AUTHOR REPLY

Dissolved organic carbon lability and stable isotope shifts during microbial decomposition in a tropical river system

N. Geeraert[1], F. O. Omengo[1,2] , G. Govers[1] and S. Bouillon[1]

[1]KU Leuven, Department of Earth and Environmental Sciences, Leuven, Belgium

[2]Kenya Wildlife Service, Nairobi, Kenya

Point-by-point reply on Reviewer #1

REF: The topic of this study fits well within the scope of BG and should be of interest for a broad range of readers. Its main strengths are the stable isotope approach used to assess the dynamics of biodegradation of riverine DOC, and the fact that the study was carried out on a river with mixed C3 and C4 sources of DOC. The data appears of excellent quality (S. Bouillon is a recognised specialist in the measurement of 13C signature of DOC), but it would be more convincing if the standard deviations of the reported averages would be available in addition to the ranges of values (see specific comments below).

REPLY: We have added the standard deviation along with the average values and the range.

REF: On a less positive note, the quality of the writing should be improved a lot. I understand that English is not the mother tongue of the authors but in several places the text would gain in clarity if reviewed by someone fluent in English. The manuscript is also very short and reports a very small dataset – although I realize that collecting water samples in Kenya is not a simple task. The discussion and conclusions would also have gained from complementary analyses of the bulk chemical composition of the DOC before and after incubation to differentiate between C3 vs. C4 decomposition and differential biochemical decomposition (optical analysis, as proposed by the authors, or FTIR/NMR analysis on freeze-dried residues). While reporting a change in the d13C signature of DOC upon bacterial degradation is novel (most studies assume that biodegradation does not lead to such changes), understanding the reason why the signatures change would have been even more enlightening. I feel that an improved version of this manuscript would be worthy of publication in BG mostly because it would report for the first time (to the best of my knowledge) changes in δ13C stable isotope signature upon microbial degradation of DOC originating from mixed C3 and C4 sources. I feel however that more DOC characterization work would have resulted a much stronger paper.

REPLY: These experiments were carried out as a side-project within a larger project to test whether it could explain some of the observations made during the first field campaign. As it was indeed very exploratory, we didn't prepare samples for complementary analyses. However, as a shift in stable C isotope ratios during bacterial degradation was not yet reported before, we found the results sufficiently exciting and novel to share them with the scientific community and to stimulate new ideas or research questions to those working with DOC mineralization.

Specific Comments

REF: 1. Page 1, lines 21-24: The concluding sentence of the abstract should be reworked; the authors probably mean that the stable isotope signature of total DOC in rivers does not necessarily reflect the relative proportion of C4- and C3-derived DOC in the catchment.

REPLY: This sentence has been reworked to make it more understandable.

REF 2. Page 4, line 2: Decomposition mechanisms were not determined in this work – only speculative hypotheses are provided in the discussion section. Reference to the mechanism should be removed since this is the paragraph that describes the work that was performed.

REPLY: We agree with this comment, and since our data do not allow to make supported statements on the mechanisms involved, we removed the reference to the decomposition mechanisms.

REF 3. Page 5, line 28 to page 6, line 2: More details should be given on the DOC-IRMS setup or a reference to published work should be provided.

REPLY: The operating principle of our TOC analyzer and a reference to the original paper where the setup was described (St-Jean, 2003) have been added to the text.

REF 4. Page 7, lines 1-7: The authors should provide a quantitative result for the differences between incubations with and without POC. What is the percent contribution of the POC bacterial pool to total degradation in each sample?

REPLY: We do not see how we should interpret this question.

REF 5. Page 7 line 10, line 11, line 20, line 21, line 23 and line 24 (and everywhere else in the text): Please provide the standard deviation whenever an average is given – giving a range of values is not sufficient.

REPLY: The standard deviations have been added whenever the average of a value is given.

REF 6. Page 7, lines 18-24: How do these degradation rates compare with literature values? The authors cite several studies reporting such rates in their introduction.

REPLY: Although not many of the studies reported the exact mineralization rates, we could compare our rates with those of Amon and Benner (1996) which also had similar initial DOC concentrations. The experiments of Moody et al. (2013) started and ended with much higher DOC concentrations, but showed a similar trend as our observations: most of the degradation occurred within 2 days. Those two examples are added with quantitative information.

REF 7. Page 8, line 10 and line 14: Please provide the significance level for the statistical test used here.

REPLY: The significance level for the test was added.

REF 8. Page 8, line 22: What statistical test was carried out to decide whether these two values are outliers? Please explain.

REPLY: While it was based on visual interpretation and the strong improvement in R^2, the calculation of Cook's distance indicated that only one of them had a distinctively large impact on the regression equation. However, based on the comments of another reviewer, we have decided to use a robust linear regression which is less affected by the outlier.

REF 9. Page 8, lines 27-27: Again, please provide the standard deviation for these averages. Are the differences between these averages significant?

REPLY: We added the standard deviation of the averages and the test (with significance level) which indeed indicated that the three of them are significantly different.

REF 10. Page 9, lines 4-5: Please provide a reference for the heavier d13C signature of carbohydrates.

REPLY: The reference to Benner et al. (1987) has been added.

REF 11. Page 9, line 24, to page 10, line 10: An alternative reason for the similar reactivity between the upstream and downstream sites could be the photo-activation of a fraction of the non-labile DOC pool (photocleavage of large biochemical into smaller, more bioavailable components. This possibility should be added.

REPLY: We had indeed neglected to mention this option. It has been added in the revised version of our manuscript. In the further discussion of this DOC source, we do argue that this may be unlikely to be an important mechanism due to the limited light penetration depth (high sediment load). Nevertheless, it indeed deserves to be mentioned and explored further.

REF 12. Pages 14-15, Table 1: The column titles should be reformatted.

REPLY: The column headings are well formatted in the online document based on the LateX file.

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.

Benner, R., Fogel, M. L., Sprague, E. K., and Hodson, R. E.: Depletion of 13C in lignin and its implications for stable carbon isotope studies, Nature, 329, 708--710, 1987.

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.

St-Jean, G: Automated quantitative and isotopic (^{13}C) analysis of dissolved inorganic carbon and dissolved organic carbon in continuous-flow using a total organic carbon analyser. Rapid. Commun. Mass Spectrom., 17, 419--428, 2003.

Point-by-point reply on Reviewer #2

Major comments and remarks:

REF: At current, I found part of the results and discussion section ('removal mechanism and origin of DOC') not to be well based on the data provided. This concerns primarily the discussion of the C3/C4 vegetation differences and how these would affect the lability of DOC. I believe that the authors need to provide more evidence here, or to cut back on their interpretation and conclusion. In this context, I wonder if there is at all any data that objectively suggests any type of relationship between stream dDOC, d13C and the C3/C4 landcover data?

REPLY: We launch the hypothesis that the shift in stable isotope ratios we observed during microbial decomposition is related to differences in the contributions of C3 and C4 vegetation, because this shift appears to be observed only in such mixed systems. This can indeed not be unequivocally demonstrated by our limited dataset. Therefore, we have reformulated the abstract by explicitly mentioning it as a hypothesis. In the Conclusions section, we emphasized that there was a different decomposition rate between DOC with heavy and light isotopes, without stating that the difference is a consequence of the mixed vegetation.

REF: Statistical methods are explained within the combined results and discussion section. I strongly suggest describing these in the methods section. At current, there are results of the stats presented, without the reader knowing what stats methods were actually used.

REPLY: We added a paragraph about the statistical methods in the methods section, stating the statistical program and the applied statistical tests.

REF: I generally prefer to have separate results and discussion sections. However, I acknowledge that this may only be my personal preference and the authors have prepared the manuscript now in the given format. Therefore, I will leave it up to the editor to decide, whether separating results and discussion is feasible and will increase the quality of the manuscript.

REPLY: In initial versions of the manuscript which had separate results and discussion sections, the results had to be repeated before interpreting them in the discussion section. To avoid this redundancy, we have decided to combine those sections.

Minor comments:

REF: P12764, L4: I was a surprised that it is stated that 'microbial consumption can take place in the entire water column'. Whereas this statement is, as such, true, there is no mentioning of the important role of the benthic system, which may also host microbial biofilms that can greatly enhance heterotroph activity. I suggest to add a sentence or two on this topic. A reference could be (Battin et al. 2003), but there are other good ones as well.

REPLY: We added a sentence, mentioning their importance for the DOC dynamics. We refer to Battin et al. (2003) and Romani et al. (2004), whereby the latter one is more relevant for larger river systems such as the Tana River. However, as the importance of the benthic compartment is very dependent on the characteristics of the river, we did not go further in detail.

REF: P12765, L14-18. It may help the reader to understand, which parts of the catchment can be considered humid and which arid (or semi-arid). This aspect may be also important for the question of how the landscape contributes to stream DOC.

REPLY: We added the geographical directions (northwestern and eastern, respectively) to the description of the study area, together with the link to the map (Fig. 1).

REF: L21: interesting approach this mixing model for the landscape C3/C4 proportions. Maybe it would help to guide the reader to why the authors apply this model. A sentence like the following could be added: "To investigate the possible effect of vegetation cover on DOC isotopic composition… we estimated C3/C4 vegetation coverage". However, before writing this, the authors may need to clarify the necessity of this vegetation cover data for the study for themselves.

REPLY: We have changed the first sentence of the paragraph in order to clarify that the vegetation can affect the riverine organic C.

REF: L25: Interestingly these d13C numbers are pretty close to those named as 'typical numbers' for d13C numbers of CO2 in soil (-23‰ and -9‰ for C3 and C4 plants, respectively) named by Clark and Fritz (1997). May be worth to note this somewhere here.

REPLY: The values we are using were those used by Still and Powell (2010) to convert the maps with %C3 and %C4 vegetation cover to maps representing the averaged vegetation $\delta^{13}C$. This has been reformulated more clearly in the manuscript.

REF: P12770, L5: please reconsider the presentation of statistical results. Were all assumptions for a t-test (normality, homoscedasticity) met here?

REPLY: We changed throughout the manuscript to the non-parametric paired Wilcoxon rank test because the normality assumption was not always met. This did not affect the interpretations about the parameters being significantly different or not.

REF: L25: as not all readers may be so familiar with the selective photochemical oxidation, I suggest adding the reference that the authors cite in the introduction.

REPLY: The reference to Opsahl and Zepp (2001) has been added.

REF: P12771, L2: The idea of selective decomposition is truly interesting. Maybe the statement that 'isotopically heavier carbohydrates were preferentially decomposed' could be evaluated and discussed a bit more. Also, it is unclear to me, based on which data the authors come to this conclusion. Please provide more detail.

REPLY: This idea is a hypothesis, since our data show the opposite of what has been observed during photochemical oxidation, i.e. an increase in $\delta^{13}C$ due to preferential decomposition of 13C-depleted lignin relative to the 13C-enriched carbohydrates (Opsahl and Zepp, 2001; Introduction P.12764 L. 23-25). This has been rephrased by stating that it is a hypothesis, which is countered in the remainder of the paragraph as this isotope shift was not observed in other river systems, with the exception of other mixed C3/C4 catchment systems.

REF: In some older literature one can read that 'bacteria prefer to metabolize the isotopically light organics and oxidizers […]' (Clark and Fritz 1997), as it is easier to break 12C-H bonds than 13C-H (or C-2H). This is generally assumed to cause the opposite effect as the one described above. So here is truly an interesting aspect to explore. But first, the reader needs some more evidence for a relationship of a DOC source and the C3/C4 story.

REPLY: It is plausible that bacteria prefer the compounds with isotopically light C, even though this has not resulted in an isotopic shift in riverine DOC in other rivers, except two other tropical rivers. We are not able to provide evidence of the linkage with C3/C4-vegetation, but we think our observations elucidated a pattern that can guide further investigations.

REF: P12772, L1: Even if I have not been to the Tana River, I am not sure these are all the potential sources of DOC to this system. You may also consider i) additions of leaf litter from riparian vegetation that can enhance POC, but also DOC for example through leaching or ii) any human activities, such as sewer inflows that may also contain organic matter. On the contrary, groundwater appears to me like an unlikely source of DOC to the river, as this is commonly considered to be low or very low in DOC, but often high in pCO2. Also, this point comes back to my first main comment.

REPLY: We expanded the list of sources by mentioning human activities, even though those are unlikely to provide significant amounts of DOC as the population density is very low and, to our knowledge, there are hardly any continuous sewage inputs. We expanded the source of ground water to 'groundwater and subsurface water inputs through leaching of DOC from leaf litter', as this is more how we interpreted the groundwater, even though it was not accurately formulated.

REF: Figure 2 and 3: They appear a bit redundant, as they show almost the same thing. I wonder if these could be combined or if one of them could be removed(?).

REPLY: Although the figures are indeed constructed in a similar way, the messages they should convey are different. In Figure 2, the focus is on the contrast between the two methods (filtered vs. unfiltered). In Figure 3, we wanted to emphasize the strong decrease in concentration and $\delta^{13}C$. The inclusion of the data of 2014 is important, because the hydrological conditions were different from 2013 (flooded vs. non-flooded).

REF: Figure 4: First part of caption reads strange. It's the percentage of change of the initial …

REPLY: The caption has been changed to: "Relative change (in %) between the initial concentration (day 0) and final concentration (day 8) in function of the initial concentration of the DOC.

REF: Figure 5 and associated results (p12770, L12-16): I believe this is a typical example, where the use of a simple regression based on least squares fitting is not a good choice. The authors acknowledge this, as they present two such regression models. However, two regressions don't make much sense here. Instead the authors should reconsider their approach and use one of the commonly used 'robust regressions' to account for the two possible outliers.

REPLY: We have reconsidered the approach, as the second regression curve indeed doesn't provide more information than the first one. We now have a robust linear regression by using an M-estimator.

References:

Battin, T. J., L. A. Kaplan, J. Denis Newbold, and C. M. E. Hansen. 2003. Contributions of microbial biofilms to ecosystem processes in stream mesocosms. Nature 426: 439-442.

Clark, I. D., and P. Fritz. 1997. Environmetal Isotopes in Hydrogeology. Lewis Publishers.

Opsahl, S. P. and Zepp, R.G.: Photochemically-induced alteration of stable carbon isotope ratios d13C in terrigenous dissolved organic carbon, Geophys. Res. Lett., 28, 2417--2420, 2001.

Romani, A.M., Guash, H., Munoz, I., Ruana, J., Vilalta, E., Schwartz, T., Emtiazi, F., and Sabater, S.: Biofilm structure and function and possible implications for riverine DOC dynamics, Microbial Ecology, 47, 316--328, 2004.

Still, C. J. and Powell, R. L.: Continental-scale distributions of vegetation stable carbon isotope ratios, in: Isoscapes: Understanding Movement, Pattern, and Process on Earth through Isotope Mapping, Springer, Dordrecht, Heidelberg, London, New York, 179--193, 2010.

Point-by-point reply on Review by A. Nordström

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 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 l. 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-1 of the global estimates 1.9 PgCyr-1 (Cole et al., 2007; Regnier et al. 2013) to 2.7 PgCyr-1 (Battin et al., 2009) is delivered to the ocean (Cole et al., 2007; Battin et al., 2009; Regnier et al., 2013)."

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.

COMMENT: P12765, line 21-25, long sentence; line 25, reformulate “. . . δ13C of respectively -27 and -12‰˙’’ as “δ13C 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 H3PO4 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 H3PO4 creates a low pH unsuitable for microbial growth and not does interfere with later analyses since H3PO4 is also added during the analytical measurement.

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

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)=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-1 day-1 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.

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 or 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 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 contradictive.

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-1. . ." as "This limited decrease in concentration can be related to the low initial concentration (<2mg L-1) …"

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-1)

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) concentration changes. This will be more interesting, and will in part justify the authors claim that DOC < 2 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.

List of relevant changes

- Updates of the statistical tests: adding the applied test and p-value when they were missing or changing the test when the data required it. The regression line (Figure 5) was changed based on the comments of the reviewers.
- Rephrasing of the results in order to make them more understandable for the readers and adding exact values instead of relative phrases.
- Expansion of the list with possible sources of DOC and a short discussion of the those sources.
- Addition of the mineralization rates in Table 1.

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Dissolved organic carbon lability and stable isotope shifts during microbial decomposition in a tropical river system

N. Geeraert[1], F. O. Omengo[1,2], G. Govers[1], and S. Bouillon[1]

[1]KU Leuven, Department of Earth and Environmental Sciences, Leuven, Belgium
[2]Kenya Wildlife Service, Nairobi, Kenya

Correspondence to: N. Geeraert (naomi.geeraert@ees.kuleuven.be)

Abstract

A significant amount of carbon is transported to the ocean as dissolved organic carbon (DOC) in rivers. During transport, it can be transformed through microbial consumption and photochemical oxidation. In dark incubation experiments with water from the Tana River, Kenya, we examined the consumption of DOC through microbial decomposition and the associated change in its carbon stable isotope composition (δ^{13}C). In 15 of the 18 incubations, DOC concentrations decreased significantly by 10 to 60 %, with most of the decomposition taking place within the first 24–48 h. After 8 days, the remaining DOC was up to 3 ‰ more depleted in ^{13}C compared with the initial pool, and the change in δ^{13}C correlated strongly with the fraction of DOC remaining. We ~~propose~~ hypothesize that the shift in δ^{13}C is consistent with greater microbial lability of DOC originating from herbaceous C_4 vegetation than DOC derived from woody C_3 vegetation in the semi-arid lower Tana. The ~~findings complement earlier data that riverine C sources do~~ results complement earlier findings that the stable isotope concentration of riverine DOC does not necessarily reflect ~~their proportion~~ the proportion of C_3 and C_4-derived DOC in the catchment: besides spatial distribution ~~, also~~ patterns of different vegetation types, processing within the river can further influence the ~~riverine~~ δ^{13}C of riverine OC.

1 Introduction

Rivers form the main connection between the terrigenous and oceanic organic carbon (OC) pools. Although rivers were previously seen as mere conduits of water and material, this is no longer the case (Cole et al., 2007; Battin et al., 2009; Aufdenkampe et al., 2011). Indeed, ~~from the~~ only 0.9 Pg C yr^{-1} of the global C input into inland waters, ranging between 1.9 Pg C yr^{-1} ~~(Cole et al., 2007; Regnier et al., 2013) to~~ and 2.7 Pg C yr^{-1} ~~(Battin et al., 2009) estimated to be globally entering inland waters, only 0.9~~ , is delivered to the ocean (Cole et al., 2007; Battin et al., 2009; Regnier et al., 2013). Of the total OC flux, 73 ± 21 % is exported as dissolved carbon (Alvarez-Cobelas et al., 2012). Despite the significant amount

of terrigenous carbon entering the river systems, relatively little organic material with a terrigenous signature can be found in the ocean (Raymond and Bauer, 2001; Bianchi and Bauer, 2011; Marìn-Spiotta et al., 2014). Therefore, it is important to have a better understanding of the changes OC is subjected to and at which point those changes occur during their transport towards the oceans. Two processes, photochemical oxidation and microbial consumption, are able either to mineralize the terrigenous OC, or transform it in such a way that its characteristics resemble the oceanic OC (Bianchi and Bauer, 2011; Lalonde et al., 2014; Marìn-Spiotta et al., 2014). When this transformation of the OM characteristics occurs during transport in the rivers, it may lead to inconsistent characteristics between the terrestrial input, what is present in the river and the OM which is delivered to the ocean, if the transformation processes are not fully understood.

Photochemical processes can occur directly, when the chromophoric dissolved organic matter (DOM) becomes excited under the influence of UV and visible light, leading to transformation within the molecules, or indirectly when free radicals are formed which react with organic compounds (Amon and Benner, 1996; Lalonde et al., 2014). Compared with carbohydrates, lignin components are found to be much more susceptible to photochemical degradation, despite their resistance to biological degradation (Opsahl and Benner, 1998; Opsahl and Zepp, 2001; Spencer et al., 2009). ~~As lignin is more depleted in than the bulk DOM, decomposition of lignin~~ A higher decomposition rate of lignin compared to the decomposition rate of the remaining constituents of the DOC pool would lead to a ^{13}C enrichment of the remaining DOC pool, as lignin is more depleted in ^{13}C than the bulk DOM (Opsahl and Zepp, 2001). An increase in the $\delta^{13}C$ signature of DOC under the influence of UV light has indeed been observed in different river systems such as the Congo, Amazon, and Mississippi rivers (Opsahl and Zepp, 2001; Spencer et al., 2009; Lu et al., 2013; Lalonde et al., 2014).

The biodegradable fraction of DOC can be quantified by the loss of DOC by microbial consumption in dark incubation experiments (Servais et al., 1989). Several experiments have combined the effect of photochemical oxidation with microbial incubation, either by exposing filtered water to UV light followed by the addition of inoculum (Amon and Benner,

1996; Lalonde et al., 2014), or by allowing both processes to act simultaneously (Benner and Kaiser, 2011; Lu et al., 2013). These studies have consistently found that photochemical oxidation ~~broke down~~ degraded a larger fraction of the DOC, compared to biological consumption (Amon and Benner, 1996; Benner and Kaiser, 2011; Lu et al., 2013; Lalonde et al., 2014). Furthermore, microbial and photochemical processes exert greater effect in combination than independently (Benner and Kaiser, 2011; Lu et al., 2013). ~~However, photochemical~~

Photochemical processes only take place in the light penetrated upper layer of a river, while microbial consumption can take place in the entire water column. Besides microbial consumption in the water column, microbial activity in biofilms can also affect the DOC concentration through consumption, production or transformation of DOC (Battin et al., 2003; Romanì et al., 2004). The influence of the benthic system on the DOC dynamics depends strongly on the characteristics of the riverine system and will here not be discussed further. Depth-integrated estimates for the Amazon River, excluding the benthic system, indicate that microbial consumption is the dominant process affecting DOC degradation, accounting for a loss of ca. 0.75 % of the DOC per day, while ~~it is~~ only 0.01 % for photochemical oxidation (Amon and Benner, 1996).

Studies have not found a significant change in δ^{13}C associated with microbial consumption of DOC, with the exception of the Congo and Parana rivers, which experienced a decrease in δ^{13}C by 1.2 and 2.5 ‰ (Lalonde et al., 2014). According to Lalonde et al. (2014), the different behavior for these rivers might be due to the importance of the C_4 carbon fixation pathway in their catchments.

In the discussion about processes affecting the stable carbon isotope signature of DOC, it is important to distinguish between isotope fractionation and selective decomposition. In processes subjected to isotope fractionation, there is a preferential use of the light or heavy isotopes of a homogeneous substrate because it is energetically more favorable. When the original substrate is not homogeneous, the isotope signature of the different components might be different. If one of those components decomposes at a faster rate than others (i.e. selective decomposition takes place), the isotope signature of the remaining substrate will

shift towards the isotope signature of the less degradable component. During the degradation of DOC in aquatic systems, selective decomposition might be the main reason for changes in isotope signatures; Opsahl and Zepp (2001) have demonstrated that the isotope shift during photochemical oxidation is caused by the preferential decomposition of lignin components, which are isotopically lighter than the remaining ^{13}C-enriched carbohydrates.

We measured DOC concentration and the corresponding $\delta^{13}C_{DOC}$ signatures at high temporal resolution at two stations ca. 385 km apart on the lower Tana River, Kenya during three different campaigns ~~,~~ in 2012, 2013 and 2014. During the first campaign (2012), we observed a significant downstream decrease in DOC concentration (from 3.30 to 2.36 mg L^{-1}). At the same time, we noticed a decrease in $\delta^{13}C_{DOC}$ (from -22.6 to -24.6 ‰). These findings suggested that significant DOC processing took place in the lower Tana River. However, quantifying decomposition rates ~~as well as understanding decomposition mechanisms requires~~ required more detailed information than can be obtained from river time series alone. Therefore, we performed dark incubation experiments during the two last campaigns (2013 and 2014) in order to assess the stability of DOC over the travel time of the water between both stations (ca. 5 days). We focused on the microbial decomposition of DOC, as light penetration was limited due to the high sediment load of the river, typically > 100 mg L^{-1} in the lower Tana (Bouillon et al., 2009; Tamooh et al., 2012, 2014). We also tested whether decomposition dynamics ~~was~~ were significantly affected by the presence of POC and suspended sediment. The presence of POC could affect microbial degradation kinetics and DOC mineralization as it may function as a source of DOC.

2 Material and methods

The Tana River catchment (95 500 km^2) is characterized by a strong variation in climate: high rainfall (up to 3000 mm yr^{-1}) and relatively low temperatures (around 10 °C) occur in the highlands of the source area of the Tana ~~,~~ (northwestern part of the basin, Fig. 1), while the area around Garissa is very warm (around 35 °C) and receives an annual amount of ca. 350 mm yr^{-1}, with high interannual variability (eastern part of the basin). The rainfall follows

a bimodal distribution resulting in high discharge from April to June and from November to January.

The variability in climate results in strong variations in vegetation composition, which likely influences the isotope composition of the riverine organic C (Fig. 1). The overall coverage of the catchment with C_4 vegetation is 59 %, based on the ~~revised isoscape map(using~~ isoscape map, which was improved by taking into account the Global Land Cover 2000 map and estimates of crop % ~~) of~~ (Still and Powell~~(~~, 2010)~~which~~ . The isoscape map, presenting the spatial distribution of vegetation stable carbon isotopes, was converted to ~~a~~ represent the ratio of C_4 vegetation cover based on a 2-source mixing scenario with as end members C_3 vegetation and C_4 vegetation with a $\delta^{13}C$ of ~~respectively~~ -27 and -12 ‰ ~~.~~ respectively (i.e. the fractionation factors used for the construction of the isoscape maps (Still and Powell, 2010)). Between the sampling sites, the river is fringed with tropical forest (whereby the total floodplain is seldom wider ~~that~~ than 5 km), while savannah and open shrubs dominate outside of the floodplain.

Water samples were taken from the lower Tana River at Garissa and near Garsen (Fig. 1). The distance along the river between the sampling points is 385 km, and the stretch has only ephemeral tributaries, which hold water during only a few days per year~~, are present along this stretch of the river. Sampling took place during the wet seasons in May–June, ~~. In the wet season of 2013 ~~and in April–May, 2014. In 2013, maximum discharge in Garissa reached 750 which resulted in~~ (May–June), the samples were taken during the decreasing limb of a large seasonal discharge pulse, resulting in extensive flooding between the two sampling ~~locations (but not upstream of Garissa), while only two small discharge peaks (up to 280) occurred in 2014.~~ sites. During the wet season of 2014 (April–May), the samples covered the full wet season which was characterized by two minor discharge peaks without significant flooding. The samples were regularly spaced throughout the campaigns as a new incubation series was started once the previous one was finished.

Grab water samples were taken from the middle of two bridges crossing the river. One 40 mL subsample was used to determine the initial DOC concentration. This water was vacuum filtered on a pre-combusted GF/F filter (pore size: 0.7 µm) and subsequently filtered

with a 0.2 µm Sartorius Minisart syringe filter. The filtration was started as soon as possible after sampling, but due to the high sediment load in the water, it could take up to 2 h before the filtration was finished. Finally, 50 µL of H_3PO_4 was added for preservation. These samples were stored in the dark and upon return from the field in the fridge, until analysis, which was within 4 months of sampling.

The remainder of the grab water sample was used to monitor DOC degradation. In 2013, the degradation rate of DOC was measured under two different treatments: with and without removal of particulate organic carbon (POC). In the first treatment, 500 mL of unfiltered water was stored in a glass bottle wrapped in Al foil. In the second, 500 mL of river water was first filtered to 0.2 µm as described above for DOC sampling. After filtration, 5 mL of unfiltered river water was added to serve as inoculum. Similar to the unfiltered set-up, the water was then kept in foil-covered glassware. To avoid large changes in temperature, both bottles were submerged either directly in the river water, or in a coolbox with water as an isolator from outside temperature changes. The water in the bottles was not stirred during the incubation. Water temperature was regularly measured and ranged between 25 and 30 °C. At days 2, 4, 6, 8 and 10, a subsample of ca. 40 mL was extracted from the incubation bottle, filtered with a syringe filter (Sartorius Minisart, pore size: 0.2 µm) and preserved with 50 µL of H_3PO_4 in glass vials with Teflon-coated screw caps. We carried out 4 series of incubation experiments (both filtered and unfiltered) at each location.

In 2014, 5 incubation series were carried out at each location, but only with filtered water (second treatment). The methodology was identical to the one used in 2013, except that subsamples were taken at days 1, 2, 3, 5 and 8 in order to provide a higher time resolution during the early stages of the incubations.

DOC concentration and isotopic signature were measured with a wet oxidation (heated persulfate) TOC analyzer (IO Analytical Aurora 1030W)~~coupled~~, coupled via a custom-made cryfocussing device with an isotope ratio mass spectrometer (ThermoFinnigan DeltaV Advantage) (St-Jean, 2003). Calibrations were based on a 2-point calibration (IAEA-C6: δ^{13}C −10.4 ‰ and an internal standard, sucrose: δ^{13}C −26.99 ‰). Based on

replicates of the standards, the error in the concentration measurements was $< 3\,\%$ and the standard deviation for the δ^{13}C measurements was $< 0.2\,‰$.

Decay rates, i.e. absolute rates of DOC loss per unit of time, over the whole incubation period were calculated based on the concentration difference between the day of in situ sampling (day 0) and day 8, which is the last common day between the measurements in 2013 and 2014. Initial decay rates were calculated over the first 2 days.

We calculated the concentration (C_{min}) and δ^{13}C signature ($\delta^{13}C_{\mathrm{min}}$) of the DOC fraction lost to mineralization during the incubation period based on a 2-source mixing scenario:

$$C_{\mathrm{min}} = C_{\mathrm{init}} - C_{\mathrm{fin}} \tag{1}$$

$$\delta^{13}C_{\mathrm{min}} = \frac{C_{\mathrm{init}} \cdot \delta^{13}C_{\mathrm{init}} - C_{\mathrm{fin}} \cdot \delta^{13}C_{\mathrm{fin}}}{C_{\mathrm{init}} - C_{\mathrm{fin}}} \tag{2}$$

The initial concentration (C_{init}) and δ^{13}C signature ($\delta^{13}C_{\mathrm{init}}$) were those measured on day 0, while the final concentration (C_{fin}) and δ^{13}C signature ($\delta^{13}C_{\mathrm{fin}}$) were those measured on day 8. ~~The seven series for which the relative error on the calculated C_{min} was higher than 50 % (due to very slow mineralization rates and hence, minor differences between C_{init} and C_{final}) were excluded from this part of the discussion of the results~~Seven series were excluded in this analysis because the total mineralization rates were so low ($\leq$0.01 mg L^{-1} day^{-1}, Table 1) that no realistic isotope signatures for the mineralized fraction were obtained. For the series that were retained (n=19), the standard error on the isotopic signature of the mineralized DOC was ~~estimated to be equal or less than 0.4~~on average $4.3 \pm 1.7\,‰$, based on standard error propagation methods of the uncertainty on the measurements.

Statistical tests were performed in R. The non-parametric paired Wilcoxon signed rank test was applied to test for differences in concentration or isotope values at different times throughout the incubation series. Average values are given $\pm$ the standard deviation. The robust linear regression was done by using an M-estimator (R-package: MASS).

3 Results and discussion

3.1 Comparison of incubation experiments with and without POC

In ~~the incubation series with relatively limited decomposition of DOC~~order to assess the influence of the POC on the mineralization process, we will first compare the two different treatments of the 8 incubation series of 2013. In the incubation series with less than 10 % decomposition of the initial DOC (n=3), the presence of POC ~~did not result in significant changes in concentration between both treatments~~ resulted in ca. 3 % less removal of DOC (Fig. 2a, Table 1). In ~~all other cases was a significant difference between the filtered and unfiltered incubation series , whereby~~ the incubation series with more than 10 % decrease in initial DOC (n=5), the final concentration of DOC was systematically higher ~~in the samples without POC~~by ca. (ca. 10 %) in the incubation series without POC. A similar pattern was observed for $\delta^{13}C_{DOC}$ (Fig. 2b, Table 1), whereby the samples with POC were ~~slightly~~ on average ca. 0.3 ‰ more depleted in ^{13}C than the ones without POC~~, with on average ca. 0.3 ‰ difference. These~~ . In contrast to our initial assumptions, these two findings indicate that there was, within the time resolution of our sampling (i.e. 48 h), no substantial net addition of DOC originating from the POC pool~~within the time frame of our sampling~~, which would have resulted in higher DOC concentrations throughout the incubation experiment. Mineralization rates ~~were slightly enhanced~~ over the first two days were on average 0.06 ± 0.06 mg L^{-1} day^{-1} higher in experiments with POC compared to those without POC (Table 1), likely due to the higher biomass of the heterotrophic microbial community in the presence of suspended matter. ~~As this enhancement was relatively minor and because~~ Because of the covariations between changes in DOC and $\delta^{13}C_{DOC}$ during both treatments and the similar range in mineralization rates at our sampling resolution (Table 1), the unfiltered incubation series is treated as equivalent to the filtered ones for the remainder of the discussion.

3.2 Change in DOC concentration during microbial degradation

The general pattern in the concentration of DOC was a reduction within the first 24 to 48 h (Fig. 3a, Supplement Table S1). After this initial period, during which up to 60 % of the DOC was lost, the concentration remained relatively stable. Initial concentrations over the whole dataset ranged between 1.35 and 5.43 $mg\,L^{-1}$, with an average of ~~2.98~~2.98 ± 1.31 $mg\,L^{-1}$. By day 8 of the incubation, this range was reduced to 1.28–4.20 $mg\,L^{-1}$, and an average concentration of ~~1.96~~1.96 ± 0.73 $mg\,L^{-1}$. Out of all series, only 6 samples from the upstream station (Garissa) in 2013 (three filtered ones and the corresponding unfiltered ones) showed minor degradation of DOC (a change of < 10 % of the initial concentration). This limited decrease in concentration can be related to the low initial concentration ~~which was for all those samples below (<~~2 $mg\,L^{-1}$ ~~and which might consist completely of)~~, which is similar in magnitude as the recalcitrant DOC ~~, which is also still present~~ at the end of the ~~series where mineralizationtook place~~ incubation series with significant mineralization.(Fig. 4, Table 1).

Paired ~~t~~ Wilcoxon signed rank tests over all the filtered series, indicated that the concentration at day 2 was significantly different (~~$p < 0.05$~~$p < 0.01$) from the initial concentration, with a mean difference of ~~0.91~~0.91 ± 0.78 $mg\,L^{-1}$. The concentration difference between day 2 and day 8 over all the filtered series was also significant (paired ~~t test, $p < 0.05$~~Wilcoxon signed rank test, $p < 0.01$), with an average decrease in concentration of ~~0.09~~0.09 ± 0.08 $mg\,L^{-1}$. The rate of decay of DOC ranged between ~~0.002 and 0.320~~0.01 and 0.32 $mg\,L^{-1}\,day^{-1}$ over the whole incubation (day 0 to day 8), with on average ~~0.127~~0.13 ± 0.10 $mg\,L^{-1}\,day^{-1}$, while the decay rate during the first two incubation days reached up to ~~1.50~~2.29 $mg\,L^{-1}$ day, with an average of ~~0.456~~0.51 ± 0.53 ~~. Similar to our experiments ,~~ $mg\,L^{-1}\,day^{-1}$ (Table 1). This is in the same order as the observations from the dark control experiments during the incubation of Amazonian waters, which had initial DOC concentrations around 9.6 $mg\,L^{-1}$ and mineralization rates of 0.12-0.16 $mg\,L^{-1}\,day^{-1}$ (Amon and Benner, 1996). However, they did not observe that the majority of the degradation took place within the first days. High initial degradation

rates were found during the experiments of Moody et al. (2013). Although the water from a peat-covered catchment had much higher initial DOC concentration (ca. 50 mg L^{-1}) compared to our measurements, they similarly observed an average decline of 47 % in DOC after 10 days dark incubation~~of water from a peat-covered catchment. They also observed that the majority of the degradation took place~~ , with high degradation rates during the first two ~~days, while degradation continued thereafter at a~~ incubation days and a much lower rate ~~(Moody et al. , 2013).~~ thereafter.

3.3 Changes in stable isotope signatures of DOC during microbial degradation

Overall, $\delta^{13}C_{DOC}$ signatures decreased consistently during microbial degradation (Fig. 3b, Table 1). However, there was considerable variation in magnitude of change between the different series. Some incubation series, mainly in 2013 at Garissa and in 2014 at Garsen, experienced hardly any change, while other series, especially in 2013 at Garsen, experienced a decrease up to 3.0 ‰. Initial $\delta^{13}C$ values ranged between -25.0 and -21.7 ‰ with an average value of ~~-23.3~~ -23.3 ± 1.0 ‰ for the whole dataset, which is in line with previous measurements for the lower Tana River (Bouillon et al., 2009; Tamooh et al., 2012). Towards the end of the incubation (day 8), $\delta^{13}C$ values had decreased significantly ~~,~~ (paired Wilcoxon signed rank test, $p < 0.05$), ranging between -25.3 and -23.3 ‰ (avg. ~~-24.2~~ -24.2 ± 0.6 ‰).

The $\delta^{13}C_{DOC}$ values at day 2 were significantly different from initial $\delta^{13}C$ values (paired ~~t~~ Wilcoxon signed rank test, $p < 0.05$) over all the filtered incubation series, with a decrease of 0.8 ‰. In contrast to the concentration, no significant change in $\delta^{13}C$ was detected between day 2 and day ~~8.~~ 8 (paired Wilcoxon signed rank test, $p > 0.05$).

3.4 Characterization of mineralized and remaining C

The change in $\delta^{13}C$ was positively related to the proportion (%) of DOC still present after incubation (Fig. 5). A ~~stronger reduction~~ large relative decrease in DOC led to a more ^{13}C depleted residue, implying that the mineralized fraction of the DOC was enriched in ^{13}C vs.

the bulk initial DOC pool. ~~The regression equation for a linear fit~~ Fitting a linear model by robust regression using an M estimator over the averaged points per incubation series over the whole dataset ~~was~~ resulted in $\Delta\delta = -$~~3.5~~ 3.87 $+$ ~~0.038~~ 0.041 $\times$ (%~~DOC remaining) (R^2 0.605). However, two series with a strong reduction in DOC, but relatively little change in isotopic signature can be seen as outliers (Fig. 5). After removal of the latter data points, the regression equation became $\Delta\delta = -4.5 + 0.048 \times$ (%~~DOC remaining), with a higher R^2 value of 0.817~~.

The isotopic ~~characteristics~~ signature of the mineralized DOC ~~were clearly different~~ was significantly different (paired Wilcoxon signed rank test, $p < 0.01$) from the initial DOC as well as from the remaining DOC (Fig. 6). The mineralized fraction of the DOC was more enriched in ^{13}C than the initial or remaining DOC, with average values of ~~-21.2, -23.1 and -24.3~~ -21.2 ± 2.2, -23.1 ± 1.1 and -24.3 ± 0.6 ‰ ~~for the mineralized, initial and remaining carbon pools for all the observations~~respectively. The concentration of the mineralized DOC was on average lower than that of the DOC which was more resistant to microbial degradation.

3.5 Removal mechanism and origin of DOC

Analogous to an isotopic shift due to selective photochemical oxidation of certain compounds ~~,~~(Opsahl and Zepp, 2001), the hypothesis can be formulated that selective decomposition during microbial oxidation ~~could be~~ is the key mechanism to explain the shift in δ^{13}C that we observed during DOC decomposition, whereby the isotopically heavier carbohydrates (Benner et al., 1987) were preferentially decomposed. However, if such a mechanism were to be generally valid, a similar shift would be expected in other types of aquatic systems (irrespective of the C_3/C_4 dominance in the catchment), yet previous studies have typically shown little or no change in δ^{13}C during microbial degradation of DOC (Lu et al., 2013; Lalonde et al., 2014). However, a similar decrease in $\delta^{13}C_{DOC}$ due to microbial degradation has been shown for both the Congo and Parana rivers (Lalonde et al., 2014). One characteristic which both river systems have in common with the Tana River, is the presence of C_4 vegetation within their catchment. A higher decomposition rate for the C_4-derived

DOC, as reported for soil organic carbon (Wynn and Bird, 2007), would indeed be consistent with the observed isotopic shift.

It is, however, not clear whether the different decomposition rates are inherently related to the different photosynthetic pathways used by C_3 and C_4 vegetation, or to the fact that in tropical regions such as the Tana River catchment, C_3 vegetation consists of shrubs and trees, which are more resistant to degradation, while C_4 vegetation consists of grass species, which are more easily degraded. The latter option would appear more likely, as a study directly comparing the decomposition of C_3 and C_4 grasses has shown a greater decomposition rate for C_3 grasses (Ross et al., 2002). More dedicated studies using a similar experimental approach as used here, but with mixtures of DOC originating from both C_3 and C_4 grasses would be required to verify the latter hypothesis. Besides the contrast between the vegetation types, a different age of the DOC from C_3 and C_4 vegetation can also be the reason for the preferential degradation of DOC with a C_4 signature. Young DOC is preferentially utilized by bacteria (Raymond and Bauer, 2001). It can be hypothesized that the DOC delivered to the river consists of young DOC from the nearby floodplains with a dominance of C_4 vegetation.

Microbial degradation was high during the initial phase (first 48 h) in both sampling sites. Considering the water travel time ($\sim$ 5 days) between these sites, the high reactivity even at the downstream site implies that there is not merely a downstream transport of DOC which is progressively mineralized, but that a replenishment of the labile DOC pool must occur. ~~Possible~~ Identifying the source of this DOC pool might assist in understanding the mineralization mechanisms. Possible DOC sources include (1) the POC suspended in the water which may release C into the DOC pool, (2) advection by the tributaries, (3) inputs from the floodplain during inundation, (4) ~~DOC~~ photo-activation of a fraction of the DOC pool, (5) human activities, such as waste water inputs, (6) DOC release from river bed sediments, and (~~5) groundwater inputs~~7) groundwater and subsurface water inputs through leaching of DOC from leaf litter. Only the first source was explicitly tested for in the experiments in 2013, and it appeared that after 48 h, there was no net effect of the presence of POC. This does not completely rule out POC as a source of DOC, as ambient conditions

in a river setting are still different than in our experimental setup or because the temporal resolution of our samples was too coarse to capture the influence of the POC. The advection of DOC by tributaries is unlikely, as their water flow is very erratic, likely even absent in 2014, while the presence of labile DOC was fairly constant. Input from the flooded floodplain will have been important in Garsen during the flooding in 2013 as DOC concentrations were significantly higher. However, it can not explain the presence of labile DOC during the wet season of 2014 when no considerable flooding took place. Photochemical and microbial processes have indeed been found to enhance each other (Benner and Kaiser,2011; Lu et al., 2013), and would need further examination in the Tana River, which has a limited light penetration depth due to a very high sediment load. Population density is very low along the area, and sewage inflow is unlikely to provide a fairly continuous DOC supply. The last two options, river bed sediments and groundwater/subsurface flow, are speculative, as no information on them is yet available. The analysis of optical characteristics of the dissolved organic matter pool (Jaffé et al., 2008; Lambert et al., 2015) could be a valuable complementary approach in order to understand the origin and processing of DOC in rivers.

4 Conclusions

Our experiments demonstrated that in the lower Tana River, a fraction of the DOC is highly susceptible to decomposition, similar to observations in other large river systems. While it was previously considered that bacterial mineralization of labile dissolved OM generally has little influence on $\delta^{13}C_{DOC}$ signatures, our results show that such a change should be taken into account, at least when considering environments with mixed C_3/C_4 inputs. ~~Differences in the rate of decomposition of the DOC originating from and vegetation~~ A higher decomposition rate of the DOC with a higher ^{13}C signature will lead to an underestimation of the C_4-derived carbon input in catchments if the residence time between the input of carbon and the sampling location allows significant microbial degradation.

In addition, the high reactivity of the DOC at both sites has raised the question about the origin of the labile DOC pool. The applied method was only able to exclude POC as

a likely source of labile DOC in the experimental setup. Further research, including optical characterization, would be required to shed more light on the dynamical replacement of the labile DOC pool in river systems.

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Table 1. Concentration and isotopic signatures at the start (day 0) and the end (day 8) of the incubation series (the full dataset including all intermediate measurements, can be found in the Supplement).

Site[a]	yr	Date[b]	~~Filtered~~Filt.[c]	Initial C ($mg\ L^{-1}$)	Final C ($mg\ L^{-1}$)	% ~~Remaining~~Rem.[d]	Initial $\delta^{13}C$ (‰)	Final $\delta^{13}C$ ~~Final~~ (‰)	$\Delta\delta$ [e] ~~$\Delta\delta$~~ (‰) [e]	Init. MR [f] (~~(~~$mg\ L^{-1}\ day^{-1}$)	F ~~(~~)mg L
GSA	2013	04/05	U	2.60	1.48	57	−22.8	−25.0	−2.2	0.46	
GSA	2013	15/05	U	1.54	1.48	96	−24.3	−24.5	−0.2	0.05	
GSA	2013	26/05	U	1.35			−24.4			−0.04	
GSA	2013	05/06	U	1.41	1.39	99	−23.9	−23.3	0.6	0.06	
GSA	2013	04/05	F	2.60	2.07	80	−22.8	−23.3	−0.5	0.28	
GSA	2013	15/05	F	1.54	1.43	93	−24.3	−24.0	0.3	0.03	
GSA	2013	26/05	F	1.35	1.28	95	−24.4	−24.2	0.1	−0.02	
GSA	2013	05/06	F	1.41	1.34	95	−23.9	−23.6	0.3	−0.02	
GSN	2013	03/05	U	5.43	3.68	68	−25.0	−25.3	−0.3	0.68	
GSN	2013	14/05	U	4.09	2.43	59	−23.5	−24.7	−1.3	0.70	
GSN	2013	26/05	U	4.00	1.80	45	−21.9	−24.8	−2.9	0.94	
GSN	2013	06/06	U	3.26	1.58	49	−21.6	−24.4	−2.7	0.77	
GSN	2013	03/05	F	5.43	4.20	77	−25.0	−24.7	0.3	0.58	
GSN	2013	14/05	F	4.09	2.69	66	−23.5	−24.4	−0.9	0.63	
GSN	2013	26/05	F	4.00	1.93	48	−21.9	−24.3	−2.4	0.93	
GSN	2013	06/06	F	3.26	1.66	51	−21.6	−23.6	−2.0	0.77	
GSA	2014	02/04	F	4.99			−23.5			1.51	
GSA	2014	11/04	F	2.99	2.23	74	−23.0	−24.7	−1.7	0.35	
GSA	2014	20/04	F	4.57	2.01	44	−22.4	−24.3	−1.9	2.29	
GSA	2014	29/04	F	2.25	1.46	65	−22.1	−23.7	−1.5	0.39	
GSA	2014	08/05	F	1.59	1.34	85	−23.1	−23.5	−0.4	0.09	
GSN	2014	06/04	F	3.91	1.81	46	−23.9	−24.1	−0.3	0.97	
GSN	2014	15/04	F	2.11	1.87	89	−24.3	−24.8	−0.5	0.10	
GSN	2014	24/04	F	2.98	2.44	82	−24.0	−24.0	0.0	0.25	
GSN	2014	03/05	F	2.40	1.91	80	−23.4	−23.4	0.0	0.21	
GSN	2014	12/05	F	2.34	1.56	67	−22.3	−24.0	−1.7	0.25	

[a] GSA, Garissa and GSN, Garsen. [b] Date of day 0 of the incubation series. [c] U, unfiltered for incubation; F, filtered with addition of inoculum. [d] Proportion of initial DOC concentration remain of the incubation. [e] Change in $\delta^{13}C$ between start and end of the incubation. [f] Mineralization rate from day 0 to day 2. [g] Mineralization rate from day 0 to day 8.

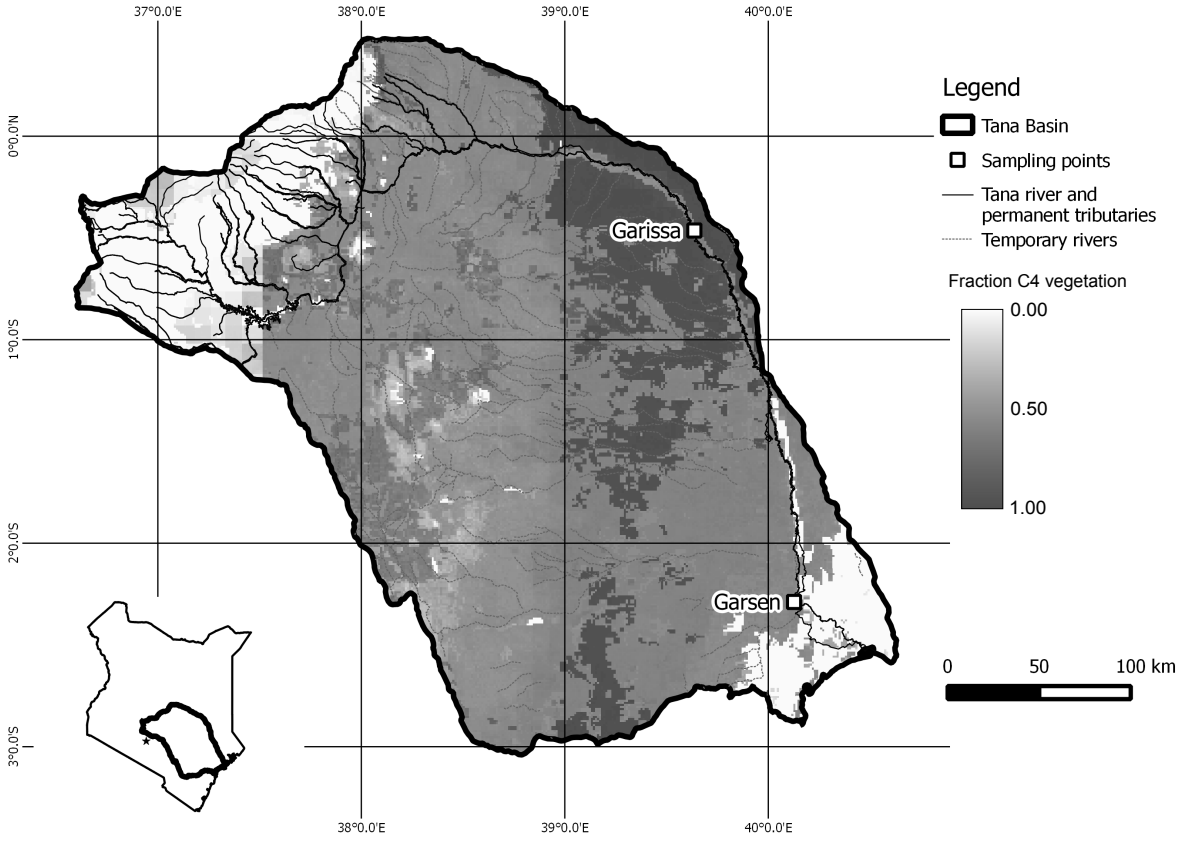

Figure 1. The Tana River with the indication of the two sampling locations in the lower catchment. The shading represents the fraction of C_4 vegetation, based on the isoscapes of Still and Powell (2010). The inset indicates the Tana River basin within Kenya.

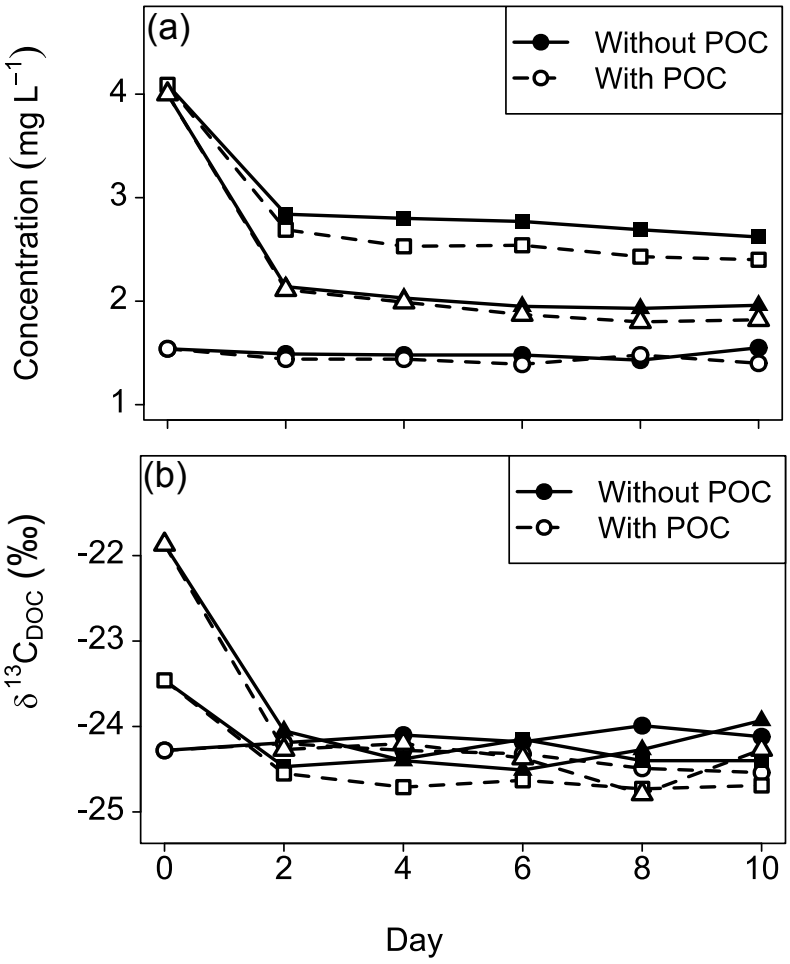

Figure 2. Comparison of evolution of **(a)** DOC concentration and **(b)** stable carbon isotope ratio of DOC ($\delta^{13}C_{DOC}$) over time, for incubations with POC (unfiltered) and without POC (filtered). The three incubation series (Garissa 15 May 2013 (circles), Garsen 14 May 2013 (squares) and Garsen 26 May 2013 (triangles)) are representative of the eight data series, as they give an example of the low, medium and high response of the DOC. No consistent difference was found between the different sampling sites. The full dataset for all incubations can be found in the Supplement.

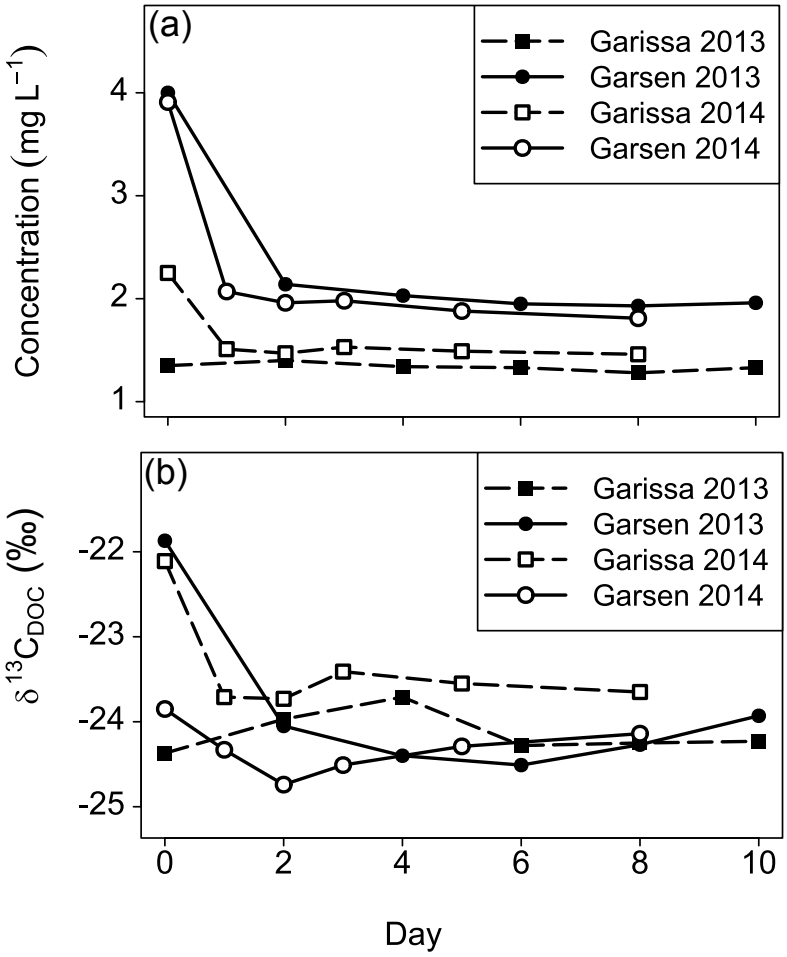

Figure 3. Evolution of **(a)** DOC concentration and **(b)** carbon stable isotope signature of DOC ($\delta^{13}C_{DOC}$) during the 8 to 10 day incubations. Both panels show data from one incubation series per sampling site and per year (Garissa 26 May 2013, Garsen 26 May 2013, Garissa 29 April 2014, Garsen 6 April 2014), providing representative examples of the low, medium and high response of the DOC. No consistent difference was found between the different sampling sites or sampling years. The full dataset and a similar figure with all the incubation series without POC can be found in the Supplement.

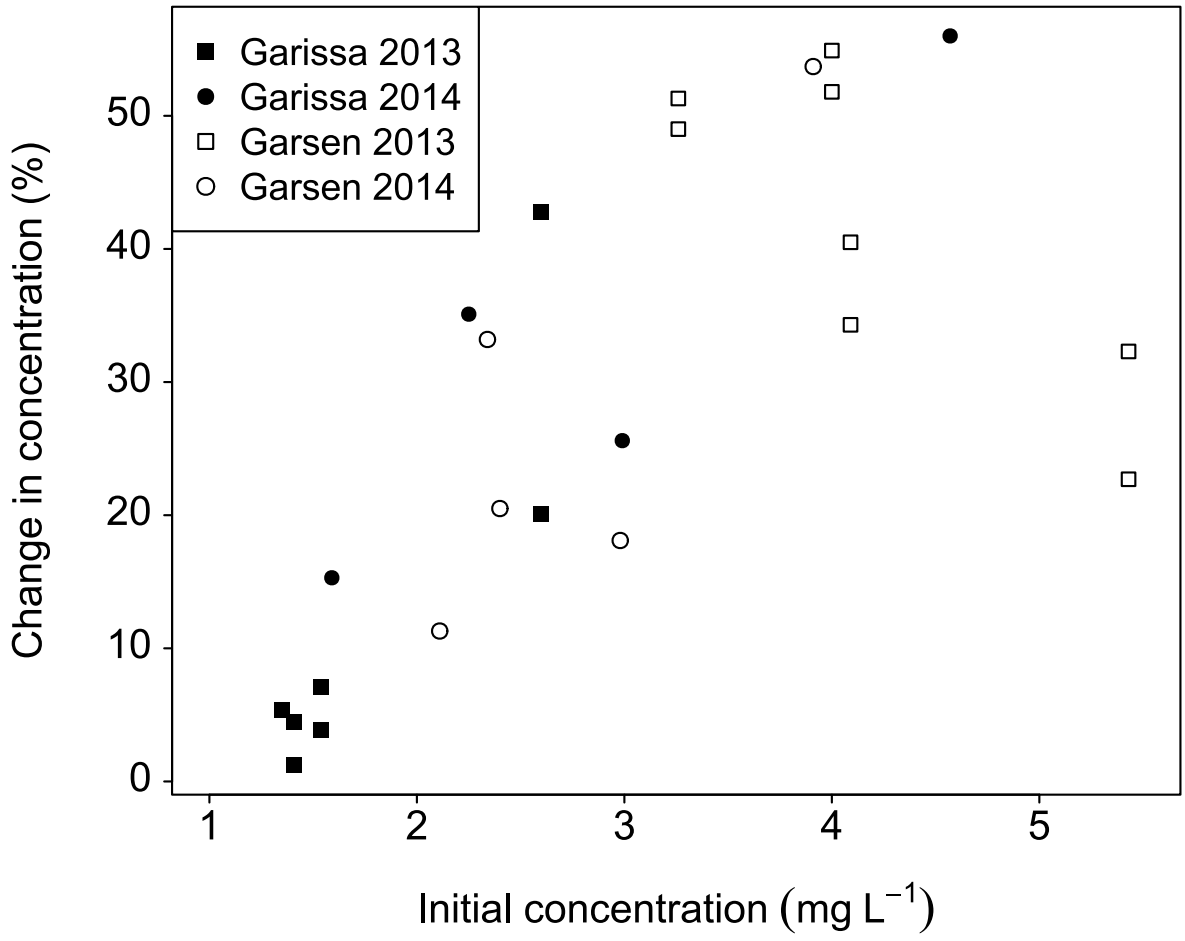

Figure 4. ~~Change~~ Relative change (in %) between the initial concentration (day 0) and final concentration (day 8) ~~vs.~~ in function of the initial concentration of the DOC. All available data are represented.

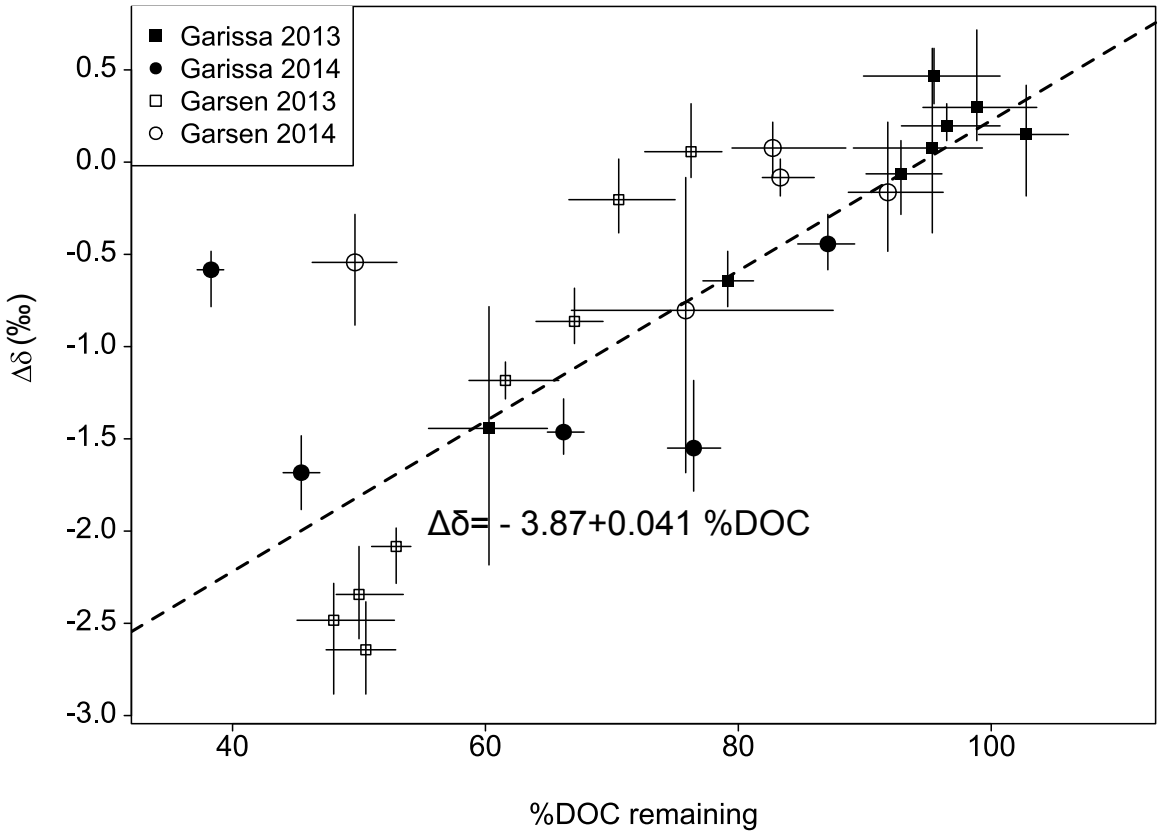

Figure 5. Change in isotopic signature between initial concentration (day 0) and later in the incubation series ($\Delta\delta$) vs. the proportion (%) of DOC remaining after incubation. The points are average values over the five measurements per series and the lines indicate minimum and maximum values within that series. ~~One~~ A robust linear regression ~~line is with two outliers included,~~ model was applied for the ~~second is without the outliers~~regression line. Both filtered and unfiltered series are represented.

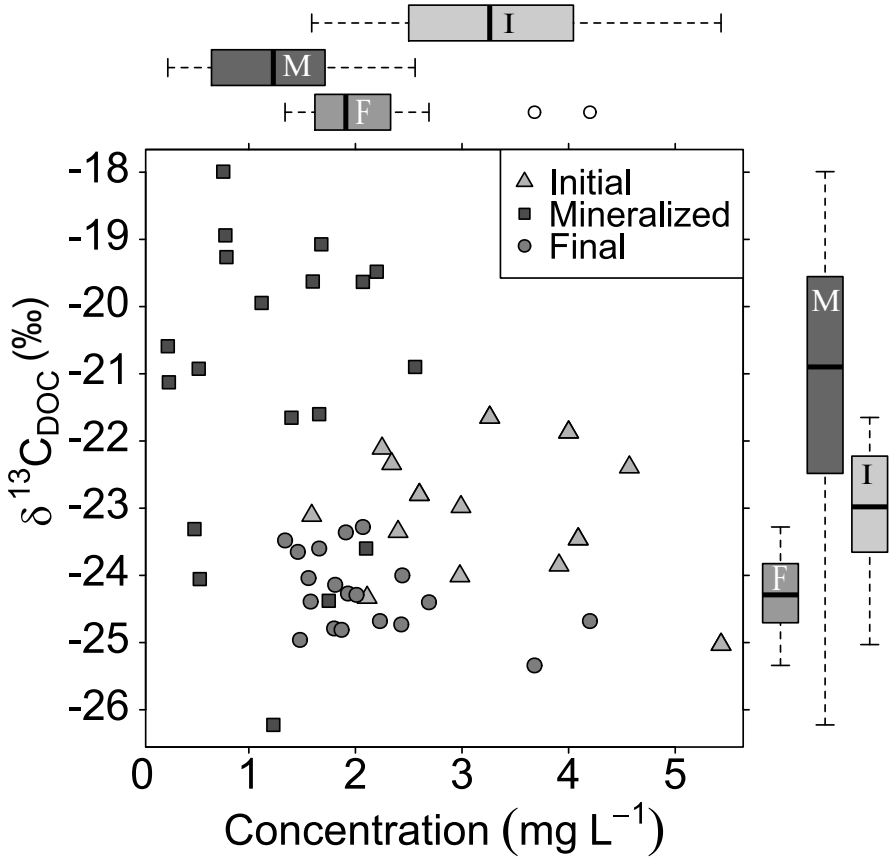

Figure 6. Calculated values of the concentration and isotopic signature of the mineralized DOC pool, for incubation series that experienced a sufficiently high loss of DOC. The horizontal box plots indicate the distribution of the concentration while the vertical ones indicate the distribution of the stable isotope signature for the initial (I), mineralized (M) and final (F) DOC. Incubation series with and without POC are represented, as long as the relative error on the concentration was less than 50 %.