

# ***Interactive comment on “Dissolved organic carbon lability and stable isotope shifts during microbial decomposition in a tropical river system” by N. Geeraert et al.***

**N. Geeraert et al.**

naomi.geeraert@ees.kuleuven.be

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We appreciate the feedback provided by A. Nordström to improve the understanding and readability of the manuscript. Replies on the comments can be found below, following each time the original comment. General comments

COMMENT: After reading the article I find that the conclusion concerning the significance of microbial degradation in removal of DOC drawn by the authors is supported by their data. However, I find the deduction of POC as a non-significant source of DOC in river water questionable. The authors measured initial fast degradation rates of DOC, and in unfiltered and filtered samples the observed concentration changes were

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relatively comparable with some differences. However, the concentration representing what the authors refer to as “recalcitrant DOC” is in most cases reached at (before) the second sampling point in the incubation series for both filtered and unfiltered samples. Thus, a possibility could be that any labile DOC released by POC could have been degraded before the second sampling of the incubation series. This would imply a too coarse temporal sampling resolution.

REPLY: We are aware that our sampling resolution might be too coarse to capture the full effect of the presence of the POC. We had mentioned this possibility in the discussion of the sources of DOC (P12772 I. 3-4) and made it even more explicit in the revised manuscript. The section where the results of the two methods are compared, also states more explicitly that we can only limit our conclusions to the time resolution of the sampling, i.e. 48 hours.

COMMENT: Overall, I think that the text have to be developed in order to clarify and strengthen arguments of the article. The authors consistently use relative descriptions when describing observed differences in their experiments (e.g. “slightly more depleted”, “slightly enhanced”, “relatively minor”, etc.). I would suggest that these descriptions is reworked and replaced with numerical measurements. For example (P12768, line 10-12), instead of writing “slightly more depleted” and later report the average difference (0.3 ‰. I would suggest the single use of the latter.

REPLY: We agree with this comment and have paid attention to render these descriptions more uniform and clear throughout the manuscript.

## Specific comments

COMMENT: P12762, line 20-23, I suggest a reformulation as “Indeed, only 0.9 PgCyr<sup>-1</sup> of the global estimates 1.9 PgCyr<sup>-1</sup> (Cole et al., 2007; Regnier et al. 2013) to 2.7 PgCyr<sup>-1</sup> (Battin et al., 2009) is delivered to the ocean (Cole et al., 2007; Battin et al., 2009; Regnier et al., 2013).”

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REPLY: We accepted the proposed formulation.

COMMENT: P12763, line 16-18; the enrichment of 13C of the remaining DOC pool would only occur if the proportion of lignin in the DOC pool decreases, in which case the lignin must be decomposed at a higher rate/preferentially compared to the remaining constituents of the DOC pool (and not simply due to the decomposition of lignin). This is partly inferred from P12763, line 14-15, but should be remarked.

REPLY: We reformulated the sentence to state more explicitly that the lignin needs to have a higher decomposition rate compared to the bulk DOC.

COMMENT: P12763, line 28, reformulate “broke down” (e.g. degraded)

REPLY: We accepted the proposed change.

COMMENT: P12764, line 3, “However” does not fit into the context. I suggest that the authors remove however and introduce a line break.

REPLY: We accepted the proposed change.

COMMENT: P12764, line 7, reformulate “.., while it is only, ..” as “while only”

REPLY: We accepted the proposed change.

COMMENT: P12764, line 8, introduce a line break.

REPLY: We accepted the proposed change.

COMMENT: P12764, line 28; what was time between each of the three campaigns? How were they distributed during the wet seasons in May-June (2013) and in April-May (2014)? From table 1 I see that there is approximately 2 weeks between each sampling date, this should be clarified in the text.

REPLY: We added the years in which the campaigns took place. In the Materials and Methods section, we added that the samples were regularly spaced throughout the campaigns as a new incubation series was started once the previous one was finished.

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COMMENT: P12765, line 21-25, long sentence; line 25, reformulate “. . .  $\delta^{13}\text{C}$  of respectively -27 and -12‰” as “ $\delta^{13}\text{C}$  of -27 and -12‰ respectively”

REPLY: We accepted the proposed change.

COMMENT: P12766, line 2-4, reformulate.

REPLY: We reformulated the sentence.

COMMENT: P12766, line 5-8, I don't find it relevant to mention maximum discharge as what the authors are implying with this is that there was flooding in 2013 (?). Maybe this can be brought up later in the text when sources of DOC are discussed (P12772, line 4-9). How were the sampling campaigns distributed in time in relation to the flooding?

REPLY: The references to the discharge were replaced by a more descriptive formulation of the hydrological conditions during the sampling. We also added that the samples were taken at a regular interval throughout the campaign.

COMMENT: P12766, line 14-16, I don't know how well  $\text{H}_3\text{PO}_4$  works as a preservative, but analysis within 4 months of sampling seems quite long. What temperature was the samples stored in?

REPLY: The most important step in the preservation of the DOC is the filtration to remove the DOC-consuming bacteria and storing them in the dark to avoid photochemical reactions. Upon return from the field, the samples were also stored in the fridge. The latter has been added to the manuscript. However, tests within our research group in other river system have revealed that the storage of the samples for several months under field conditions did not affect the results of the DOC measurements. The addition of  $\text{H}_3\text{PO}_4$  creates a low pH unsuitable for microbial growth and not does interfere with later analyses since  $\text{H}_3\text{PO}_4$  is also added during the analytical measurement.

COMMENT: P12766, line 26-27; were the incubation bottles stirred during the experiment?

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REPLY: Although we had tried to find a possibility to create turbulence in the bottles, especially those with POC, by placing them in the river flow, this didn't create the expected stirring effect. As this attempt to keep the water in motion was logistically not straightforward and the effect was limited, we decided to keep the bottles in water-filled coolboxes. However, if we would have the chance to further investigate this topic in a more systematic manner, we would certainly include stirring of the bottles.

COMMENT: P12767, line 25; How did the authors calculate the relative error? Why did the authors choose 50% relative error as a "reason to exclude"? Looking at the supplementary data, I think that more could be said concerning why the slow mineralization rates were measured. The initial DOC concentrations are close to what the authors describe as "recalcitrant DOC", wherefore the degradable DOC would have been minimum in those samples and therefore a slow mineralization rate is calculated.

REPLY: The error was on the concentration measurements was estimated at 3% or less, based on the replicates of the standards. The absolute error on each measurement can then be calculated as the concentration times 0.03. The error of a subtraction ( $z=x-y$ ) is then calculated as:  $e(z)=\text{SQRT}[e(x)^2+e(y)^2]$ , which can then be expressed as a percentage of the result of the subtraction.

The calculation of the isotope signature resulted in unrealistic values for 6 series (and another was excluded because of missing values), and those had a relative error above 50%. This is indeed due to the very low mineralization rates. As we added the mineralization rates to Table 1, we now use a mineralization rate below 0.01 mg L<sup>-1</sup> day<sup>-1</sup> as criterium. This will indeed be more straightforward for the readers.

COMMENT: P12768, line 1, how many series in total were retained?

REPLY: At this position in the text, it were 19 series, which we indicated by adding "(n=19)". Throughout the manuscript, we added information about the number of series that are described.

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COMMENT: P12768, line 4, some kind of introduction to the results and discussion section must be given. This is partly due to the first line in section 3.1 (P12768, line 6) where the authors make an immediate distinction between (1) incubation series with relatively limited decomposition of DOC, and (presumably, 2) incubation series with relatively high decomposition of DOC referred to as “all other cases”. What is a relatively limited decomposition of DOC? Which are the all other cases? This should be clarified

REPLY: We added an introductory sentence outlining the reason why we compare the two different treatments. We also rephrased the section to give more exact values instead of relative qualifiers and to explain some concepts more explicit.

COMMENT: P12768, line 8, insert “, there” (“In all other cases, there was a significant. . .”)

REPLY: We accepted the proposed change.

COMMENT: P12768, line 9-10, reformulate, e.g. “. . . the final concentration of DOC was systematically 10% higher in the samples without POC”

REPLY: We accepted the proposed change.

COMMENT: P12768, line 14-15, refer to the table/figure where the reader can find the mineralization rates.

REPLY: The mineralization rates were not explicitly reported, but we added them to Table 1 and also refer to the table whenever the rates are discussed.

COMMENT: P12768, line 17, what is meant by “relatively minor”?

REPLY: It means that you wouldn't be able to identify the series with and without POC if you see a graph with only one of them. This has been reformulated as: “there is a similar range in mineralization rates at our sampling resolution”.

COMMENT: P12768, section 3.1; The authors investigated POC as a potential source

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of DOC in the river, and found that there was a “. . . significant difference between the filtered and unfiltered incubation series, whereby the final concentration of DOC was systematically higher in the samples without POC by ca. 10%.” (P12768, line 8-10). Later on, the authors state that “. . .” the unfiltered incubation series is treated as equivalent to the filtered ones for the remainder of the discussion” (P12768, line 18-19) due to a “relatively minor” enhancement of mineralization rates in POC samples (P12768, line 17). I find the use of words contradictory.

REPLY: If the series are compared pair-wise, there is a statistical difference which is significant. However, the filtered and unfiltered series can't be differentiated when not comparing data as pairs, because the range and temporal trend is similar. Therefore, we included the unfiltered series in the rest of the analysis.

COMMENT: P12769, line 5-6 reformulate “This limited decrease in concentration can be related to the low initial concentration which was for all those samples below 2 mg L<sup>-1</sup>. . .” as “This limited decrease in concentration can be related to the low initial concentration (<2mg L<sup>-1</sup>) . . .”

REPLY: We accepted the proposed change.

COMMENT: P12769, line 7-8, in which series was mineralization observable? (insert reference)

REPLY: Those were all the series except for the 6 which had been discussed . We added the reference to the table where the values of the final concentration can be found.

COMMENT: P12769, line 14-16, the calculated rates of decay of DOC should be “per day” (day<sup>-1</sup>)

REPLY: The superscript (-1) has been added to the units.

COMMENT: P12769, line 16-20, when comparing the results to the results from Moody et al. 2013, I think it is better to compare absolute instead of relative (percentage)

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concentration changes. This will be more interesting, and will in part justify the authors claim that  $\text{DOC} < 2 \text{ mg L}^{-1}$  is recalcitrant (if Moody et al. 2013 have similar values).

REPLY: We added a reference to the experiments of Amon and Benner (1996) who had similar concentrations and mineralization rates as our experiments, although the strong decrease was not observed during the first days. We added the absolute values of Moody et al. (2013), but still gave most attention to the similar temporal pattern as our results.

COMMENT: P12770, line 10. What is meant by a stronger reduction? Is it enhanced decay rates or greater absolute degradation? Reformulate “stronger”. (Same at line 14)

REPLY: This formulation was indeed confusing. We rephrased this as: “a large relative decrease” and “one series with a large percentage loss in DOC”.

COMMENT: P12770, line 21, reformulate “. . . of -21.2, -23.1, and -24.3‰ for the mineralized, initial and remaining carbon pools for all the observations.” as “. . . of -21.2, -23.1, and - 24.3‰ respectively.”

REPLY: We accepted the proposed change.

## References

Amon, R. and Benner, R.: Photochemical and microbial consumption of dissolved organic carbon and dissolved oxygen in the Amazon River system, *Geochim. Cosmochim. Ac.*, 60, 1783–1792, 1996.

Moody, C. S., Worrall, F., Evans, C. D., and Jones, T. G.: The rate of loss of dissolved organic carbon (DOC) through a catchment, *J. Hydrol.*, 492, 139–150, 2013.

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