Dear Mr He and Profs Yang and Cui,

Thanks again for your replies for comments and suggestions raised during the first review of your manuscript and for the revised version. Following the splitting of your manuscript and focusing the present manuscript on the pyrolysis oxidation part, I have sent your manuscript for another review. We have received three reviewer reports this time, including one reviewer who has reviewed this paper before. All reviewers are very positive about your manuscript and I agree with their feedback. Based on their comments and suggestions, only minor revision is required, which will further improve your manuscript. Therefore, I invite you to address the feedback that has been received before I make a final decision.

Thanks for these changes and looking forward to your revised manuscript.

Kind regards
Dr Sebastian Naeher
Associate Editor, Biogeosciences

RE: We sincerely appreciate your assistance in processing our manuscript and are grateful for your consideration of our work for publication in Biogeosciences. We have carefully addressed all the specific suggestions and concerns raised by three reviewers. Please find our point-to-point replies to each reviewer in the response file and the revised manuscript.

Referee #1

This revision of the MS very successfully clarifies that the MS is about carbonate removal methods prior to RPO analysis. The text is much clearer and reads well with some editorial changes needed. Specific suggestions are included below.

RE: We sincerely appreciate your positive feedback and constructive suggestions. Please find our point-to-point replies to your specific suggestions below.

Late in my review I read the response letter and learned that the TOC and carbon isotope data might be published later. That is great. I have an issue with Line 52 of the MS which states "Using RPO analyses supplemented with bulk measurements (TOC and δ13Corg), we assess the potential…" – BUT the only actual mention of carbon isotope data is in Table S2. There is no discussion of the carbon isotopic data in the manuscript. The issue can be solved by REMOVING "supplemented with bulk measurements (TOC and δ13Corg)," from Line 52. If the portion of the above sentence is removed, the data can be left in the MS even though it is not discussed.

RE: We agree with your comments and believe that TOC and δ^{13}Corg results would be interesting as well in a future paper. In the sense of current paper, we decided to focus on RPO result, which is not closely related to TOC and isotopic data. Therefore, we have removed the misleading expression in Line 54 according to your suggestion.

In a future paper I would like to see the statement seen in the original MS Conclusions that rinsing of high carbonate samples with liquid acid was the best pretreatment for carbon isotopic analysis of organic carbon. I actually referred people to the pre-print with this in mind. I would like a paper to easily refer people to in the future.

Jason Curtis, Sept 17, 2025

RE: Thank you for your thoughtful consideration and positive evaluation of our work. Although the detailed discussion about TOC and isotopic results is not included in the current paper, we think with some other additional analyses it will make a great scientific contribution accordingly.

Line 7 - Remove "The" – very first word in the abstract

RE: Corrected in Line 7.

Line 8 – Be clear as to what "characterizing sedimentary organic carbon" means.

Concentration? Labile versus refractory? Isotopically? Etc.

RE: Thank you for reminder. It has been revised to "characterizing sedimentary organic carbon provenance and reactivity".

Line 8 – Same as above. “bulk carbon and molecular-level analyses” Bulk what, molecular what analysis. See similar comment for line 101.

RE: We have revised it to “bulk carbon isotopic measurement and molecular-level biomarker analyses”.

Line 15 – “Notably” not quite the right word. Maybe “Generally, results from…”

RE: Done as suggested in Line 16.

Line 37 Remove - “Moreover”

RE: Done as suggested in Line 38.

Line 68 – “additionally” should be removed

RE: Done as suggested in 69.

Line 69 needs work “and temperatures, the potential influence of heating” Maybe “…and drying temperature to understand potential influence…”

RE: We intended to convey the potential influence of reaction temperature rather than drying temperature. To resolve this, we have revised this sentence to “and temperatures, heating during decarbonation and prolonged exposure to concentrated acid.”

Line 70 “furthermore” is not the right word. Use “Additionally” or similar

RE: Corrected in Line 71.

Line 79 – “to be neutralized” should be “until neutralized”

RE: Corrected in Line 80.

Line 80 – what mass of sample?

RE: For acid fumigation, we basically used the same sample mass as acid rinsing to ensure sufficient sample for measurements. The phrase “(> 200 mg)” has been added to state the sample mass we used.

Line 83 remove “beneath”

RE: Corrected in Line 84.

Line 84 “This is based on the former practice that” - should be “this is because previous studies have shown …”

RE: Corrected in Line 85.

Line 88 and further – Comment – I still contend that freeze drying, air drying, or oven drying fumigated samples does not remove the HCl. It simply removes the water from the sample. It leaves residual HCl. And this residual HCl is strong. This is rarely noted in papers.

RE: We agree with your comment. We have added some discussion about this point in Section 2.2 and Section 3.2 (Line 95 and 242).

Line 94 – "Aside" is awkward. Maybe "alongside" but this is not perfect either.

RE: Now has been revised to "were oven dried with sodium hydroxide flakes being placed alongside".

Line 98 – What does the "§" symbol refer to?

RE: Now has been added in Table. 1.

Line 98 – remove "to be" – not needed

RE: Done as suggested.

Line 101 – What does "bulk" analysis refer to? Bulk %CaCO3?, bulk total lipids, bulk cholesterol? I think that it means bulk d13C but this needs to be clear

RE: Thank you for suggestion. Now has been revised to "bulk carbon isotopic measurement and RPO analysis".

Line 102 – Again, what does "bulk carbon measurement" mean. Should be "bulk carbon isotopic measurement".

RE: Corrected in Line 106.

Line 102 – change "placed" to "placed into"

RE: Corrected in Line 107.

Line 104 – IRMS should probably be a Thermo Electron. Corporation name in China might be different than I am used to. Check this.

RE: Thank you! We have checked the instrument information and corrected it.

Line 105 – Call for table 2 should be Table S2 – CHECK calls for tables throughout.

RE: Corrected in Line 110.

Line 106 – change to "measurement of usgs40"

RE: Corrected in Line 112.

Line 110 – "with thermocouples mounted". "mounted" seems unnecessary.

RE: Corrected in Line 116.

Line 115. If the 5% O2 is 95% Helium and 5% O2 just write this. Don't simply use "diluted" which does not tell anything about what it was diluted with. I later learn from the Sup info and the diagram that it is O2 mixed with He.

RE: The 5% O_2 used in our RPO system is mixed with N_2 rather than He. Information about gas composition has been added in Line 122.

Line 115 continued – I don't see how the gas inputs in the text match up with the gas inputs in Fig S1.

RE: Thank you for pointing this out. As shown in Figure S1, there are two gas inlets. The first one ($He+O_2$; at the top) is introduced directly into the upper quartz reactor, while another flow ($He+O_2$) is supplied to the lower part of the reactor through the outer quartz tube. During conventional analysis, we normally use a gas composition of 27 mL min^{-1} He + 3 mL min^{-1} 5% O_2 for the upper reactor and another 5 mL min^{-1} O_2 directly to the lower furnace. However, for any unconventional analysis, the gas composition can vary depending on situations, given the installation of both O_2 and He supplies in upper and lower furnaces.

Line 116 – define "sub-oxidation mode" – probably what was described before but not clear enough

RE: The phrase "sub-oxidation mode" means that the O_2 concentration (0.5%) used in this study is lower than the concentration of oxidation mode but still higher than pyrolysis (O_2% = 0). Information about O_2 concentration has been added in Line 122.

Line 116 – "adopted" maybe should be "used"

RE: Corrected in Line 122.

Line 122 – "Notably, the residual chloride in sediments" should be "Residual chloride in acidified sediment samples"

RE: Corrected in Line 131.

Line 124 – "This consideration was of no concern to" should be "Residual chloride was of no concern for because it was already removed"

RE: Corrected as suggested in Line 131.

Line 124 "rinsing" should be "rinsed"

RE: Corrected in Line 131.

Line 125 – "counterparts" should be "samples"

RE: Corrected in Line 133.

Line 125 – "surged the risk" – should be "increased the possibility of damage to catalytic wires"

RE: Corrected in Line 133.

Line 126 “as the standard” probably not needed

RE: Now has been revised to “we ran an in-house standard sample” in Line 134.

Line 130 – “the in-house standard (Irati T2)” should be “Irati T2”. End the sentence here

RE: Corrected.

Line 130 – Start a New sentence. Change “…by assuming that chlorine gas is generated…” to “We assume that chlorine gas generated…” (no need for “is”)

RE: Corrected.

Line 138 – “Based on aforementioned” should be “Using the aforementioned”

RE: Corrected in Line 147.

Line 158 – “gradient of HCl concentrations” should be “gradient of HCl concentrations used for sample acidification”

RE: Corrected in Line 170.

Whole sentence from Line 156 to line 158 – “Although variations” Remove “Although” - Then later – “and S4), thermographic patterns” should be “and S4) PERIOD then start a new sentence. “Whereas thermographic patterns”. (split sentence into two. This will help to emphasis the second sentence.)

RE: Now corresponding changes have been made.

Line 159 – “To illustrate the inherent consistency between thermograms and HCl concentrations being applied” remove “inherent” and change “being applied” to “HCl Conc used to acidify samples”

RE: Corrected in Line 171.

Line 169 “In contrary” should be “In contrast”

RE: Corrected in Line 181.

Line 170 “has been” should be “was”

RE: Corrected in Line 182.

Line 183 – Use “as aggregates”

RE: Corrected in Line 194.

Line 190 – Include which metal are isotopically fractionated.

RE: Now has been included in Line 203.

Line 215 – “forms” should be “formed”

RE: Corrected in Line 228.

Fig 2 – "(a), (b), (c) and (d) represent subsamples" Maybe change to "Panels (a), (b), (c) and (d) are subsamples…"

RE: Corrected.

Line 224 – "Powders" adds confusion. I suggest using "Samples"

RE: Corrected in Line 237.

Line 241 – "other" should be "rather"

RE: Corrected in Line 255.

Line 259 – "held by" should be "found in"

RE: Corrected in Line 273.

Line 264 "Former studies" should be "Previous studies"

RE: Corrected in Line 278.

Line 268 "overlain together" is repetitive. "Overlain" is sufficient.

RE: Corrected in Line 282.

Supplementary

Fig S1: "The" schematic – remove "The"

RE: Corrected.

Fig S1 "and the corresponding thermogram" should be "and a representative thermogram"

RE: Corrected.

Fig S2: Suggested changes "Normalized thermograms of the standard sample (Irati T2) analyzed before and after analyses of acid-fumigated subsamples. No obvious shifts are observed when comparing the thermograms."

RE: Corrected.

Fig S4 – Initially I had no idea what FD means. Determined that it is Freeze dried. No clue what heat is if it is not oven dried. Define FD, OD, and heat in the caption.

RE: Now all definitions have been added.

Fig S5: The "shot" should be the "Image" or the "photograph"

RE: Corrected.

Fig S5 Continued – "The black remnants are likely sourced from the incomplete" should be

“The black remnants are likely a result of incomplete”

RE: Corrected.

Fig S6 – I suggest using “Raw sample” or “original unmodified sample” instead of “raw aliquot”

RE: Corrected.

Table S2 – I would suggest adding to the caption something like “See Table S1 for definitions of different experiment conditions (EC numbers)”

RE: Now has been added in the caption. All specific comments have been addressed. We appreciate your valuable suggestions.

Referee #3

Review of He et al. “Technical note: Assessing pretreatment approaches for serial pyrolysis-oxidation analysis of sedimentary organic carbon” (egusphere-2025-701)

Overall, I find this manuscript to be well written and the results clearly presented. I am satisfied with most aspects of the study and believe it is suitable for publication.

RE: We sincerely appreciate your positive feedback. Please find our point-to-point replies to your specific comments below.

I would only suggest the author to consider addressing two minor points:

Table 1: I was a bit confused. In the Method sections, the authors mentioned several different dying methods, temperatures, but only some are shown here. Probably explain why were these particular conditions chosen as the main experimental setup? From my experience, the oven drying is still a common method for treating these acidified samples?

RE: Thank you for the comments. We would like to state that we have considered different conditions (including oven drying and different temperatures) as you mentioned. In the first round of review, all different experimental setups were included in the main text. However, we felt it a bit lengthy to put all conditions and discussion together in the main text. Thus, main experimental conditions (acidification method and aqueous HCl concentration) were determined after we thoroughly compare all results. According to your and another reviewer’s comments, we have added a brief discussion on other conditions that were not included in the main text starting from Line 167.

Section 3.3: Besides concluding that acid rising is more effective to maintaining sediment pristine conditions, there appear to be substantial differences between low concentration acid rising the pristine samples. I suggest adding more discussion on this point.

RE: We fully agree with your idea that samples rinsed with low concentration HCl were not completely identical with the pristine samples. This can be attributed to: 1) overestimating thermogram of raw sample due to decomposition of carbonates at low temperature (<450°C), 2) OC loss during acid fumigation. We have further added some discussion on this point in Section 3.3.

With these small clarifications, I fully support acceptance of the manuscript.

RE: We have addressed all your specific comments. Thank you for your valuable suggestions.

Referee #4

Assessing pre-treatment approaches for serial pyrolysis-oxidation analysis of sedimentary organic carbon

Summary

He et al. present an evaluation of different sample pre-treatment methods for ramped-temperature oxidation/pyrolysis analysis. Considering that ramped-temperature oxidation/pyrolysis is becoming more widely used, the information presented in this manuscript are useful and timely. I found the results of the experiment with CaCl2 addition particularly interesting. The manuscript is well written and organised, and properly referenced. There are some aspects that can be improved significantly:

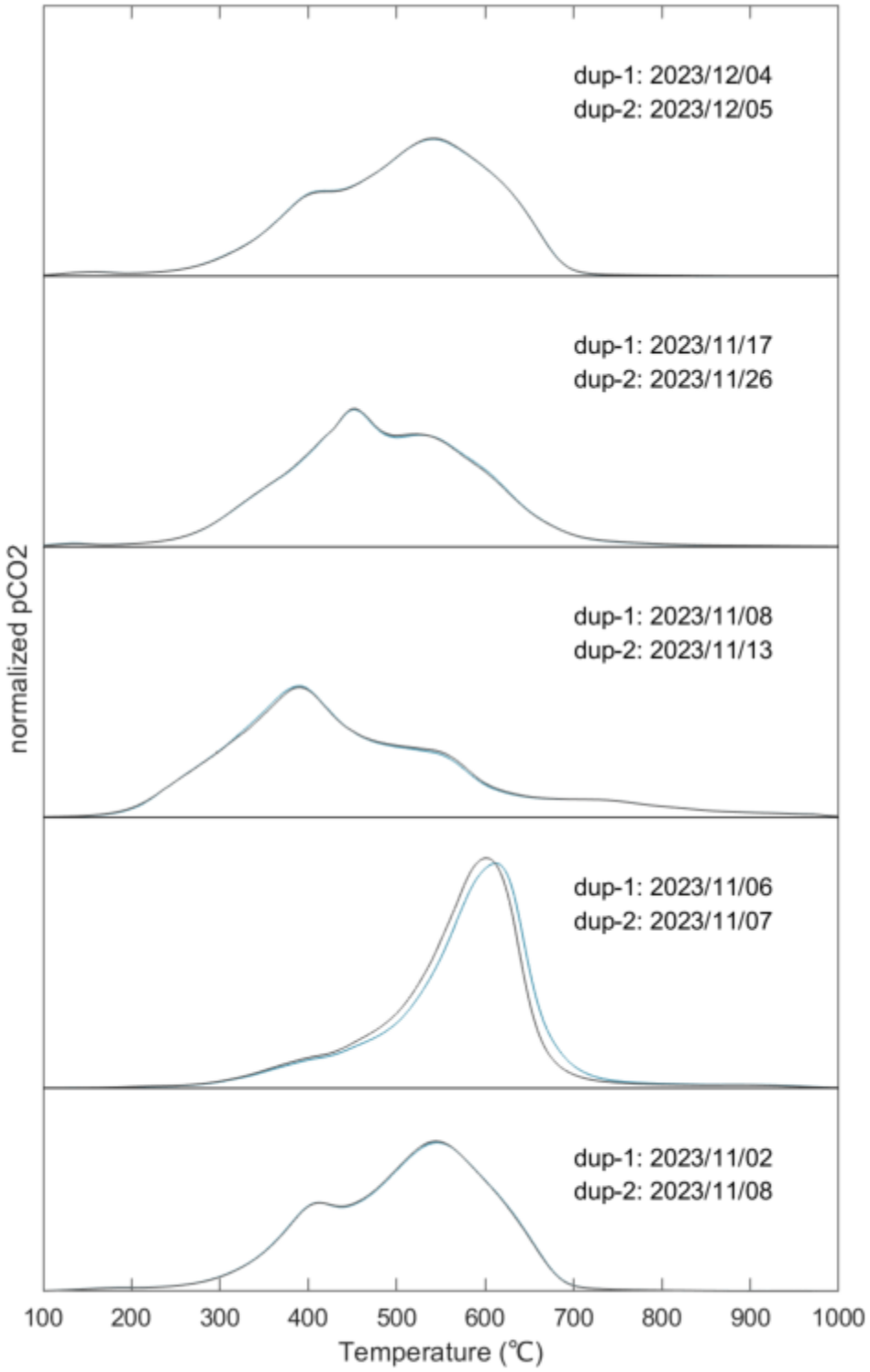
RE: We sincerely appreciate your positive feedback. Please find our point-to-point replies to your specific comments below.

1. The title can be misleading. All samples were analysed using oxidation mode only. Do the authors expect to observe similar results when using pyrolysis mode? Perhaps they can include a clarification/suggestion in the conclusions.

RE: Thank you for your insightful comment. We have added a clarification in the main text (Line 306) and would like to add some explanations here about the title of this study. Although we only used oxidation mode in this study, the same observations and main conclusions are expected to be identical or similar when using pyrolysis mode. Secondly, O_2 concentration in direct contact with the sample is much lower than previous studies that applied oxidation mode. Therefore, given the trace oxygen level maintained throughout the analysis, reactions in the quartz reactor are more similar to pyrolysis process (though it is indeed not pyrolysis). When vaporized OC is carried out of the reactor by the carrier gas, it will be instantly oxidized by pure O_2 and be converted to CO_2. In conclusion, this is the pyrolysis-oxidation process mentioned in the title. We have also added the annotation "pyrolysis" in Supp Fig. S1 for readers to understand the reactions happened in the reactor.

2. Lack of replication. It would recommend a strong justification of why replicate analysis were not carried out. Can the authors include data in the supplemental material that justifies not running replicates?

RE: In fact, we ran replicates throughout the analyses of each batch of samples, of which the point was not included in the main text. Here we provide a figure to exhibit the replicate results.



As shown in the figure, thermograms of most replicates are entirely overlapped, suggesting great reproducibility throughout the analysis. However, this is not included in the main text as the reproducibility of replicates are indicated with the in-house standard sample (Irati T2) in the supplementary file. In fact, the Irati T2 standard sample was measured periodically to secure the reliability and reproducibility of data.

3. There should be a separate results and discussion section for the results of reaction time,

drying methods and drying temperatures. These results are important and should be discussed accordingly. If the authors consider that they do not have enough data to do so, perhaps they can be removed them from the manuscript.

RE: We agree with your idea that other conditions are important in processing samples. A very brief discussion has been added in Section 3.1 to show the results (Line 167). Unfortunately, the detailed discussion concerning these factors has been removed after the first round of revision to streamline the overall content. We would like to incorporate them into another future paper.

4. Activation energies are not discussed. I think the authors are missing the opportunity of discussing the impact of the pre-treatment methods on the activation energies of the samples (both mean and standard deviation), and from each activation energy intervals (or temperature fractions). It is possible that although there are both carbon loss and chemical alteration during treatments, they result in similar activation energies (within uncertainties). Then the questions are whether to work with the thermograms or activation energy distributions, and the impact on radiocarbon results for each fraction.

RE: Thank you for your insightful consideration. We completely agree with you that activation energies (E) are very important parameters in a paper that primarily focuses on RPO analysis. There are three main reasons for not including too much discussion in the paper. (1) Thermogram is a more straightforward way to reveal changes compared to activation energy. For example, two completely different thermograms can result in identical mean E value. However, any small change can be clearly exhibited by thermograms. Additionally, it would make the paper lengthy by including too much discussion on activation energies. (2) Parameters of activation energies are comparatively more sensitive to noise. A drift of 10-20 ppm in the baseline at high temperature can mask any nuanced shifts in low temperature. In contrast, thermograms are more resistant to any noise in the system. (3) Thermograms are more easily to be scaled, facilitating the direct comparison between raw samples and acidified samples as shown in Section 3.3. In comparison, distributions of activation energies are affected by different reaction kinetics of carbonates (also see reply below), inhibiting direct comparison when including raw samples. Overall, it is more reasonable to focus simply on thermograms when carrying out discussions. Meanwhile, we argue that the optimized pretreatment conditions as suggested in this study would benefit the broader application of activation energies in future studies.

Radiocarbon results for RPO fractions are as important as RPO thermograms. It is a pity that we did not carry out radiocarbon analysis of different RPO fractions in this study. It is especially interesting to compare the fractional radiocarbon data for acid-rinsed and acid-fumigated samples, given the significant differences between thermograms in our study. Some previous

studies have preliminarily studied on the methodological impacts on radiocarbon analysis (Bao et al., 2019; Plante et al., 2013). Therefore, future studies can merit on previous and current study to systematically probe the mechanism.

5. Line 236. What are the mechanisms in which chlorine can influence thermochemical reactions of organic matter?

RE: Chloride is shown to react directly with catalytic wires, which further impacts the efficiency of oxidizing C to CO_2. Furthermore, it is likely that chloride is converted to reactive chlorine during heating that accelerates OC decomposition. However, the reaction complexity is beyond the scope of this study.

6. Line 271. Does the activation energy value of 182.48 kJ/mol correspond to a non-acidified sample that contains carbonates? If that is the case, please check Hemingway et al. (2017) about the limitations of calculating activation energies with the rampedpyrox package. I believe first-order kinetics model should not be used when carbonate is present.

RE: We appreciate your expertise and insightful suggestions. It is misleading to use the mean activation energy of unacidified sample as carbonate decomposition does not follow the same reaction kinetics as OC decomposition. To resolve this, we revised the expression to "*relatively lower T_{max} value of raw Sed2 sample compared to the corresponding acidified samples*".

7. Line 288. Please specify the concentration of HCl or range of concentrations. Using the term "diluted" here is inappropriate.

RE: Now has been revised to "1 N" in Line 306.

8. Are the results of Fig S2b post-fumigation and rinsed? Please specify

RE: We conducted a systematic comparison of Irati T2 before and after analysis of each acid-fumigated, oven-dried sample. Specifically, samples are analyzed in such order: Irati T2 (pre-fumigated samples) → SR1 (acid fumigation) → Irati T2 (post-fumigated samples). It is designated to capture any noticeable changes after running acid fumigated samples. Accordingly, Fig. S2 is not the results of post-fumigation and rinsed, but the comparative results of Irati T2 before and after running acid fumigated samples.

We have addressed all your suggestions and concerns. Thank you for your valuable suggestions.

References cited

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413, https://doi.org/10.1017/RDC.2018.125, 2019.

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