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Reply on RC2

Julia Gensel et al.

Author comment on "Origin, transport, and retention of fluvial sedimentary organic matter in South Africa's largest freshwater wetland, Mkhuze Wetland System" by Julia Gensel et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-172-AC2, 2021

Overall, we thank anonymous reviewer #2 for taking the time to comment on our manuscript and provide feedback. We hope that we can address his/her concerns with the detailed responses below. General

1. The manuscript is generally very well written and focusses on an interesting and important topic, the characterisation of the organic matter input from Mkhuze Wetland System in South African to Lake St. Lucia. The first problem that struck me when reading the introduction was, that aims and questions are very vague., It is not clear what is meant by assessing the status of the wetland (see below). Is the hydroglogical status meant (drained, undrained) or the soil degradation status or the degradation status of OM? Also using $\delta^{13}C$ *and* $\delta^{2}H$ *of n-alkanes to characterize sinks and sources is no doubt a fore front method, but for sure not matured enough, to draw conclusions on plant type communities and degradation status of wetlands.*

- a) We will describe the objective of the study in more detail for clarity. Our study is the first to investigate OM and thus carbon storage within the Mkhuze wetland system and aims to identify the carbon cycling within the Mkhuze Wetland System by identifying sources and sinks and ultimately the transport pathways of particulate OM transported by the Mkhuze River.
- b) Plant wax-derived *n*-alkanes are refractory tracers that are well preserved in sediments, even over geologic time. By analyzing their carbon and hydrogen isotopic composition, additional information can be derived about the hydrologic growth conditions exhibited by the plants, as well as the photosynthetic pathway (distinguishing them into C_3 and C_4 plants, respectively). In our study, the degradation status of OM is mainly inferred from the results of Rock-Eval analyses. The combination of conservative molecular tracers (*n*-alkanes) and analyses of OM degradation (Rock-Eval) shows that *n*-alkanes, although representing a small fraction of total OM, follow the same trends as bulk OM properties determined by Rock-Eval. The information on OM degradation derived from the results of the *n*-alkane analyses only consolidates Rock-Eval analyses.
- c) For clarity and better understanding, we will include a new section in a revised version of the manuscript to explain our approach and the methods used in more detail.

2. As such, compared to such ambitious aims, the introduction is very general. I totally *miss discussion of state of the art on $\delta^{13}C$ and δ^2H organic matter tracing and what it can tell us about sinks and sources. The same holds true for stability of n-alkane concentrations when used as indicators for sediment or organic source attribution.*

- a) Interpretation of *n*-alkane concentration in terms of degradation is used only to support the results from Rock-Eval analyses, which are the main method for determining OM degradation in our study.
- b) As mentioned earlier, we will introduce a new section to further explain the approach and methods used.

3. The description of the sampling concept is totally missing. All it states is, that "ten samples where collected." However, Figure 1 displays around 30 sampling sites, so I assume that ten samples for each sub-environment was taken? This is totally unclear. A detailed map of vegetation communities is presented (Figure 3) but it is not at all clear, if all these communities were sampled as possible sources and if so, how many samples, which plant species etc....If the aim is, to track OM in the lake back to these communities, the detailed sampling scheme has to be described.

- a) The manuscript contains a subsection "2.2 Sampling" (132ff), which describes, among other things, how many samples were collected and also which plant species were collected. The names of the collected plant species are additionally mentioned in the manuscript in the corresponding results section (subsection "3.3.1 Plant Samples" and the corresponding figure (Figure 8 and its caption).
- b) The sentence quoted by reviewer #2 originally reads "Ten **plant** samples were collected." (line 134), while in line 140 the information is given that "a total of 41 surface sediment samples [...] were collected [...]".
- c) We will add a reference to Figure 3 in line 136.

4. The results are mainly a listing of all measurements done with differences in numbers and sizes. There is no real information gain for the reader, as none of these results are set into perspective and the discussion does not give a clear link back to these data descriptions. Not even the indices and parameters used are in any way explained in the results section (and only very briefly in the discussion). Variability and differences are hard to assess, as sampling numbers and possible errors are not described. It is not clear if error bars indicated in-field heterogeneity or analytical uncertainty. Figure 8 states that error bars might be intra-laboratory long-term errors.

- a) We agree that we have presented the results in the "Results" section without interpreting them, as it is our understanding that interpretation is part of the "Discussion" section. It is important for us to distinguish between the measured values and our interpretation of these values.
- b) The indices and parameters used are explained in the "Materials and Methods" section of the manuscript and literature references are provided for all indices and parameters mentioned.
- c) The equations used to calculate the carbon preference index (CPI) and average chain length (ACL) are given in the subsection "2.4 Distributional parameters of *n*-alkanes".
- d) The hydrogen index (HI) is introduced in line 167ff in subsubsection "2.3.1 Bulk organic matter analyses".
- e) R- and I-indices are introduced in line 170f within subsubsection "2.3.1 Bulk organic matter analyses".
- f) The meaning of the indices and parameters is given in the respective parts of the "Discussion" section and, in addition, a link back to the results is provided (also in numbers, if applicable); see e.g. lines 383f, 401ff, 427ff, 447ff. Otherwise, a cross-

reference to the respective figure is given (see, e.g., lines 340, 350, 353, 384, 389, 400, 406, 412, etc.).

- g) However, for better understanding of our approach and the methods used, we will additionally include information on the parameters and indices used in the newly planned "Approach" section.
- h) The respective subsections of the "Materials and Methods" section contain information on replicate measurements and sample replicates. We have chosen to always present the largest of all possible errors (natural heterogeneity exceeds analytical errors). If the analytical errors are smaller than the long-term error within the laboratory or a replicate measurement was not possible due to limited sample size, the long-term error was assigned to more adequately reflect reality.
- i) When multiple errors can be represented, such as in the analysis of plant samples, we refine the corresponding caption, i.e., Figure 8.

5. The discussion is more a descriptive qualitative narrative of differences found in parameters within and between different sub-ecosystems. Indices for evaluation are not adequately introduced and partly interpreted in a wrong way (e.g., that CN ration of OM would be a general indicator of chemical stability). As such, I can not follow conclusions drawn and can not judge if these conclusions adequately assess the results. One example would be the conclusion "Sedimentary OM in the floodplain and swamp exhibit high variability in their source signatures and degradation status reflecting environmental diversity, with samples from the floodplain characterized by a mixture of degraded OM from the hinterland and fresh OM."(line 575-577). With clearly high ongoing and very variable degradation of OM in these systems, concentrations of organic substances can not be used as a conservative tracer.

- a) To the best of our knowledge, the present study is the first to examine OM properties in the Mkhuze Wetland System. Therefore, no quantitative comparison with previous studies is possible. Intra-system comparison of different sub-areas leads to a qualitative assessment of whether certain characteristics of OM are found to a greater or lesser extent in other sub-areas.
- b) We will introduce a new "Approach" section to improve understanding of our approach by explaining our general approach and methods used, as well as the indices provided and their meaning (see also responses "b", "c" to comment #4).
- c) We will delete the sentence about the C/N ratio providing information about chemical stability, since in this case we did not intend to evaluate the stability of chemical bonds. We agree with Referee #2 that the wording is unfortunate and thank him/her for the comment. Nonetheless, the results of the C/N measurements serve as supporting information, since the main method for deriving information about OM degradation in our study is the Rock-Eval analyses.
- d) We will split and edit the mentioned sentence (line 575-577) into two sentences to improve readability and comprehensibility.
- e) *n*-Alkanes are refractory molecules that are well preserved over geologic time. Therefore, they can be used as conservative tracers, as is commonly done. In addition, we refer almost exclusively to relative concentrations of *n*-alkanes rather than absolute concentrations. Absolute concentrations of *n*-alkanes occur only in statements used to support the results of Rock-Eval analyses. *n*-Alkanes accumulate in sediments because of their chemical stability (now actually defined as stability of chemical bonds) when OM is degraded, and labile parts of OM are removed and utilized by microbes. Therefore, comparison of absolute *n*-alkane concentration normalized to dry weight or organic carbon can serve as an indicator of OM degradation (performed in our study only as additional information to our main Rock-Eval method).

6. Regarding the isotope tracers used, no un-mixing was done and the values were interpreted in a qualitative way, which is, from my perspective not leading to meaningful *conclusions.*

- In the early stages of the manuscript development, we attempted to apply a Bayesian un-mixing model, but at a certain point had to accept that the quality parameters of the statistical model were insufficient, as convergence could not be achieved. The reason for this is most likely that these models require either a larger sample size or endmembers with larger differences to be statistically reliable. Therefore, we opted for a qualitative approach by identifying marker compounds (groups of specific *n*-alkanes) that are present in certain plants and absent (or only present in negligible amounts) in others. These marker compounds define clusters of plant types (e.g., aquatic, grassy) that are segregated from each other and thus can be used to identify predominant sources of vegetation. This approach will be explained in more detail in a revised version of the manuscript in a new section "Approach".

7. All in all, I would judge this work as containing highly valuable and interesting data and results. But description of sampling concept is inadequate and interpretation of data is qualitive with numerous assumptions I am not sure can be hold.

- a) We thank Reviewer #2 for his/her comments attesting that the data presented are of high interest and value.
- b) We believe that our study contributes to a better understanding of the carbon cycle and carbon storage in the Mkhuze Wetland System. As discussed in detail in the "Discussion" section, our finding that OM is sequestered under current conditions in the swamp area of the wetland system studied may also be found in other wetland systems, suggesting that carbon sequestration in such systems is primarily hydrologically controlled.

Introduction

8. Generally, well written and interesting to read about the Mkhuze Wetland System. However the aim of "assessing the current status" is very vague to me (line 36). Which status do you mean? Hydrological? Soil degradation? Nutrient status?

- We will refine the description of the objectives of our study (see answer "a" to comment #1)

9. I totally miss discussion of state of the art on $\delta^{13}C$ and $\delta^{2}H$ organic matter tracing and what it can tell us about sinks and sources. Also, what about the stability of n-alkane concentrations in these systems? Are you sure you can use these as conservative tracers?

- a) We will include the requested information and references in the newly planned "Approach" section.
- b) Yes, we are sure that *n*-alkanes can be used as conservative tracers. Because of their chemical stability and resistance to microbial attack, they are well preserved (e.g., Eglinton and Hamilton, 1963), which is why they are commonly used as refractory tracers over geologic time (e.g., Eglinton and Eglinton, 2008).
- c) In addition, the high CPI values indicate that *n*-alkanes weren't subject to advanced degradation processes.

10. 40-42 this assumes that you have species specific tracers

- We agree with reviewer #2 that plant wax-derived *n*-alkanes and their stable carbon and hydrogen isotope compositions are not capable of identifying specific plant species, but rather provide information about vegetation types. Combined with knowledge of the occurrence of (dominant) plant species in a given area, inferences about the most likely source can be made even at the plant species level.

11. 42-45 how can you assess the vegetation type (do you mean plant community?) with $\delta^{13}C$ of nalkanes?

- The newly planned "Approach" section will also include an explanation of the use of *n* -alkane-$\delta^{13}C$ to distinguish plant types with different photosynthetic pathways.

12. 45 hydrological conditions of what? Of the regime under which the plants grew? Of the soil? E.g., wetland, upland, drained? Not sure you can achieve this with $\delta^{2}H$?

- a) We will include the requested information in the new "Approach" section.
- b) Briefly, δD of *n*-alkanes preserves information about hydrological conditions experienced by plants during their growth phase. There are several possible interpretations, as several factors can affect the δD of plant wax (will be explained in the "Approach" section.

13. 51 this is a big aim, to the assess the status of the wetland systems in terms of its filter function and influence on Lake St. Lucia!

- a) We agree.
- b) See also response "a" to comment #1.

Methods

14. 133 what do you mean by "Ten samples were collected." Of what? Ten repetitions within a site?

- a) The original sentence reads, "Ten plant samples were collected." (line 133). This means that we sampled a total of ten plants. The number of replicates of each species is given in lines 136ff.
- b) Further, line 140 indicates that a total of 41 surface sediment samples were collected.

15. 137 – 139 these plants were not collected? But all others were? Or these are the ones which you did collect?

- We absolutely agree that the English word "this" at the beginning of the sentence in line 137 refers to the subordinate clause mentioned earlier. We thank Referee #2 very much for pointing out this incorrect reference and will, of course, reword the sentence accordingly. However, we sincerely hope that most readers of the discussion paper have concluded from the context that we sampled the number of plants indicated in each case, rather than sampling all but one or two plants in the area.

16. Figure 4 is basically describing standard analysis and could be moved to supporting information.

- Figure 4 will be removed and the sample preparation information will be provided as plain text.

Results

17. 252 what is HI value?

- a) The Hydrogen Index (HI in mg HC/g TOC-1) is one standard parameter determined by Rock-Eval analyses. It is calculated by integrating the amounts of hydrocarbons released during thermal cracking of OM between defined temperature limits (line 167ff). Depending on the application of Rock-Eval analyses, different information can be derived. When examining sedimentary rocks, the degree of thermal maturity and kerogen type can be inferred. When examining organic matter in soils or sediments (as in our study), the hydrogen index is used to infer the origin of organic matter based on the difference in predominant biomacromolecules in aquatic organisms and algae (rich in lipids and proteins) compared to terrestrial plants (rich in carbohydrates).
- b) We will include a statement in the new "Approach" section that provides a more detailed introduction to the methods used and the information they provide, rather than just a literature reference.

18. 256 what is R-index?

- a) The R-index calculates the relative contribution of the most thermally stable HC pools (line 170f) and the I-index calculates the ratio between thermally labile and resistant HC pools (line 171f). Details can be found in the given reference at line 172, Sebag et al., 2016. Briefly, both indices use the integration of certain areas (A1-A5) under the S2 curve between certain temperature boundaries (for the exact boundary temperatures, the reader is referred to the given reference). These integrated areas relate to the differences in thermal stability of the biopolymers in the OM. Basically, one could say that the higher the R-index (R = refractory), the more pronounced are the thermally refractory pools within the OM and the higher the I-index (I = immature), the more pronounced are the thermally labile parts of the OM.
- b) In addition, as derived from their mathematical construct, these two indices (R-index and I-index) are inversely correlated when OM stabilization (R-index increasing) results from progressive decomposition of labile organic compounds, and relative enrichment in refractory ones. Then, in the I/R diagram, a "decomposition regression line" describe the decreasing labile pools and concomitant increase in more thermally stable pools, was observed in compost by Albrecht et al. (2015) and soils by Malou et al., (2020), Masseroli et al. (2021), Matteodo et al. (2018), Sebag et al. (2016), and Thoumazeau et al. (2020). However, situations with OM mixture from different sources may generate a distribution in the I/R diagram aside the "decomposition line", i.e. a poorly related I–R indices.
- c) A brief explanation of the two indices will also be included in the new "Approach" section to improve understanding of the methods we use.

19. 260 what is I index?

- See answer to comment #18.

20. For all errors it is not clear from how many reps they are produced, if repetition at all or if this is analytical error.

- See answer „d" and „e" to comment #4.

Discussion and Conclusions

21. Paragraphs 343 -349 versus 332 – 338: I am not sure I understand you correctly, but this makes not much sense to me. First, you describe the differences in n-alkane patterns within plants, within sites and between different ecosystems, but then you assume that you can take literature values from generally well studied plants, such as trees as source values to be characteristic for your sites?

- We will refine subsection 4.1 in the discussion section to make it clearer why we used the respective *n*-alkanes for each identified cluster. Also, we will replace the word "end-member" with "marker compound". In short, we identified marker compounds, i.e., specific *n*-alkanes that are present in certain plant clusters while absent (or present only in negligible amounts) in others. We selected one of the marker compounds per cluster (the higher concentrated one across all samples) to represent the respective trends. The *n*-alkanes we selected to represent the cluster labeled "woody" were C_{29} and C_{31}. We agree that these *n*-alkanes are not produced exclusively by woody plants but are present in almost all plants. We mention this limitation in line 347ff. We will delete the term "woody" as a cluster name and instead explain that these particular *n* -alkanes are more of an integrated signal of all plants to avoid confusion. We will restrict the term "woody" to subsection 4.2.1.

22. The discussion in 4.1. mainly compares the n-alkane concentrations determined in this study in comparison to literature values. But what is the message behind this paragraph?

- a) Comparison of the relative *n*-alkane concentrations determined in our study with the literature provides the rationale for our choice of specific *n*-alkanes to serve as marker compounds. Furthermore, limitations of the approach known from the literature (line 333ff) are mentioned and why we are nevertheless convinced that the approach remains valid despite these limitations.
- b) As mentioned in answer "a" to comment #25, the subsection will be refined.
- c) In addition, although more as a side effect, the placement of our results in the previously published literature should help the reader assess the credibility of the data presented.

23. Paragraph 4.2.: what is is this telling me regarding the aim of your study, the status of your system, what is the aim of this paragraph? What is the connection to your results?

- a) The first paragraphs of subsection 4.2 were originally intended to provide information applicable to all subsequent subsections (4.2.1 - 4.2.4).
- b) We thank Referee #2 for bringing to our attention that this has caused confusion. We will embed the information provided here in the new "Approach" section.

24. 369 the C/N ratio is an indicator of chemical stability? I do not think so?

- See answer "c" to comment 5.

25. Paragraphs 4.2.1. to 4.2.4. describe the variation of the different measured parameters in each of the sub-ecosystem types and tries to induce state of degradation of OM or plant origin. This discussion is qualitative and not really set in perspective to literature values.

- a) The mentioned subsubsections provide the interpretation of the measured variables in terms of OM properties and forms the basis for the following inferences about OM transport pathways within the system. We are convinced, as mentioned above, that the methods used are fully capable of providing the necessary information and not just "trying" to do so.
- b) Internal comparison of subareas does not provide quantification, but rather an assessment of "more", "less", "similar", or "different" compared to upstream areas.
- c) To our knowledge, there are no previous studies of OM in the system, so a comparison to previously observed characteristics is not possible, nor is an assessment of whether OM is more or less degraded than in another wetland plausible.

26. Section 4.3. (line 467 – 480) starts with a general description of Mkhuze Wetland System which might be transferred to the methods or the introduction. Or are these statements conclusion from your data? If so, please make the link to your results.

- a) The first sentence refers to the previous subsection (4.2), where interpretations of OM properties from measured parameters are given, and introduces the following conclusion on OM transport pathways.
- b) The second sentence extends the interpretation of OM to sediments in general and justifies this with literature references.
- c) The following sentences (lines 472ff) are the essence of the interpretation of OM properties and inferred transport pathways. These are related to the available literature and any discrepancies that arise are discussed.

27. 494 – 499 is this general knowledge of literature or is this a conclusion from your results? Please make the link to your data

- It is a conclusion from our results, as shown by the respective sentence beginnings "Therefore, it can be concluded [...]" (line 494) and "The identification of the Mkhuze Swamps as [...]" (line 494).

28. Some conclusions might be considered speculative.... e.g., from the result that "....the higher hydrogen isotope signature of the sedimentary n-alkanes in the lake probably resulted from a dominant contribution of lakeshore vegetation" (line 464 – 465) the general conclusion is drawn, that "OM in the surface sediments of Lake St. Lucia originates primarily from lakeshore vegetation" (line 501). There is no unmixing of possible source signatures, no quantitative evaluation. This is just one example, which leaves the impression, that conclusions drawn are based on rather qualitative assumptions and might even be speculative.

- a) The Rock-Eval analyses clearly show that in the lake area, "These results differ drastically from the OM results of the upstream sub-environments, but they do not reflect aquatic autochthonous contributions (as indicated by the low HI). Although the sources of this OM are probably terrestrial, it is not a detrital (allochthonous) OM reworked from the catchment area, but rather a proximal (para-autochthonous) contribution." (line 449ff).
- b) Analyses of plant wax-derived *n*-alkanes and their stable carbon and hydrogen isotopic compositions indicate that the vegetation source in the lake area is quite similar to the upstream environments (similar $\delta^{13}C$ and relative alkane concentrations), while the significant offset in δD indicates a different water source that was available to plants for growth (see lines 455-463).
- c) To point out that our study, like most if not all scientific studies, cannot provide 100% certainty in interpretations, we used the word "probably", "mainly", etc.

- d) However, the combined interpretation of both methods suggests that the shoreline of the lake as the origin of the OM is the most reasonable explanation (line 464f).
- e) We agree with reviewer #2 that our study is mainly a qualitative study comparing different characteristics of OM obtained by a combination of methods. But to put it simply: If we do not find the upstream OM characteristics in the lake area, but instead find completely different characteristics, then in all likelihood the OM must have come from somewhere other than upstream.