Letter of Responses

Author Note: Comments from editors and reviewers are in black and the responses follow in blue. Line numbers in the responses are those in the marked-up revision. Reviewers' comments have been numbered for the easy reading.

Dear Dr. Liang,

Thank you for your careful response to the original reviewers (original R1 & R2) which are appreciated by current R2 and the editorial team (original R1 was not available to reply). As you can see, R3 (current R1) has presented new and valuable comments on your revised manuscript. Could you please now provide a response to R3?

Best regards,
Kate Buckeridge

Response: Dear Dr. Buckeridge, we greatly appreciate the constructive comments from the editor and three knowledgeable reviewers. We are also grateful to you for offering the opportunity to us to revise the manuscript. Detailed point-by-point responses to comments of reviewers are below. We hope you will find our revision satisfactory.

Report #2
Anonymous referee #1:
Comment: I want to thank the authors for their thorough and comprehensive revision of this manuscript. Excellent work! I recommend to accept as is.

Response: We appreciate the reviewer finds our revision satisfactory. We are grateful for the reviewer's constructive comments during the peer-review process.

Report #1
Anonymous referee #3:
Overall Comments:

Note that I am newly reviewing this paper after the first round of revisions. This paper uses isotopically labeled glucose additions to investigate POM and MAOM formation from dissolved C. It finds evidence for dissolved C contributing to POM, in opposition to the widely cited and evidenced two-pathway model (Cotrufo et al., 2015). This is an exciting finding and I think the topic of this paper is very timely and addresses an important uncertainty in the SOM literature. However, I felt some of the methodological choices require greater justification and the presentation of the results could be more clear:

Response: We appreciate the reviewer's recognition and constructive comments on our manuscript. Based on the reviewer's suggestions, we have revised the manuscript thoroughly. Please find the point-by-point responses below. We hope that the reviewer will find our revision satisfactory.

Comment 1: Particularly, it was unclear to me why the authors focus on heavy POM but do not carry out a fractionation scheme that provides a heavy POM pool. I see this is addressed in the response to reviewers but it is important that the authors make it clear why they did this in the methods.

Response: We appreciate the reviewer's suggestion to add justification for our choice in the Methods. In the revision, we have added more details about why we isolated heavy-POC in the modeling analysis (lines 143-147) but not in the incubation experiment (lines 117-122):

Line 117-122: "*The experimental design was a trade-off between the incubator's space (which determines the jar volume and soil samples in jars) and the total number of jars. With the limited samples, we decided to do the size separation (i.e., POC vs. MAOC) instead of the density fractionation because we anticipated that the size separation might provide more insights into SOC dynamics and is more related to microbial processes according to the literature (Lavallee et al., 2020). Despite that, both light-POC and heavy-POC were included in the following modeling analysis to broaden the implication of the experiment.*"

Line 143-147: "*Models designed based on the data of soil C pools and CO_2 emission fluxes in the incubation experiment. Here, it is important to note that in both soil C models, POC is divided into two pools: the light-POC and the heavy-POC. This is because heavy-POC is a plant residue-microbial product-soil mineral complex, which is more likely to be the destination of dissolved C inputs than light-POC, which only comprises plant residues (Samson et al., 2020). Therefore, the two POC pools were modeled separately.*"

Comment 2: Additionally, I am confused why the authors carry out DOC and DOC+POC addition experiments in their model – it is unclear what question this is addressing. This requires justification so it is more clear what the reader is supposed to learn from this work.

Response: We set up those two C input scenarios based on the incubation experiment as well as the natural C input conditions. Firstly, the "DOC input only" scenario was set to be consistent with our incubation experiment, which only included dissolved C input (i.e., glucose). In the absence of structural C inputs, newly formed POC only originates from dissolved C input, which allows us to determine directly how the process of dissolved C flow to POC impacts C sequestration. Secondly, the "DOC+POC input" scenario was set up to mimic the natural C input in the field. This scenario illustrates that even with structural C inputs, the process of dissolved C flow to POC can still have a significant impact on C sequestration. In the revision, we added more description of these two C input scenarios:

Line 180-183: "*For each model, we set up two C input scenarios. To fit with the incubation experiment including dissolved C input only, we set up the scenario of "DOC input only." To make the prediction closer to the natural C input in the field, we set up the scenario of "DOC+POC input.'*"

Comment 3: In the title, abstract, discussion, and conclusion, there is large emphasis on the impacts of long-term C sequestration but is unclear if the study evaluates long-term C sequestration. Without this evaluation, I don't believe this claim is supported and it would be useful to use more cautious language and consider that sequestration implies storage over a long period, rather than solely new formation of soil C. Throughout the paper, C sequestration can often be replaced with C pools or stocks for concentrations and area-based estimates, respectively.

Response: We appreciate reviewer's suggestion to be careful in using the term *C Sequestration*. We agree that C sequestration is a long-term process. We are aware that results from the incubation experiment may not be appropriate to demonstrate the change in C sequestration. That is one of the reasons we used the model analysis, in which we ran the model to the equilibrium (i.e., the steady state after 500 years).

We do value the reviewer's suggestion. In the revision, we have revised thoroughly to avoid C sequestration when describing the results of incubation experiment and replace it with new C formation. In the modeling analysis, we hope the reviewer would agree that using C sequestration is appropriate to describe the long-term SOC simulation. Adding the process of dissolved C flow to POC leads to a net increase in the predicted SOC stock.

Comment 4: Finally, the main claim of the work, that dissolved C contributes significantly to

POC, seems to be undermined by the result that added DOC represented less than 1% of total POC pool – this should be addressed more clearly to ensure the conclusions in the paper hold true to the data.

Response: Glucose-derived POC is indeed only a small part of the total POC pool in the incubation experiment. because the experiment was designed based on the annual C input relative to soil microbial biomass in the studied sites, which is a widely used methodology (e.g.,Ghee et al. (2013); Zhang et al. (2016)). Compared with the initial SOC, glucose is added in a small amount, 0.4 mg C g^{-1} soil, which only accounts for 0.67% ~ 5.70% of the initial SOC content (varying with sites). Even though glucose has a great transformation rate, the C sequestration amount is relatively small in the experiment. Similarly, when we focus on the amount of glucose derived-MAOC as a percentage of total MAOC (0.26% ~ 1.46%), this is also a small value because it is constrained by the limited C input. Therefore, we consider that newly formed C accounts for only a small fraction of the total POC is reasonable.

Compared to the ratio of glucose-derived POC to total C pool, we focus more on the proportion of glucose derived-POC to total glucose-C, because it fully represents the distribution of the newly added C between the different C pools. This proportion is relatively larger and more pronounced, i.e., 1.58% ~ 28.00% of the dissolved C input enters the POC pool. Therefore, we consider that this data is sufficient to support our conclusion that dissolved C contributes significantly to POC.

Specific Comments:

Comment 5: Line 37: I believe the authors mean “coarse MAOC”, not “course MAOC”.

Response: We appreciate the reviewer pointing out spelling errors. We have revised it as suggested.

Comment 6: Lines 43-45: This sentence is unclear. I think the authors mean to say that heavy POC may be a precursor to MAOC, given heavy POC is a combination of plant residues, microbial products, and soil minerals?

Response: Yes, our intent is consistent with the reviewer's understanding. We have rewritten the sentence to more clearly describe the process from heavy-POC to MAOC in the revision (lines 35-41).

Line 35-41: "*During the gradual decomposition of plant residues by microorganisms, the surface of fresh litter combines with soil minerals in the presence of microbial products (which act as binders in the soil), forming heavy-POC (or coarse-MAOC, >53 μm and >1.6 – 1.85 g cm^{-3}) (Samson et al., 2020). The plant-soil interface in heavy-POC promotes the formation of soil aggregates directly, becoming the hotspot for SOC formation (Witzgall et al., 2021). Meanwhile, given that heavy-POC is a combination of plant residues, microbial products, and soil minerals, and that heavy-POC has a similar C:N ratio to MAOC, it is reasonable to hypothesize that the fragmentation of heavy-POC promotes the formation of MAOC and is the precursor for MAOC (Samson et al., 2020; Zhang et al., 2021).*"

Comment 7: Line 46: Note that MEMS 2.0 no longer contains this pool so I am not sure this line should remain in the manuscript. See Zhang et al., 2021. Zhang, Y., Lavallee, J.M., Robertson, A.D., Even, R., Ogle, S.M., Paustian, K., Cotrufo, M.F., 2021. Simulating measurable ecosystem carbon and nitrogen dynamics with the mechanistically defined MEMS 2.0 model. Biogeosciences 18, 3147-3171.

Response: We appreciate the reviewer for reminding us that the heavy-POC pool is no longer included in MEMS 2.0. We have removed the sentence as the background of our research in the revision.

Comment 8: Line 47: Dissolved C input from living roots are included as rhizodeposits right?

Response: We agree with the reviewer that dissolved C input from living roots is included as rhizodeposits. We have revised the text in lines 48-49: "*Dissolved C input from living roots (i.e. rhizodeposits), which has a dominant effect on the net formation of SOC, is considered approximately 2 to 13 times more efficient than litter inputs in forming SOC.*"

Comment 9: Line 70 and throughout: I believe this should be "fenced and grazed grasslands" to be grammatically correct?

Response: Revised as suggested. In the revision, we replaced "fencing and grazing grasslands" with "fenced and grazed grasslands".

Comment 10: Line 83: I am not following this last hypothesis – increased microbial C use efficiency from microbial use of DOC that then becomes POC or of POC that becomes MAOC?

Response: The final hypothesis has been modified to focus more on modeling analysis results instead of microbial C use efficiency, which would lead to a clearer and less ambiguous statement (lines 79-80). We hope this will help the reader better understand our hypothesis.

Line 79-80: "*Finally, neglecting the process of dissolved C flow to POC leads to an underestimation of SOC sequestration.*"

Comment 11: Lines 112 and 135: I am confused by the authors use of a size fractionation when they seem to be interested in heavy POC dynamics, given the modeling. As noted above, there should be justification for this choice in the methods.

Response: We appreciate the reviewer's suggestion to add justification for our choice in the Methods. As response to Comment 1 above, we have added the justification in the revision.

Comment 12: Line 164: Were the two modeled POC pools summed together for calibration?

Response: Yes, since we only measured the size of total POC, heavy-POC pool and light-POC pool were summed together for the calibration.

Comment 13: Line 173: It is unclear why the authors choose these 100 parameter sets for further work. It is my understanding that the calibration step explained above this line provides the parameter values.

Response: In the modeling experiments, we randomly choose 100 sets of parameters is to reduce the cost of computation. During the calibration, we set up 30,000 times of simulations. Based on the reception rates at different sites, the model ended up receiving about 9,000 to 15,000 sets of parameters. In the later simulation experiments we run the model for 500 years until the steady state, but using all parameter sets would be time-consuming. Therefore, we randomly select 100 sets of parameters in the subsequent predictions. Randomly selecting a subset of parameters is proven to be representative to reproduce the original parameter distributions and also improves the simulation efficiency (Xu et al., 2006; Liang et al., 2018).

Comment 14: Line 174: It is unclear why there are two input scenarios – what question is this testing?

Response: We appreciate the insightful comment. The two scenarios we set were to test how the process of dissolved C flow to POC affects soil C sequestration under the both experimental and field condition. In the revision, we added more description of these two C input scenarios. Please see our detailed response in Comment 2 above.

Comment 15: Line 178 and throughout: As noted above, it is unclear how the authors are testing "long-term SOC sequestration". Are the models being run forward for many years? If so, how much trust can we put in models that are so specifically parameterized? I think the models in this work can help us understand mechanisms but given parameterization was done for individual sites and treatments, the models are not very generalizable. Please consider the definition of sequestration in Don et al., 2023 when using this term.
Don, A., Seidel, F., Leifeld, J., Kätterer, T., Martin, M., Pellerin, S., Emde, D., Seitz, D., Chenu, C., 2023. Carbon sequestration in soils and climate change mitigation—Definitions and pitfalls. Global Change Biology, e16983.

Response: Based on Don et al. (2023), we have carefully re-examined the use of *C sequestration* in the manuscript. In the modeling experiment, models were run for 500 years to reach a steady state after calibration. The results show a net increase in the SOC prediction after adding the process of dissolved C flow to POC, which is consistent with the definition of C sequestration. Meanwhile, we agree that our models are too specifically parameterized to be generalizable. However, given that the data from all 10 sites had similar results in the model, we hope the reviewer would agree that it is reasonable to state that it can help us understand mechanisms of the dissolved C flow to POC on C sequestration.

Comment 16: Line 203: As noted above, less than a percent is a small contribution – does this not undermine the conclusions that DOC flows to POC and contributes to C sequestration?

Response: We appreciate the constructive comment. Please see our response to Comment 4.

Comment 17: Line 209: I recommend creating a modeling results section and making the above section (3.2) an incubation results section.

Response: We appreciate the reviewer's suggestion on creating a new section for the modeling results. In the revision, we moved the results of modeling analysis to Section 3.3, *modeling analysis of soil C dynamics*.

Comment 18: Line 213: The absence here is the comparison of Model 1 to Model 2, correct? Please clarify.

Response: Yes, the absence of f_{HB} here is the comparison of Model I to Model II. To clarify, we rewrite the original sentence in revision (line 226): "*Compared with Model II, the absence of the* f_{HB} *in Model I affects other parameters differently.*"

Comment 19: Line 234: Is this 36% of the glucose C in POC and MAOC? Line 203 suggests a much smaller amount of the total C pool is glucose-derived POC. Also, I would say this is POC and MAOC formation – sequestration requires evaluating POC and MAOC over time (again see Don et al., 2023).

Response: In the previous marked-up revision, 36.49% in the Line 234 was calculated through $\frac{glucose\ derived-POC}{glucose\ derived-POC\ +\ glucose\ derived-MAOC}$. It is not same with the proportion in the Line 203, with is calculated through $\frac{glucose\ derived-POC}{total\ POC}$. We were attempt to show the proportion of 36.49% to illustrate that the pathway from DOC to POC accounted for a significant proportion of the SOC sequestration. In the revision, to make the statement clearer, we have removed this sentence.

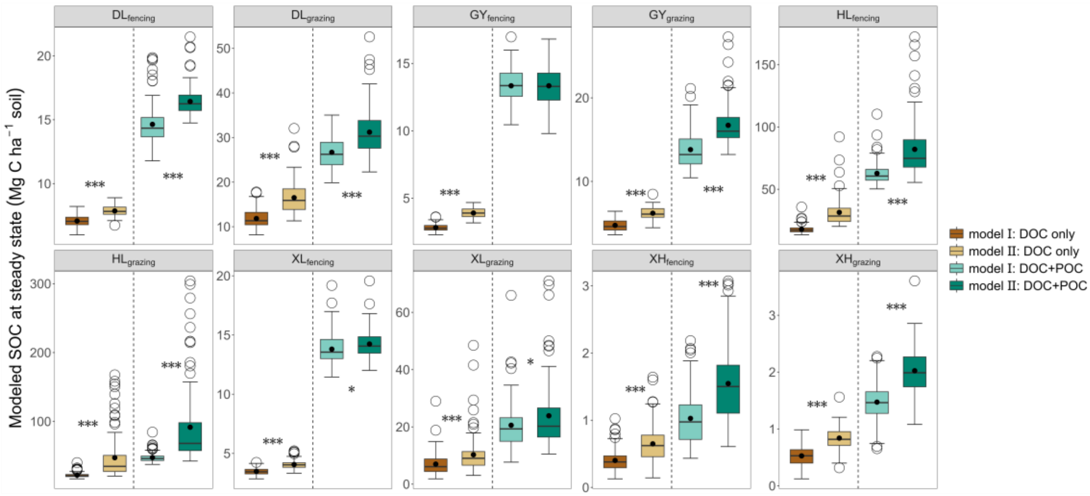
Meanwhile, we agree that changes in the C pool observed in short-term incubation experiment belongs to C formation rather than C sequestration. In the revision, we have revised thoroughly to avoid using the term C sequestration to describe the results of incubation experiment.

Comment 20: Line 262: I would say "potential usefulness" rather than "necessity". I don't think one experiment justifies adding this process to SOC dynamic models.

Response: Revised as suggested (line 273).

Comment 21: Figure 4: Please make the outliers for each boxplot more visible, either by outlining in black or making them black dots.

Response: In the revision, we have marked the outliers in Figure 4 and Figure S8 in black, hoping to make it clearer to the reader.



Revised Figure 4: Modeled SOC content at steady state under two types of C input conditions. The two different C input scenarios for each site are separated by a dotted line. The upper and lower ends of boxes denote the 0.25 and 0.75 percentiles, respectively. The solid line and dot in the box mark the median and mean of each dataset. The open circles denote outliers. Asterisks represent significant differences between Model I and Model II (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Reference:

Don, A., Seidel, F., Leifeld, J., Kätterer, T., Martin, M., Pellerin, S., Emde, D., Seitz, D., and Chenu, C.: Carbon sequestration in soils and climate change mitigation—Definitions and pitfalls, Glob. Change Biol., 30, https://doi.org/10.1111/gcb.16983, 2023.

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Lavallee, J. M., Soong, J. L., and Cotrufo, M. F.: Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century, Glob. Change Biol., 26, 261-273, https://doi.org/10.1111/gcb.14859, 2020.

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Zhang, H., Ding, W., Luo, J., Bolan, N., Yu, H., and Zhu, J.: Temporal responses of microorganisms and native organic carbon mineralization to 13C-glucose addition in a sandy loam soil with long-term fertilization, European Journal of Soil Biology, 74, 16-22, https://doi.org/10.1016/j.ejsobi.2016.02.007, 2016.

Zhang, Y., Lavallee, J. M., Robertson, A. D., Even, R., Ogle, S. M., Paustian, K., and Cotrufo, M. F.: Simulating measurable ecosystem carbon and nitrogen dynamics with the mechanistically defined MEMS 2.0 model, Biogeosciences, 18, 3147-3171, https://doi.org/10.5194/bg-18-3147-2021, 2021.