Overall response to Reviewer #2's comments:

We sincerely appreciate your positive feedbacks and constructive suggestions, which significantly improve the overall clarity of this manuscript. After carefully considering your and Reviewer#1's suggestions, we decide to split this MS to 2 separate papers, with the current revised MS mainly focusing on the optimal pretreatment approaches for RPO analysis. Accordingly, some of the suggested changes concerning the sections or statements of bulk OC and $\delta^{13}C$ would not appear in this revised version, but will be incorporated in a second paper specifically on acidification protocols for bulk measurements. In response, we have made extensive revisions to address all the concerns as listed below.

(1) First, we have highlighted the ramped-temperature pyrolysis/oxidation technique and strengthened its connection to acid pretreatment. The introduction section has been reorganized to comprehensively illustrate the methodological impacts on RPO results caused by different acid pretreatments. The title of this manuscript has been changed to "**Technical note: Assessing pretreatment approaches for serial pyrolysis-oxidation analysis of sedimentary organic carbon**". Detailed discussion regarding bulk OC and $\delta^{13}C$ has been removed to streamline this manuscript and to highlight the pretreatment for RPO as the primary focus of this study. Additionally, the schematic of RPO instrument has been added in the supplementary file for readers to get a quick understanding of this technique.

(2) Second, we have carefully improved the overall quality of the manuscript by reorganizing and rewriting. We extensively rewrote the Abstract, Introduction, and Conclusions sections to emphasize the main focus of this study is the optimal acid pretreatment for RPO analysis. The overall length of this manuscript has been significantly reduced by removing the introduction and discussion on bulk OC and $\delta^{13}C$ measurements as well as moving Table 1 and 2 into the supplementary file. Language and clarity throughout the manuscript have been significantly improved through careful editing. The complex sample IDs and method codes have been thoroughly rephrased to more accurate and direct expressions.

We believe these revisions would adequately address reviewers' comments while preserving and highlighting the merits of our work. Now this manuscript presents more clarified and well-organized version with coherent narrative throughout. We are grateful for your thorough suggestions and for considering this manuscript for publication in Biogeosciences.

General comments

I think this is a good study but could do with more information such as C/N, FTIR etc. And I would have liked to see the results of what was lost (since you did talk about the supernant)

RE: Thank you for your positive feedbacks. According to your and R1's comments, we decide

to split the original submission into two papers, with the current revised version focus specifically on how acidification affects RPO results. The rest part, especially bulk data, will be supplemented with additional data (e.g., C/N and FTIR) for the preparation of a second paper on the optimal pretreatment protocols for bulk measurements. Accordingly, we fully agree that other proxies (e.g., C/N and FTIR) can be very informative to reveal what was lost during acidification. As the bulk results are no longer the main content in the current revised MS, we decided to include potential more proxies/measurements in the second paper to better support our bulk measurements. Now this revised manuscript highlights the optimal pretreatment conditions for RPO analysis.

The paper is very long and hard to follow.

RE: Thank you for the general comment concerning the length and clarity of this manuscript. After splitting this manuscript into two separate papers, we substantially revised this manuscript to align better with the objectives of this current study. Specifically, we:

(1) Removed the discussion on the bulk results (Section 3.1) and focused on the RPO part.
(2) Revised Table 1 to only include major variables (Acid fumigation and acid rinsing as well as the HCl concentration for rinsing) in the MS. Other minor variables have been moved to supplementary material to complement our overall conclusions.
(3) Moved the second Table (Table 2) to the supplementary material as bulk parameters are no longer the main focus of this paper.
(4) Shortened/Revised the Abstract, Introduction and Conclusions to highlight our main results.
(5) Moved methodological details of $CaCl_2$ addition experiment to the Method part in order to streamline the Discussion section.

I would suggest reducing and making concise, and bringing the RPO and acid experiment together instead of dealing with them separately that makes it hard to evaluate.

RE: The manuscript has been intensively shortened after being split into two separate papers. Unnecessary expressions have been revised/removed according to your specific comments. The discussion on bulk results has been removed to make the structure more concise. In addition, we emphasize this paper is mainly about impact of acidification methodology on the RPO result to reduce likely misunderstanding.

R1's suggestion of 2 papers seems like a good idea to me. There are good results here but I think the paper itself is confusing and hard to read due to sample codes, jumping back and forward for different methods, and the lack of linkage between the acid/RPO experiments.

RE: We appreciate your and R1's suggestions to separate this MS into 2 papers. Upon careful consideration of your comments, we decided to have this MS focus only on RPO results. The

structure of the paper has been correspondingly reorganized to avoid confusing sample codes and jumping discussions.

Nonetheless, the last part of the discussion and the TOC-based normalization are dependent on the bulk data (i.e., TOC) of each individual sample. Considering this, we provided the bulk data (Table 2, now as Table S2) in the supplementary material.

I have commented on the attached pre-print.

RE: Thank you for your thorough examination and careful comments. We have carefully addressed all your suggestions/concerns. All comments in the attached file have been organized in order and responded in the line comments below.

Line comments

p1, L29, "Not the best wording to use here".

RE: This sentence has been eliminated in the revised MS.

p2, L31-32, "such as - some examples and references needed here.".

RE: This statement has been eliminated in the revised MS. Detailed introduction of examples have been included in the introduction of the revised MS.

p2, L36, "Some studies use EDTA to reduce OC loss.".

RE: We respectfully appreciate your suggestion that some studies use EDTA. However, we have not incorporated this point in the revised MS for a few reasons. First, it is not a common protocol for acidification in most studies and is not within the scope of this paper. Second, EDTA is a widely used chelating agent for metals while its role in stabilizing OC and reducing OC loss at low pH has not been well illustrated. Third, some previous studies found that addition of EDTA can lead to extra loss of fulvic compounds (Jez et al., 2021). Thus, to avoid underlying misinterpretation, we do not include this point in the revised MS.

p2, L36, "Get rid of semi colon here. These are two sentences.".

RE: Now has been revised according to your suggestion.

p2, L39, "may or can".

RE: "might" is changed to "can".

p2, L40, "These references are all quite old. There are recent studies available, https://scholar.google.com/scholar?as_ylo=2021&q=poc+acid+radiocarbon+isotopes&hl=en&as_sdt=0,5 a quick search. Druffel particularly

References old throughout"

RE: Thank you for your suggestions. Some recent papers on acidification effect and RPO have been added throughout the MS.

p2, L40, "thought to. The language throughout is a little clunky, better words could be used for readability. Small edits for this though."

RE: Done as suggested. The language of the MS has been improved according to your comments.

p3, L67, "I agree with the other reviewer that the sample names are very confusing, as are the EC labels.".

RE: Now has been addressed throughout the context. According to your and R1's suggestions, sample IDs are not specified. SR1/2 and Sed1/2 are used to present four samples. The methods and the Table 1 in the main text have been refined to mainly focus on major variables in the discussion section. EC labels have been removed and replaced by direct expressions throughout the MS.

p3, L71, "how? by hand or in a proper machine that really does homogenize?".

RE: Two sediments were ground by agate mortar and pestle until no coarse grains were observed. Two sedimentary rocks were ground by zirconic puck mill. Throughout the experiments, samples were homogenized by hand for several times to avoid artificial biases before acidification. Machines were not used due to limited amount of sample and anticipated loss during processing. To verify our findings, a part of samples (e.g., SR2 acidified by 2N acid rinse) were processed parallelly 2-3 times during a one-month interval. The reproducibility of RPO thermograms is always good, suggestive of insignificant bias from sample heterogeneity.

p3, L87, "Did you analyse the supernatant? Ignore if below - it would be nice to say what happened with the supernatant afterwards. Maybe you could have counted the loss of OC in the supernatant, with your methods there was bound to be OC in it. If you did this below state something here. If not, it's maybe something to think about for a repeat experiment (but not essential really for this paper IMO, Ed's decision).".

RE: Thank you for your valuable suggestions. It is of great significance to analyze the supernatant to indicate the amount and properties of OC leached to the solution. It can be a very good perspective to compare the residual OC in the acidified sediments with soluble OC in the supernatant (to see if the loss of labile OC increases with HCl concentration). A couple of previous studies showed very interesting results about the supernatant as well.

The analysis of supernatants has not been conducted by far for the processed samples. Although it is improper to include all the results (sediments and supernatants, bulk and RPO) in this paper, the supernatant results can be expanded to another comprehensive study with bulk parameters

and other analyses as you suggested. Therefore, we agree with you about the merits of analyzing OC in the supernatant but decide not to include the result in this paper.

p4, L107, "Another word that isn't suitable here.".

RE: The word "impotent" has been revised to "inefficient". The sentence has been rephrased according to another reviewer's suggestion.

p4, Table 2, "Without more obvious codes that shows what the samples are, this is way to hard to really interprate without going back and forth."

RE: The sample codes have been simplified according to your and R1's suggestions. As the MS has been revised to focus on RPO results, Table 2 is moved to the supplementary material. Without confusing sample codes and too much data displayed, the clarity and readability of this paper can be improved. After revisions, the interpretation of the data would be basically based on RPO thermograms in the figures without too much effort to move back and forth.

Also, you present the RPO stuff here, when most of the above is about Acid methods. I agree with R1 that this should maybe should be two papers, or else change it to balance the acid methods with the RPO methods."

RE: This paper has been reorganized to mainly focus on RPO results. It is necessary to emphasize that bulk and RPO analysis were conducted concurrently for each acidified sample. For instance, ~ 500 mg of a raw sample was acidified, dried, and ground into powder. 20 mg of acidified sample was used for TOC and $\delta^{13}C$ analysis while 100 mg of the same, acidified sample was used for RPO analysis. Therefore, bulk and RPO results present the OC properties of the same sample. In tandem, two different analytical tools can reveal the impacts of acidification on OC properties from different aspects.

Despite the discussion above, we fully agree with your and R1's comments that two parts in the original MS (bulk data and RPO data) were not well joined and transition from TOC and $\delta^{13}C$ to RPO data interpretation lacks a smooth connection. One of the problems is the introduction and method sections were primarily about acidification and bulk parameters. To address it, the introduction section was rephrased to focus more on acidification and the understudied impacts of acidification on RPO data interpretation. We also greatly appreciate your insightful suggestions to incorporate bulk results and more analytical tools into another paper in the future.

p7, L131, "It's not pyrolysis then it's ramped combustion. which is fine, and probably a good choice, but you need to change the term."

RE: Thank you for the reminder. Yes, it is ramped combustion with oxygen supplied in this study. The term "charring" here highlights that the RPO system in this study is supplied with oxygen to avoid charring as observed in other studies using pyrolysis. Yet, it is not clearly

clarified in the MS. To prevent confusion, we have revised the sentence and added another relevant reference to improve the clarity, as:

"This sub-oxidation mode was consistently adopted in this study to circumvent possible charring during pyrolysis as illustrated by previous studies (Huang et al., 2023; Stoner et al., 2023; Williams et al., 2014)."

p9, Fig 1, "Have the labels changed? Again, it's hard to read this without going back and forth. I actually have two copies open so I can look at them without going back and forth, it's really not easy to interpret - and with so many methods maybe another reason to spilt the paper."

RE: The labels have been changed as mentioned above. We fully agree with you that the labels and methods here are confusing and hard to follow. As the focus of this paper is about RPO results, this figure has been removed due to limited discussion on it. For the rest of this paper, sample and method codes have been consistently revised to enhance readability. We agree with your idea that splitting the paper may be a good choice.

p10, L201, "I would actually would have liked to see C/N results to show what changes in that would be. It would show more about what is lost. Again, looking at the supernant or what was lost would have been good.".

RE: We fully agree with your suggestions. Detailed elemental composition (e.g., C/N) of acidified sediments can be more informative than TOC and $\delta^{13}C$. The further analysis of residual sediments and the supernatant would be a very interesting part to see in the next stage of the experiment. Hopefully, it can be expanded into another comprehensive study. In this specific manuscript, this part of discussion has been removed. Nonetheless, considering your constructive suggestions, some discussion about the bulk parameters has been incorporated into the implications in the revised MS.

p10, L211, "I would have expected this as the labile OC would have been mostly lost with acid treatment that would not really have been caught by bulk isotope results, which are a mean of many mixtures so not really informative.".

RE: Thank you for the insightful comment. The loss of labile OC is expected during acid treatment, which is supported by the TOC data. However, the changes in bulk isotope values are not significant in this study, since the bulk isotopes are a mean of many mixtures as you suggested. This point has been mentioned in the introduction and conclusion sections to highlight the merits of utilizing RPO analysis to reveal what is lost during acidification. In addition, conducting analysis of supernatant can provide essential information about the amount and the properties of acid-soluble OC.

p11, Fig. 2, "codes again, I've forgotten which samples are what again. The caption could

be more informative.”.

RE: The legends have been revised throughout to be more informative (e.g., 1 N rinse instead of EC-1) to directly relate thermograms to experiment conditions.

p11, Fig. 2, “It's also hard to see the difference in colours, especially if printed out in black and white if anyone does that anymore.”.

RE: We agree with you the differences in colors are insignificant, making it hard to distinguish. All figures in the revised MS have been refined with more contrast colors to highlight the differences.

P13, “The paper is very long, at this point I had to take a break to get back to is. I do think this should be reduced or split into two papers.”.

RE: We agree with you this paper is too long to follow. As suggested above, we have split this paper into two and mainly focus this revised version on RPO results. Accordingly, some introduction and discussion of bulk parameters have been removed to improve the flow of this paper.

p13, Figure 3, “As someone who is lightly colourblind I can't see the differences in these lines at all.”.

RE: The colors of the lines in Figure 3 (now Figure 2) have been changed to blue, orange, and purple. The selected colors are expected to be colorblind-friendly with sufficient contrasts.

p14, L291, “I thought you said above that it didn't affect it with the catalysts in the furnaces?”.

RE: This point was exactly mentioned in Sect. 2.4 but in a different way. For samples after acid rinsing, most of $CaCl_2$ was removed through repeated water rinsing. On the other hand, $CaCl_2$ remained in sediments after acid fumigation. According to the experiments in this study and the results of other tests in our laboratory, too much $CaCl_2$ in sediments (basically for acid fumigation) is corrosive for the catalytic wires. There were several lines of evidence to support it. First, the lifespan of catalytic wire in RPO system significantly decreases from several months to less than a few days after processing $CaCl_2$-containing samples. Second, the yield of CO_2 declines significantly after the corrosion of catalytic wires, which was also mentioned in the paper. Third, as shown in the supplementary material, there were some black matters (likely black carbon due to incomplete combustion) adhering to the quartz reactors after processing acid fumigated samples. Overall, $CaCl_2$ does have impact on the catalytic wires, which should be noticed and carefully considered in RPO analysis.

p14, L291, “If it does really effect the method (I'm actually surprised that you got graphite from some of these) this is an impact that people should know about. It's buried here and

if papers were separate it would be easier to see.".

RE: Thank you for your insightful comments. As you suggested, this is an important issue that should be noticed. Since there were detailed discussion on the corrosive effect of $CaCl_2$ in the main text (e.g., L310-316), it would be clear after the paper is split as you suggested.

p14, L294, "FTIR or some other method to show what was actually in the sample would be informative".

RE: Thank you for the valuable suggestion. FTIR analysis would be very useful to reveal the OC composition and organo-mineral interactions in the sediments and is likely informative to link the laboratory observations to specific mechanisms. We would like to incorporate this point in the second paper in the future.

p14, L302, "I think that the paper jumps around too much, this is back to methods again. I think even with two papers, this needs to be cut down and reorganised."

RE: We agree with your concern regarding the coherent narrative of the structure. The detailed description of the $CaCl_2$ addition experiment has been moved to the methods part as subsection 2.6.

p14, L315, "so basically don't use RPO on samples treated by acid.

Or is there a solution with changing the catylist? Like Ag for S?"

RE: Acid treatment is basically needed for RPO analysis to remove inorganic carbon. Considering the likely influence from inorganic carbon on data interpretation (e.g., Sed2 in this study, Supp Fig. S5), acidification is typically adopted to remove IC. According to the comparative experiments in this study, acid rinse with 1 N HCl is generally a good approach to maintain the relatively pristine OC properties. Acid fumigation is not recommended for RPO analysis as too much $CaCl_2$ would bias the result.

Changing the materials of the RPO catalytic wires may be an alternative choice. Yet, it is hard to say whether the performance of the new catalytic wire and the robustness of results can be improved significantly. Additionally, the effect of chlorine on catalysts is evident and the possible solution to this is tricky though. Therefore, the good choice at present is to improve the acidification and other pretreatment protocols for RPO analysis. We suppose the influence of Cl or S on the catalytic wires can be illustrated by SEM or other analyses. The advance of RPO system is largely dependent on the detailed analysis.

p17, L346, "what about the same for RPO?".

RE: The conclusion part has been revised to focus more on RPO. Besides, we incorporate some insightful points (something like implications) according to your comments. The conclusion

part has been further revised according to your and R1's suggestions.

All your suggestions and concerns have been carefully considered and addressed. Thank you for your time and effort invested in reviewing our MS.

References cited

Jez, E., Bravo, C., Lestan, D., Gluhar, S., Martin-Neto, L., De Nobili, M., and Contin, M.: Changes in organic matter composition caused by EDTA washing of two soils contaminated with toxic metals, Environ. Sci. Pollut. Res., 28, 65687–65699, https://doi.org/10.1007/s11356-021-15406-z, 2021.