-2} yr^{-1}. Assuming that GHG emissions (CO_2 + N_2O) from upland evergreen oak and beech soils (54% and 38% of the catchment, respectively) are similar to other Mediterranean regions (oak: 19 – 1240 g C m^{-2} yr^{-1}; Asensio et al., 2007; Barba et al., 2016; Inclán et al., 2014); beech: 214 – 1182 g C m^{-2} yr^{-1}; Guidolotti et al., 2013; Kesik et al., 2005), then riparian soils (6% of the catchment area) can contribute between 16 – 22% to the total catchment soil GHG emissions. Although these estimates are rough (i.e. we assumed that riparian soils emit the same rate of GHG that our study site), our results clearly pinpoint that riparian soils can be potential hot spots of GHG emissions within Mediterranean catchments. These findings contrast with the common knowledge that water limited soils are powerless GHG sources to the atmosphere (Bernal et al., 2007; Vidon et al., 2016) and stress the importance of simultaneously consider several GHG emissions (i.e. CO_2, N_2O, CH_4) to get a whole picture of the role of riparian soils in climate change.

5 Conclusions

Mediterranean riparian zones are dynamic systems that undergo spatial and temporal shifts in biogeochemical processes due to changes in both soil water content and substrate availability. In a first attempt to simultaneously quantify CO_2 and N_2O emissions from Mediterranean riparian soils, we showed that most of GHG emissions occur in form of CO_2, even in the wet soils located near the stream. In addition, our results clearly illustrate a strong linkage between riparian hydrology and the microbial processes that produce GHG. Deep groundwater tables fueled large respiration rates in the relatively dry soils near the hillslope, while denitrification mostly occurred in the wet zones located near the stream channel. As occurred at spatial scale, riparian soil water content was a primarily control of the temporal patterns of CO_2 and N_2O emissions. Soil dryness diminished respiration rates during summer, while a fast soil N cycling promoted high N_2O emissions after a rewetting event in spring. Overall, our study highlights that future variations in catchment hydrology due to climate change can potentially affect the riparian functionality in Mediterranean zones, as well as their contribution to regional and global C and N cycles.

Author contributions

Sílvia Poblador, Santiago Sabaté, and Francesc Sabater designed the experiment. Sílvia Poblador and Anna Lupon carried them out. Sílvia Poblador performed all laboratory analysis. Anna Lupon and Sílvia Poblador analyzed the data set and prepared the manuscript, with contributions from Santiago Sabaté and Francesc Sabater.

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Tables

Table 1. Mean annual values (± standard deviation) of soil water content (volumetric), soil temperature, soil pH, soil redox capacity (Eh), soil organic matter, soil molar C:N ratio, soil carbon (C) and nitrogen (N) content, and soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations for the three riparian zones. For each variable, different letters indicate statistical significant differences between riparian zones (*post-hoc* Tukey HSD test, $p < 0.05$).

	Near-stream	*Intermediate*	*Hillslope*
Soil water content (%)	29.58 ± 7.55 ^{A}	19.36 ± 6.00 ^{B}	19.81 ± 6.24 ^{B}
Temperature (ºC)	11.37 ± 5.39 ^{A}	11.82 ± 5.90 ^{A}	12.01 ± 6.34 ^{A}
Eh	170 ± 111 ^{A}	184 ± 103 ^{B}	184 ± 95 ^{C}
pH	6.66 ± 0.42 ^{A}	6.31 ± 0.50 ^{A}	6.68 ± 0.53 ^{A}
Organic matter (%)	4.41 ± 0.71 ^{A}	7.98 ± 2.88 ^{B}	9.53 ± 1.99 ^{C}
C:N ratio	14.25 ± 3.64 ^{A}	14.09 ± 1.78 ^{A}	13.63 ± 1.18 ^{A}
C (mg kg^{-1})	2004 ± 1038 ^{A}	4007 ± 1785 ^{B}	4923 ± 1428 ^{B}
N (mg kg^{-1})	160 ± 44 ^{A}	330 ± 135 ^{B}	418 ± 107 ^{C}
NH_4^+ (mg N kg^{-1})	1.88 ± 1.21 ^{A}	5.58 ± 3.48 ^{B}	3.90 ± 2.07 ^{B}
NO_3^- (mg N kg^{-1})	0.75 ± 0.58 ^{A}	4.66 ± 4.25 ^{B}	5.30 ± 4.20 ^{B}

Table 2. Results from the mixed-model analysis of variance (ANOVA) showing the effects of riparian zones and seasons on soil water content, soil temperature, soil pH, soil redox capacity (Eh), soil organic matter, soil molar C:N ratio, soil carbon (C) and nitrogen (N) content, and soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations. Plot was treated as a random effect in the model whereas riparian zones, seasons and their interactions were considered fixed effects. Values are *F*-values and the p-values are shown in brackets. P-values < 0.05 are shown in bold.

	Riparian Zone	*Seasons*	*Zone × Seasons*
Soil water content	**18.6 [< 0.001]**	**100 [< 0.001]**	**13.6 [< 0.001]**
Temperature	0.33 [0.721]	**2117 [< 0.001]**	0.42 [0.906]
pH	1.97 [0.182]	2.43 [0.060]	2.73 [0.052]
Eh	1.34 [0.247]	3.53 [0.062]	1.88 [0.084]
Organic matter	**27.8 [< 0.001]**	2.77 [0.053]	1.62 [0.144]
C:N ratio	0.99 [0.400]	**10.9 [< 0.001]**	1.72 [1.118]
C	**27.1 [< 0.001]**	1.86 [0.132]	0.77 [0.630]
N	**39.7 [< 0.001]**	1.22 [0.311]	0.63 [0.746]
NH_4^+	**12.4 [0.001]**	2.71 [0.051]	1.52 [0.176]
NO_3^-	**22.4 [< 0.001]**	**5.63 [< 0.001]**	**4.09 [< 0.001]**

Zone = near-stream, intermediate, hillslope.
Season = February, April, June, August and November.

Table 3. Mean values (± standard deviation) of potential denitrification rates (in mg N kg^{-1} d^{-1}) after anoxia (DEA_{MQ}), carbon addition (DEA_{C}), nitrogen addition (DEA_{NO3}) and carbon and nitrogen addition (DEA_{C+NO3}) treatments for the three riparian zones during the study period. For each zone, different letters indicate statistical significant differences between treatments (*post-hoc* Tukey HSD test, n = 15, $p < 0.01$).

Potential Denitrification Rates (mg N kg^{-1} d^{-1})				
	DEA_{MQ}	**DEA_{C}**	**DEA_{NO3}**	**DEA_{C+NO3}**
Near-stream	0.31 ± 0.41^{A}	0.26 ± 0.27^{A}	0.42 ± 0.42^{A}	0.63 ± 0.85^{A}
Intermediate	1.01 ± 1.12^{A}	1.88 ± 1.59^{A}	2.28 ± 3.57^{A}	2.40 ± 2.45^{A}
Hillslope	1.34 ± 1.33^{A}	2.35 ± 1.97^{AB}	1.73 ± 1.43^{AB}	3.82 ± 2.78^{B}

Table 4. Summary of the partial least squares (PLS) models produced for CO_2 and N_2O emissions at the riparian site (n = 75). Values are the coefficients from PLS models which describe the relationship (direction and relative strength) between explanatory variables and gas emissions. The variance inflation factor (VIF) of each explanatory variable, indicative of collinearity, are shown in brackets. Bold values indicate the most influencing variables (variable importance in the projection (VIP) >1.0).

	X-variable	*Acronym*	*CO_2*	*N_2O*
Soil Properties	Soil water content (%)	SWC	**-0.235 [1.72]**	**0.205 [1.32]**
	Groundwater level (cm b.s.s.)	GWL	---	-0.157 [1.24]
	Temperature (C)	Tsoil	**0.599 [1.45]**	---
	pH	pH	---	---
	Redox potential (mV)	Eh	---	---
	Bulk density (g cm^{-1})	BD	---	---
	Coarse texture (%)	% Sand	---	---
	Organic matter (%)	SOM	---	---
	Total Carbon	C	---	---
	Total Nitrogen	N	---	---
	Molar C:N ratio	C:N ratio	---	---
	Ammonium	NH_4^+	0.167 [1.61]	---
	Nitrate	NO_3^-	0.066 [1.80]	-0.060 [1.47]
Soil N processes	Net N Mineralization	NNM	---	---
	Net Nitrification	NN	---	---
	Denitrification	DNT	---	**0.449 [1.09]**
R^2Y			**0.71**	**0.40**
Q^2Y			**0.66**	**0.34**

Acronym is used in Figure S2 for PLS loading plots.

Figures

Figure 1. (a) Plot layout for the studied Mediterranean riparian forest showing the three riparian zones and the location of the chambers (n=5 for each riparian zone) (b) Conceptual approach of the influence of riparian hydrology on soil microbial processes across a Mediterranean riparian zone. Soil water content decreases from the near-stream to the hillslope zones due to changes in groundwater table, increasing unsaturated soil column and oxic conditions. Anaerobic processes (denitrification) occur under anoxic conditions while aerobic processes (respiration) are optimized under a moderate range of soil water content.

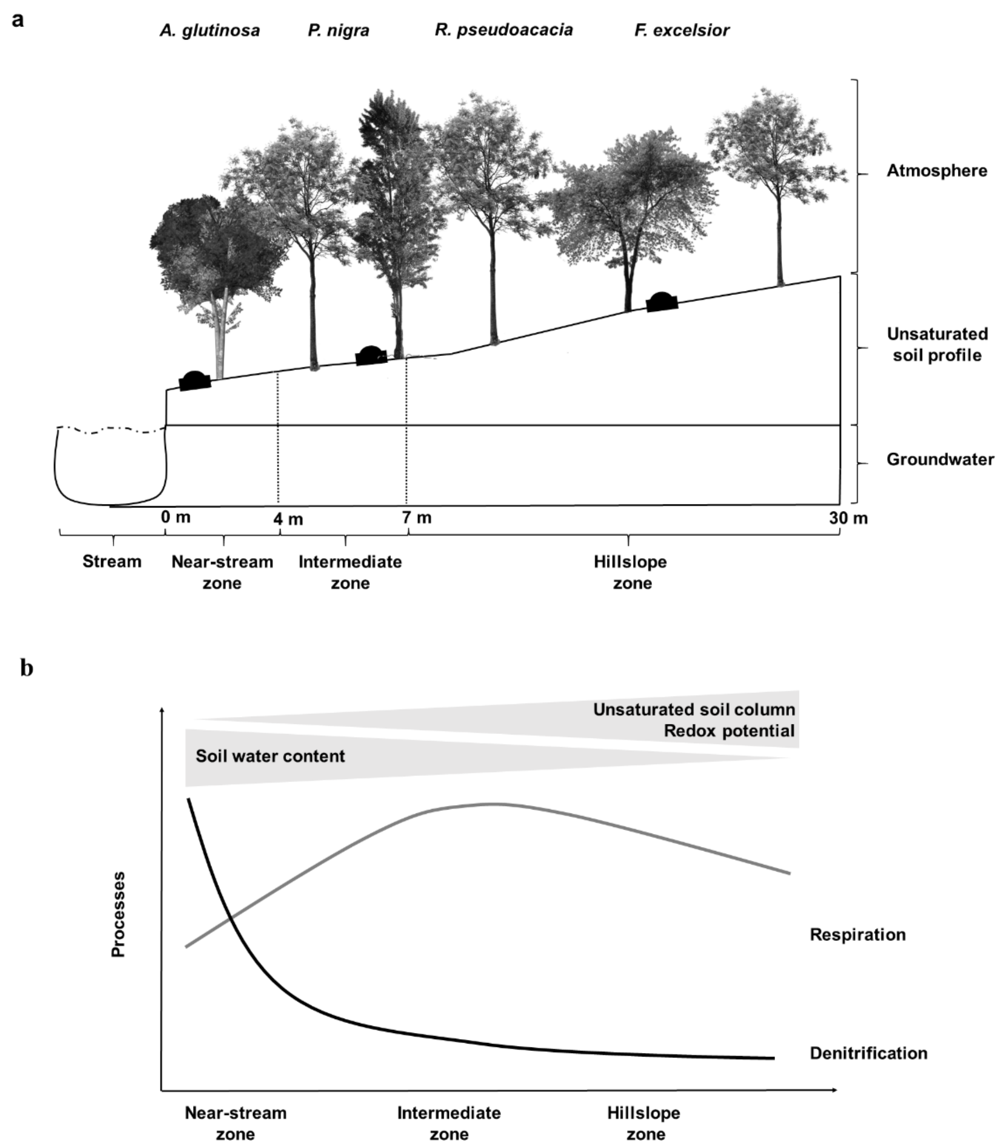

Figure 2. Temporal pattern of (a) mean monthly precipitation and (b) biweekly groundwater level at the studied riparian site during the year 2013. Circles are mean values of groundwater level at the near-stream (white), intermediate (grey), and hillslope (black) zones. Precipitation data was obtained from a meteorological station located at ca. 300 m from the studied riparian site. At each riparian zone, groundwater level was measured in 3 PVC piezometers (32-mm diameter, 1–3 m long) with a water level sensor (Eijkelkamp 11.03.30).

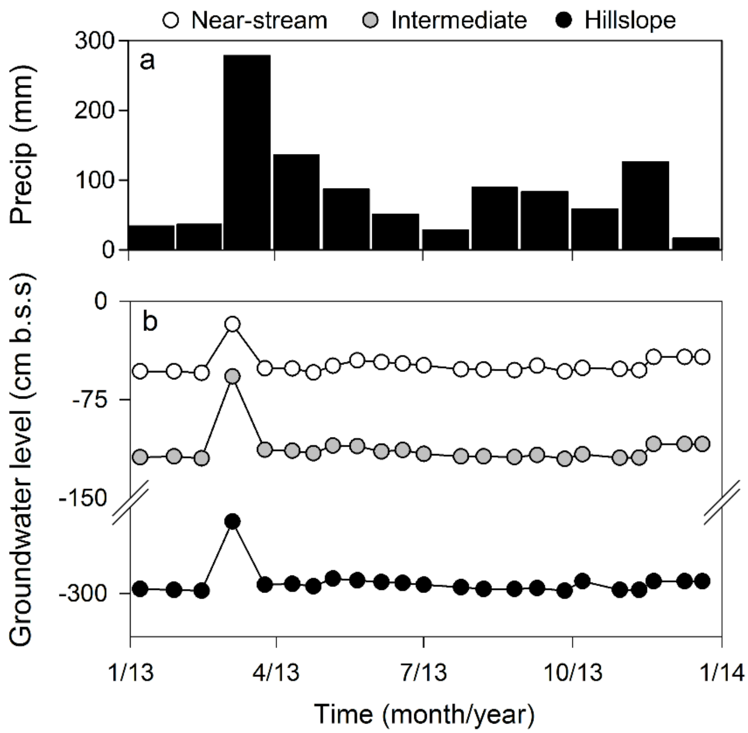

Figure 3. Temporal pattern of (a) soil water content, (b) soil temperature, (c) soil C:N molar ratio, and (d) soil nitrate concentration at 10-cm depth. Data is shown for the near-stream (white), intermediate (grey), and hillslope (black) zones during the study period. Circles are mean values and error bars are standard deviations.

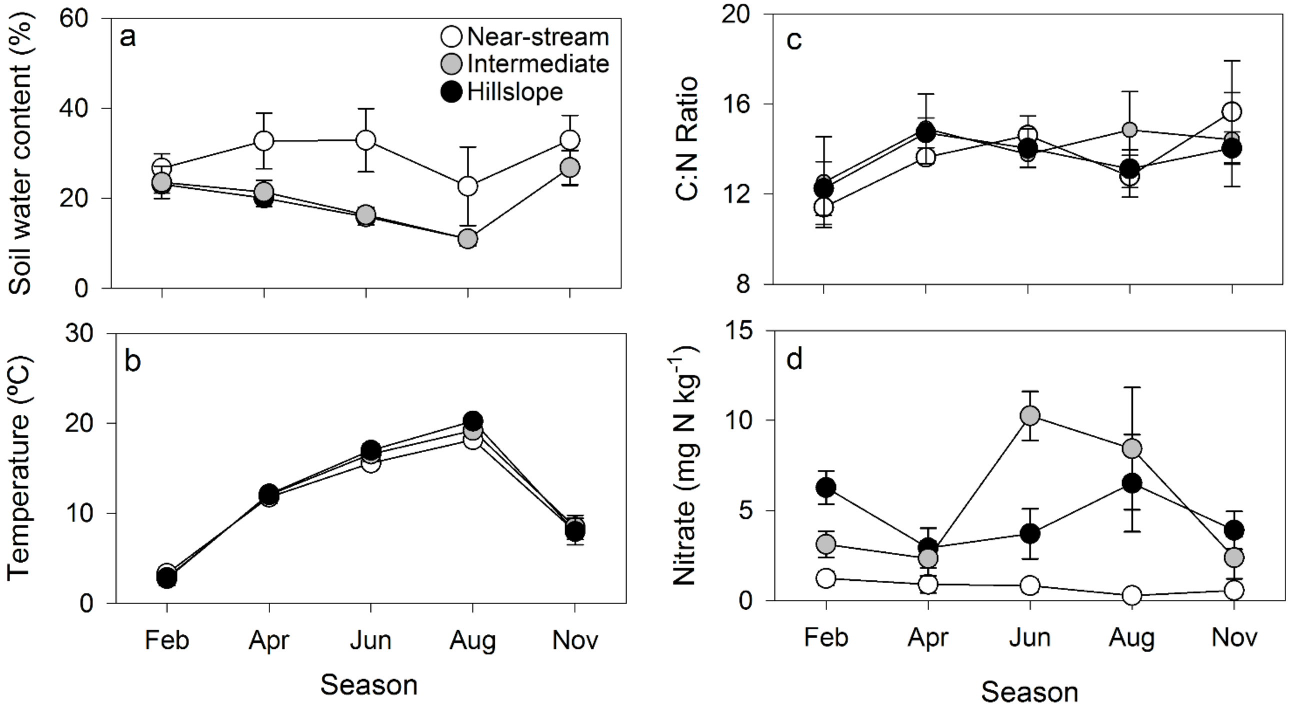

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Figure 4. Temporal pattern of (a) soil net N mineralization, (b) net nitrification and (c) denitrification rates at the near-stream (white), intermediate (grey), and hillslope (black) zones during the study period. Bars are mean values for each section and error bars are standard errors. For each season, different letters indicate significant differences among sections (mixed-model ANOVA, $p < 0.05$).

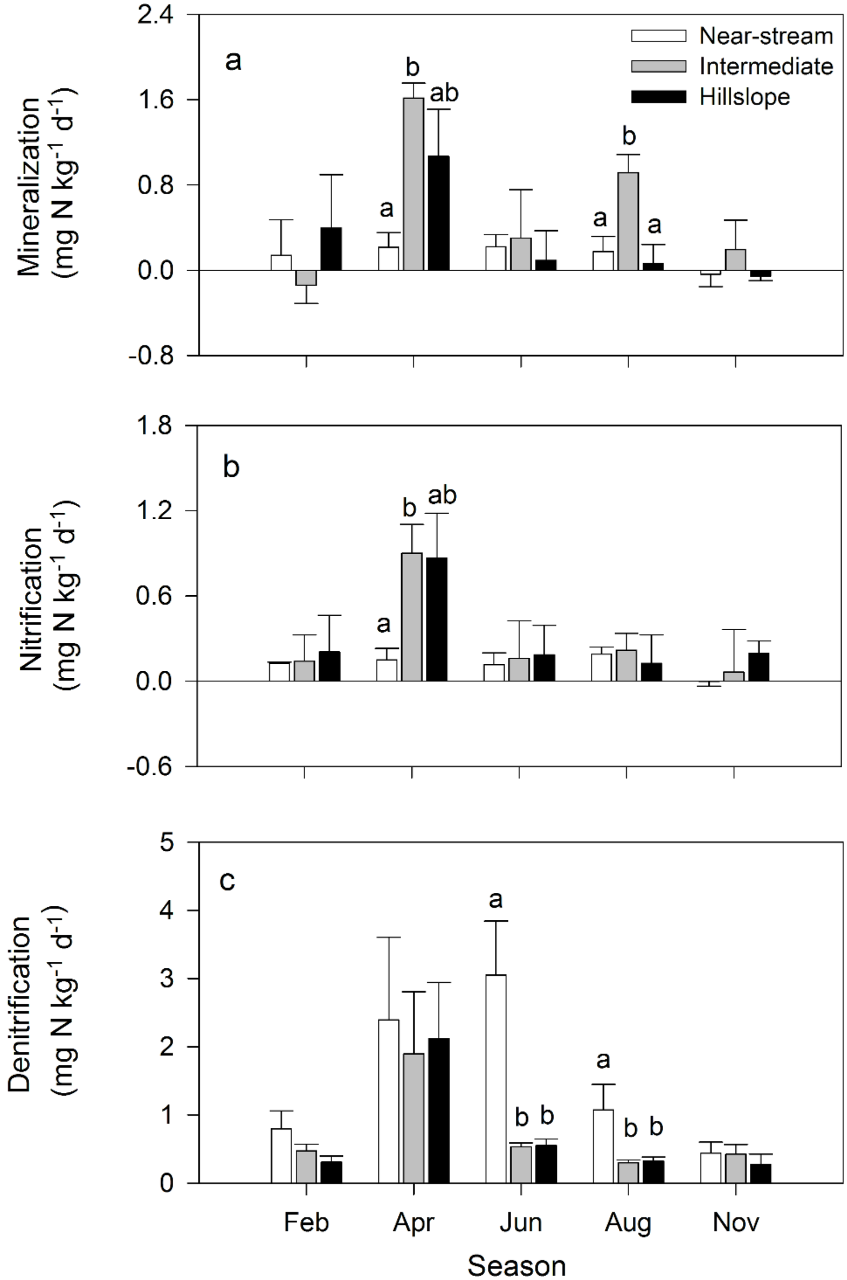

Figure 5. Temporal pattern of soil (a) CO_2 and (b) N_2O emissions at the near-stream (white), intermediate (grey), and hillslope (black) zones during the study period. Bars are mean values for each section and error bars are standard errors. For each season, different letters indicate significant differences among sections (mixed-model ANOVA, $p < 0.05$).