We would like to thank Peter Douglas and Dailson Bertassoli for the time and effort taken to provide constructive and insightful comments on our manuscript. Below, we have copied their original suggestions and comments in full, followed by a response to each of the issues raised and how these will be addressed in a revised version. Should we be invited to revise the manuscript, a more detailed reply detailing the actual changes to the manuscript will be provided subsequently.

RC1: 'Comment on egusphere-2024-2213', Peter Douglas, 05 Sep 2024

<u>Ref</u>: This is an interesting article that tackles an important but understudied topic, namely the role of (tropical) lakes in modulating inland water carbon fluxes. I'm glad to see the focus on carbon sources and fluxes upstream and downstream of the lake. There is a huge wealth of data contained in the paper, and for the most parts the authors do a solid job of analyzing these data coherently. Ultimately I think it will be a valuable contribution.

However, there are a number of issues that need to be addressed before this should be published in Biogeosciences. I would characterize these as major revisions. I summarize major issues below, and then provide line by line comments.

Peter Douglas

Introduction: I found the introduction to be somewhat unfocused, and a clear rationale for the study does not emerge. I think the authors should put more attention to the importance of this study in a global context, and how this site is representative of globally important ecosystems. What questions in global carbon cycling and inland water carbon fluxes does this study help us address? The discussion of environmental impacts of sediment from soil erosion is interesting, but given the topic of this study it seems the introduction should be more focused on carbon cycling. Also, it seems part of the expressed motivation of the study is to interpret paleo-proxy data, which I think is tangential. Again I think these data are more interesting in terms of modern day carbon cycling and that should be a larger focus. See cited paper Cole et al., 2007 and Regnier et al., 2020 for examples of key questions in inland water carbon cycling.

Regnier, Pierre, et al. "The land-to-ocean loops of the global carbon cycle." Nature 603.7901 (2022): 401-410.

<u>Reply:</u> We appreciate this clear request to reformulate the context and objectives – this also falls in line with similar suggestions by Ref#2. We can certainly focus more here on the carbon cycling aspects and global relevance of (sub)tropical lakes; as this is indeed likely what many readers will be interested in. Nevertheless, we do wish to point out that we do not consider the importance of this study in terms of paleo-proxy data interpretation as tangential – this was in fact the main driver of conducting this work. In the broader framework of the project which this study is part of, we examine various proxies in floodplain, marsh and lake sediment cores – and any interpretation of these requires a basic understanding of the

biogeochemistry of the lake itself, and a solid characterization of the inputs and outputs. A manuscript is being finalized on those sediment data, and the conclusions from the current study are an important element guiding the sediment proxy interpretations. Hence, we will on the one hand better explain this part of the rationale, while at the same time better express the global inland water C cycle context.

<u>Ref:</u> Sampling Design: I think the selection of sampling sites and the sampling design needs more clarification and elaboration. How were inflowing rivers chosen and why were some sites sampled at a higher frequency? How were the lake sampling sites chosen, and importantly what depth was water sampled from? Lake sampling is not really described at all. How far downstream of the lake outlet was the outflowing river sampled? It seems to me the marsh sampling should be discussed in the same section as the water sampled and not terrestrial plants or phytoplankton, which are also probably important OM sources? The point of the cores from the marshes is not that clear- maybe explain a bit more the rationale for collecting cores as opposed to surface samples, and what information is provided by the depth profiles.

<u>Reply:</u> We will add details providing all relevant information on sampling in the revised version. Briefly, inflowing rivers were sampled along the entire lake area (when there was sufficient water flow), with a selection made for regular sampling based on their accessibility for collaborators on site and that these would have flow year-round. Lake sampling took place along a N-S gradient covered by boat, all water samples were taken with a Niskin bottle from the surface (approx 0.5 m depth).

<u>Ref</u>: Analytical Methods: This section is very dense and hard to follow. I would suggest splitting into subsections if journal allows. Some reorganization is needed. PCO2 measurements should proceed the estimates of DIC concentration since they are used for that. There is no mention at all of the CH4 measurements that are presented, which is a major oversight.

<u>Reply:</u> We will bring more structure into the Methods section as suggested. CH_4 methodology will be added.

<u>Ref</u>: Results: This section is very dense and hard to follow. If possible to break into subsections that would make reading easier.

<u>Reply:</u> As for the section on analytical methods, we will break this up into subsections as suggested.

<u>Ref</u>: Vegetation data: As mentioned above, having d13C values of local terrestrial plants and phytoplankton, or at least estimates, would really be helpful and complement the data shown in Table 3. The 13C values of subsoils are referenced but the actual numerical values

should be summarized, and it is unclear why topsoils are not included. In line 448 there is discussion of a mixing model based on plant isotopic values, but this is quite unclear and hard to follow. More details are needed, and the relative abundance of different plant types should be specified, ideally in a table.

<u>Reply:</u> Samples of terrestrial vegetation were taken and reported in Razanamahandry et al. (2022, <u>https://doi.org/10.5194/bg-19-3825-2022</u>). We will refer to this in the revised version. Topsoil data (from the same study) can also be cited again. Regarding phytoplankton, we sampled the total suspended matter pool in the lake (as discussed in the next comment); but see no way of sampling pure phytoplankton in such settings. As discussed in the manuscript, suspended matter in lake surface water seemed to be mainly composed of phytoplankton.

<u>Ref:</u> Phytoplankton 13C calculation: I'm afraid this is a gross oversimplification. This fractionation factor can very hugely and is sensitive to biological and environmental variables like growth rate and light, as well as differences between taxa. Use of a single value of 20‰ is too simplistic, and based on my quick review is on the high end, as opposed to an average value. So I don't think this is appropriate. In addition the uncertainty in this estimate needs to be accounted for. There is an extensive literature on this, but see for example:

Burkhardt, Steffen, Ulf Riebesell, and Ingrid Zondervan. "Effects of growth rate, CO2 concentration, and cell size on the stable carbon isotope fractionation in marine phytoplankton." Geochimica et Cosmochimica Acta 63.22 (1999): 3729-3741.

De Kluijver, A., Schoon, P. L., Downing, J. A., Schouten, S., & Middelburg, J. J. (2014). Stable carbon isotope biogeochemistry of lakes along a trophic gradient. Biogeosciences, 11(22), 6265-6276.

The Fry et al citation is quite outdated. Including more uncertainty in this calculation is required, and likely has effects on the inferred contribution of phytoplankton.

<u>Reply:</u> We fully agree that our approach is a simplification, and indeed there is a wealth of knowledge on isotope fractionation in phytoplankton and the controlling factors. However, since we have no information on the phytoplankton community composition, we cannot make full use of this knowledge and are, in our opinion, limited to a coarse estimate. However, we will look into the literature to screen for more recent approaches used by others, and include an uncertainty evaluation on the phytoplankton δ^{13} C estimates by using a range of likely values of fractionation factors compiled from literature.

<u>Ref:</u> Consideration of hydrodynamic processes: Differences in %POC between the TSM and soils was suggested to indicate a different, more organic rich source. However, this could also reflect sorting and selective settling of eroded soil that could lead to greater %POC in the lake. For example, soil minerals may selectively settle and deposit in

sediments, whereas OM rich material may be more likely to be suspended. I think this is worth consideration. This could also potentially explain the higher %POC in the lake vs the inflowing rivers.

<u>Reply:</u> We will add some literature and discussion on this – indeed importantaspect. One would expect, however, that the fraction ultimately transported to the lake itself would be the finer clay fraction – which typically has %OC lower than 5%, thus substantially lower than those observed in lake suspended matter.

<u>Ref:</u> More detailed implications: I think it would be really valuable to see a bit more discussion of the implications of this work at the end of the discussion. What does this mean for the role of lakes in riverine carbon transport and emissions? Does the presence of lakes in (tropical) rivers lead to a net increase or decrease in emissions, and does it change the overall source of C that is being respired? Do your results have implications for the carbon cycle effects of current anthropogenic changes in the catchment and the lake? Will greater erosion and/or rice production lead to changes in the GHG fluxes from this system or the downstream export of carbon? I think addressing some of these questions will enhance the impact of the paper.

<u>Reply:</u> This suggestion is in line with earlier suggestions on the introduction (context & objectives), so indeed it would be logical to come back to this in the Discussion. Note that some of the questions raised can only be addressed broadly and might be somewhat speculative, but we see the use of raising the questions and providing some pointers. We will refer to a recent synthesis by our group (Borges et al. 2022, <u>https://doi.org/10.1126/sciadv.abi8716</u>) that showed that emissions from lentic systems were marginal to lotic systems at the scale of the African continent. The same seems to hold from the Amazon basin (Chiriboga et al. 2023, <u>https://doi.org/10.1007/s00027-023-01039-6</u>). Both studies are based on field measurements upscaled at larger scales with GIS so provide quantitative estimates albeit admittedly uncertain.

<u>*Ref: Line by line comments:*</u>

Line 38: this phrase about degassing is not clear to me.

<u>Reply:</u> This will be reformulated – we also expect degassing of CO_2 once the riverine water (oversaturated in CO_2) enters the open lake waters, where higher turbulence due to wind fetch increases the gas transfer velocity.

<u>*Ref:*</u> L61: This sentence is not clear. If the C fixed is not buried or emitted where does it go? Exported as DOC downstream?

<u>Reply:</u> This will be corrected – should have read "20 times higher than organic C burial" – thus indicating fast recycling of phytoplankton biomass.

<u>*Ref: L70: Be more clear why data from Madagascar is valuable in a global sense.*</u>

<u>Reply:</u> We will expand on this aspect.

<u>*Ref:*</u> L90: Specify how the wetlands have been altered.

<u>Reply:</u> We will elaborate on this in the revised version, but the main alteration is conversion to rice paddies.

<u>Ref:</u> L105: This is an important point and isn't totally clear here. Based on the Broothaerts paper there is a huge amount of sedimentation in the floodplain (100x greater than the lake) and a lot in the wetland (10x greater than the lake), leading to very low sedimentation in the lake itself.

<u>Reply:</u> We will expand this section. It is indeed an important aspect that we come back to in the Discussion, and in a follow-up paper that discusses our elemental and stable isotope data in floodplain, marsh, and lake sediment cores in which our data suggest that sediments from hillslope erosion have been trapped mostly in the floodplain and marshes.

<u>*Ref:*</u> *L*130: This sentence is redundant with the earlier part of the paragraph.

<u>Reply:</u> These will be merged.

<u>*Ref:*</u> Figure 2: Is it possible to add how this hydrological difference affects lake level?

<u>Reply</u>: We will verify if historical lake level data can be found; otherwise see Figure 3 where water levels for the outflowing river (Maningory) are shown for 2018-2019; this should be a reasonable proxy for lake water levels (and nicely matches the longer-term outlet discharge in terms of timing and shape).

<u>*Ref: L176: Awkward phrasing- try to rewrite.*</u>

<u>Reply:</u> Point taken, we will rephrase this.

<u>*Ref:*</u> L195: Much more details on lake sampling needed.

<u>Reply:</u> More details will be added in the revised version.

<u>Ref:</u> L298: There is not really much on d15N in the paper. Was it used at all? I recognize the authors are providing a wide range of data that was not used in the paper. Perhaps methods for these analyses should be in a supplement so that they can be used later but do not distract in the main text.

<u>Reply:</u> Indeed, we found no clear use for them in this manuscript but feel it important to put the δ^{15} N data out (as well as some other parameters), as they

might still prove to be useful for other researchers e.g. for data syntheses or meta-analyses. We will modify the Methods to explicitly mention the methodology (measured in the same run along with OC, PN, and δ^{13} C).

<u>*Ref:</u>* Figure 6: The cause of the gaps in data are not clear to me, make clearer here or in the methods.</u>

<u>Reply:</u> pCO₂ was measured in situ (LICOR-820, headspace equilibration) during our own field campaigns, but not by the team performing the regular monitoring for logistical reasons. CH₄, in contrast, was analysed on discrete samples preserved on site and measured later in the lab – hence, for this parameter a more complete dataset is available. For logistical reasons, not all of the rivers sampled during our own field campaigns were monitored regularly, this was only the case for a sub-set of rivers.

<u>*Ref:*</u> L358: It is interesting that %POC is lower in the outflow than the lake. What is the source of inorganic TSM in the outflow?

<u>Reply:</u> This is an interesting point – we will look into this more closely. Note however, that the Maningory data (outflow) include data from regular sampling throughout the year, while the lake samples were taken during a short time periods of the full field campaigns. The CH₄ data indicate lateral inputs from riparian wetlands to the Maningory, which might be one possible hypothesis.

<u>Ref:</u> Figure 9: Is any information on the age-depth relationships in the cores available. The core data in general is not that informative, so maybe it could be more simply summarized as a source of OM.

<u>Reply:</u> Yes, ¹⁴C dating was performed on the M3 core, these are reported in Broothaerts et al. (2022); we will add these data in the text of the revised version. We will carefully weight the options to summarize the core data or keep its current presentation form (the full depth profile) – see also last comment by Ref#2 who asks whether some interpretation of the depth trends can be provided.

<u>*Ref:*</u> L454: Give numerical values (i.e. average plus standard deviation) for the lakes in East Africa being compared to.

<u>Reply:</u> These data will be summarized and added in the revised version.

<u>Ref:</u> L469: In addition to the issues discussed above, if there is high C fixation this can lead to enrichment of d13C in the water column (i.e. a Rayleigh distillation effect) and potentially lead to erroneous estimates based on the fractionation factor.

See for example: Van Dam, Bryce R., et al. "CO2 limited conditions favor cyanobacteria in a hypereutrophic lake: an empirical and theoretical stable isotope study." Limnology and Oceanography 63.4 (2018): 1643-1659.

<u>Reply:</u> In line with the earlier comment on isotope fractionation for phytoplankton, we will re-examine this aspect of the study and include a more critical discussion of factors influencing isotope fractionation between DIC and phyto biomass.

<u>*Ref:*</u> L474: This begs the question: are the differences between the inflow water and the lake/outflow water significant?

<u>Reply:</u> Yes, indeed – for some reason we have not mentioned this explicitly; we will do so in the revised version and will consider an additional graph in the supplement whereby the seasonal data from inflowing rivers and outflow are plotted jointly.

<u>*Ref:*</u> L513: Again, provide numerical values for these other lakes.

<u>Reply:</u> will be included in the revised version.

<u>*Ref:*</u> L517: Need a citation for these data from the Congo.

<u>Reply:</u> Reference will be added.

<u>Ref:</u> L524: I think a more detailed explanation for the connectivity causing high pCO2 and pCH4 is needed.

<u>Reply:</u> We will expand this section of the Discussion in the revised version.

RC2: <u>'Comment on egusphere-2024-2213'</u>, Dailson Bertassoli, 16 Oct 2024

<u>*Ref: General comments:*</u>

This study investigates the carbon biogeochemistry of Lake Alaotra, Madagascar, by analyzing variations in carbon pools, CO₂, CH₄, and other parameters over a complete hydrological cycle. It offers valuable and much-needed data that contribute to advancing discussions on the role of tropical lakes as "biogeochemical reactors." While the authors have made an important effort to underpin their discussion, I believe that some broad generalizations weaken certain key findings of the research. Therefore, I believe this manuscript should undergo major revision or be resubmitted before publication.

The introduction offers extensive details about the study area but lacks sufficient emphasis on the main research objectives, making it difficult to fully understand the rationale of the study. The authors should reconsider the level of attention given to 'lavakas' (l12, l83–l86, l124) and paleoenvironmental interpretations (l108–112), as these topics are not directly connected to their main findings. Additionally, the manuscript would benefit from clarifying the gaps in the carbon cycle that this research aims to address, providing a stronger rationale for the study.

<u>Reply:</u> This suggestion is in line with those of Ref#1, see response there – we will re-organize parts of the Abstract and Introduction (and Discussion) to frame the study more in the context of global/regional C cycling and the role of (sub)tropical lakes. The paleo-environmental proxy context was and remains an important justification for us to have conducted this work, but we will express this better and place it more in the background and link to published and submitted companion papers; we understand the link might not be obvious in the current manuscript.

<u>Ref:</u> The authors mention a "selection of rivers," but the criteria for choosing these two rivers and their representativeness regarding the overall water balance of Lake Alaotra are not clearly explained. Additionally, highlighting these rivers on the map in Figure 1 would improve visualization. There is also some ambiguity about how many rivers were sampled during the high- and low-water field trips. Overall, the sampling scheme and the methodologies used are somewhat unclear and lack key details. For example, what were the water depths at the sampling sites? How were the sampling points in the lake selected? While coordinates are provided in the Supplementary Data, important implications regarding the sampling strategy should be discussed in the main text. For instance, CH_4 concentrations in lakes can vary significantly depending on proximity to the margins. Addressing these aspects would greatly enhance the study.

<u>Reply:</u> These are mostly in line with suggestions from Ref#1 and will be taken into account: more details will be added in the Materials and Methods section, and rivers where sampling was done regularly will be indicated with a separate symbol on Figure 1.

<u>Ref:</u> The results section would be much more reader-friendly with the inclusion of additional graphs and tables. Biplots, in particular, could greatly aid in comparing the ranges of organic carbon across soil, lake, and river samples. In the discussion, although the evidence suggesting that the increase in %POC in the lake is likely driven by phytoplankton input is relatively solid, the text is structured in a way that makes the argument somewhat unclear. A clearer and more focused presentation of this idea would improve the coherence of the discussion. Also, the authors may get interesting perspectives on erosional patterns by delving deeper into the TSM changes.

<u>Reply:</u> Thanks for this suggestion. We will work on some additional plots and consider whether they would fit best in the main text or in the Supplement. In line with comments from Ref#1 on discussing possible changes in %OC during transport of suspended sediments, we will work to improve the line of reasoning to the conclusions in the importance of phytoplankton within the lake.

<u>Ref:</u> My main concern, however, lies in section 4.2. It is important to emphasize that the separation between POC and DOC fractions, based on size, represents an "instrumentalist" approach that overlooks key factors. Most importantly, a primary control on %POC and TSM, the energy of the environment, was severely neglected in the discussion. This, naturally, has significant implications for some of the interpretations. Similarly, degradation, which is also size-dependent, probably play a critical role in shaping the OM δ 13C signatures observed in the system but was not adequately addressed. Additionally, although the conclusion that DOC and POC sources are uncoupled is reasonable, the entire discussion regarding DOC sources seems to oversimplify the system and is not fully supported by the data presented in the article.

<u>Reply:</u> The distinction between POC and DOC is indeed operationally defined. We assume the comment on the energy of the environment is similar to the comment of Ref#1 regarding hydrodynamics influencing particle composition (deposition, resuspension, particle sorting, etc.) -see our reply there. Degradation may indeed have small effects on δ^{13} C – we can include this into the Discussion of the revised version although we do not see that this will fundamentally change our conclusions. However, we are unsure what the reviewer refers to with the statement that the discussion on DOC sources is not fully supported by the data presented – some more details would have been welcome. Either way we will revisit and amend the relevant sections critically.

<u>Ref:</u> Lastly, it would be great if the authors could place the obtained results within a broader regional context, highlighting their implications for the current understanding of the tropical carbon biogeochemistry cycle.

<u>Reply:</u> This suggestion is in line with those of Ref#1, see response there – we will re-organize parts of the Abstract and Introduction (and Discussion) to frame the

study more in the context of global/regional C cycling and the role of (sub)tropical lakes. The paleo-environmental proxy context was and remains an important justification for us to have conducted this work as well as for submitted or published companion paper, but we will express this better and place it more in the background.

<u>Ref:</u> Specific comments:

L12-14: As I read these lines, I thought the main focus of the article to be different. Consider focusing the beginning of the abstract towards the main target of this article.

<u>Reply:</u> See response to previous comment.

<u>*Ref: L26: Not necessarily 'surprising'*</u>

<u>Reply:</u> It was surprising to us. The vast majority of lakes we have studied so far show little or no change in DOC concentrations (and δ^{13} C) from the inflowing rivers. See Discussion on lines 504-517 in the initial version of the manuscript. Unless this is considered a critical point, we would prefer to leave this as it is.

<u>*Ref:*</u> L31: I'm not sure if "was expected" is the right expression here. Do you mean "was found," as in: "δ13C data indicated that marsh vegetation was the main source of net DOC inputs, while phytoplankton likely contributed to POC in the lacustrine waters."?

<u>Reply:</u> Indeed, bad choice of words: we will rephrase.

<u>Ref:</u> L100: I recognize that this study is present in the literature, but its conclusions don't seem well-supported by the findings. I'm not sure if continuing to reference it truly benefits the advancement of science.

<u>Reply:</u> Fair point – we indeed agree that their conclusions are not supported by subsequent studies (and we feel this is clear from the rest of the sentence and the following one); but we can for sure consider to remove it, or to rephrase to make it more clear that this is a disputed claim.

<u>*Ref:*</u> L108-L112: I am not sure how this directly relates to the main subject of the article.

<u>Reply</u>: We feel this is an important justification or aspect of the context of this paper: the lake is situated in a region that has experienced major anthropogenic disturbance and deforestation, and as stated in the introduction one of our aims is also to use lake sedimentary records to reconstruct some of these changes. We would thus prefer to keep this sentence.

<u>Ref:</u> Figure 1: Please consider highlighting which rivers were measured monthly and which were not measured during the dry season.

<u>Reply:</u> We should be able to accommodate this, by using a separate symbol for the rivers that were measured monthly.

<u>*Ref:*</u> Figure 9: Are there any considerations regarding changes in the parameters for different depths that can contribute to your discussion?

<u>Reply:</u> We acknowledge that the data in Figure 9 are currently not discussed in detail: that will be part of a broader manuscript in preparation that discusses similar data from across the floodplain-marsh-lake gradient; and we mainly presented them here since they show %OC and δ^{13} C values from what we consider to be a potentially important 'end-member' source of OC to the lake. Ref#1 suggested to replace this Figure with a summary, rather than present the full profile. We feel there are good arguments for both options (summarize the data. versus keeping the full profiles but add some more interpretation/discussion), and will carefully weight these during our revisions.