

Summary

The main finding of this manuscript is there is abrupt microbial community shifts at Lake Magadi over the last 456 ky, alternating between periods with prominent methane cycling and periods without. The authors use multiple organic geochemical techniques, specifically isoGDGT indices, leaf waxes, and bulk organic matter d13C values, alongside previously published information on lake levels and hydrothermal inputs to make these claims. Intervals with strong methane cycling are associated with low hydrothermal inputs while intervals with weak methane cycling are associated with greater hydrothermal inputs.

General comments

Overall, the research findings are new and interesting, and most of the methodology is sound. Exploring biomarkers in soda lakes (and other such non-freshwater lakes) is certainly useful for testing biomarker applicability in a wider range of environments. However, the application of leaf waxes and the discussion would benefit from further development.

Here are my general comments:

- 1) Despite different lines of evidence being used, the discussion was very GDGT-reliant. Leaf wax data were included but the extent to which they were considered in the context of the paper was limited. For leaf waxes, the main measurements used were ACL and CPI. There was a heavy reliance of ACL and CPI as an indicator of terrestrial sourcing or C4 vegetation, but ACL and CPI alone are insufficient as determinations of either. Pollen records were cited (L 489-491) and would be useful for tracking C4 grassland abundance downcore alongside OM d13C (e.g., are C4 grasslands only abundant in interval 1?). If possible, a better metric of C4 vegetation would've been d13C of individual long-chain n-alkanes (e.g. C27, C29, and C31), rather than just bulk OM. Additionally, were there any patterns in changes of alkane or FAME abundances (both total and for individual chain lengths) downcore?
 - **After some consideration, we have decided to remove the *n*-alkanes from the paper as well as the FAMEs. This was, in part, due to the reviewers' comments stating that the extent of consideration was limited. While the *n*-alkanes provide some context to the core, they are not the main focus of the paper and will be removed.**
- 2) While the figures used as visuals do their job, modifications to current figures and additional figures would better support the main text and push discussion forward. For example, how do plots of CPI, ACL, and bulk OM d13C compare downcore? If C17 FAMEs are being used as an indicator for SRB, how do abundances compare downcore? What about C17 FAME abundance plotted alongside pyrite appearance and methane cycling indices?
 - **A plot was created to compare the CPI, ACL, and bulk 13C measurements. The C17 FAMEs have been removed from the paper after reconsidering the lack of overlap of C17 FAMEs and pyrite in the core.**

What about plotting [2]/[3] data from Rattanasriampaipong et al. (2022) alongside values from this study for a visual comparison of overlap?

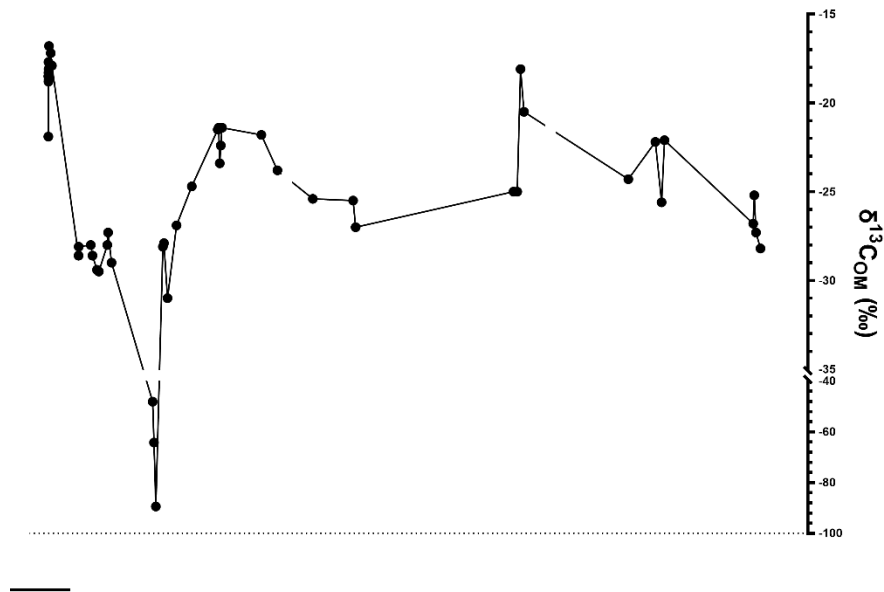
- **More information has been placed in the text (Section 2.3.2) to better contextualize the results from the different samples and localities in Rattanasriampaipong et al. (2022) and how they relate to the research we presented in this paper.**

As for current figures, consider simply removing Fig. 2 as knowing the structure of the GDGTs being used don't contribute to an increase in understanding the findings of the manuscript, particularly since Fig. 2 is currently cited in Sect. 4.1 where visuals of GDGT structure are not very relevant.

- **After discussion with co-authors, Fig. 2 will be removed from the paper and simply cited as the reviewer notes.**

Fig. 3d shows OM d13C downcore, but the < -40 permil values in interval 2 makes it difficult to compare d13C values across intervals (it currently just looks like a straight line for every interval besides 2). One possible modification is to have the full d13C record as an inset graph and a larger graph excluding just the < -40 permil values.

- **The scale has been changed on the graph from a starting $\delta^{13}\text{C}$ of 0 to -15 ‰. A scale break has also been added between -35 and -40 ‰. The figure below shows the changes. The y-axis has also been scaled based on the scale break with the lower part scaled to 30% and the upper part scaled to 70%.**

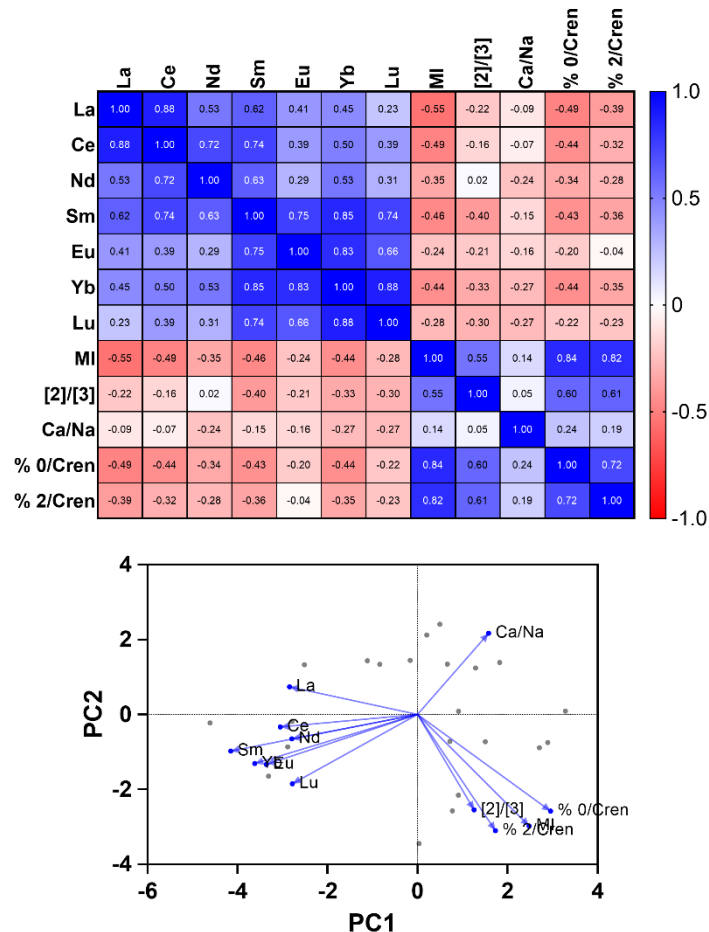


For Fig. 4, the link between hydrothermal inputs and MI, from the graph alone, is not immediately obvious. Based on information from the main text, increased hydrothermal input is indicated by low Ca/Na and high %REE. If low MI occurs when there's more hydrothermal input, I expected to see low Ca/Na and high %REE in the blue MI-off intervals, but this doesn't seem to be the case.

- **We agree with the reviewer that it is not immediately obvious from Fig. 4 alone, this is why we decided to add a PCA plot and accompanying Spearman correlation matrix to help disentangle some of the complex relationships between REEs and methane indices. While Fig. 4 shows an overall trend of increased aridity and thus an increased proportion of hydrothermal flow to Lake Magadi steadily decreasing, but not a 1:1 relationship of Ca/Na from Interval 5 to Interval 1 (which is discussed further in the next general comment below), it does not detail the relationship between REEs as clearly which is why we chose to perform a PCA and Spearman correlation. The PCA loads the methane indices and REEs and shows an anti-correlation between these two groups. This anti-correlation can also be seen in the Spearman correlation matrix. So, while Fig. 4 does not show these trends as clearly, we hope that the PCA at least clarifies the relationship between REEs and methane indices.**
- **We have also clarified this by adding Ca/Na to the PCA and correlation matrix (shown below) further solidifying the relationships between methane indices and**

proportionally more hydrothermal inputs to Lake Magadi. Tb has been removed from the REEs as it had a very low number of samples where it was found ($n=4$) and was thus skewing interpretations in the core since other REEs and indices were represented more robustly ($n \geq 32$). The Ca/Na is anti-correlated with REEs in both the PCA and correlation matrix. Since the proportion of Ca/Na decreases when REEs increase, we can say that statistically, when it is drier (and thus proportionately more hydrothermally influenced) the Ca/Na decreases, REE values increase, and the methane indices are suppressed.

- Ca/Na is loaded positively on PC1 and PC2, while the methane indices ([2]/[3], MI, %0/Cren, and %2/Cren) are loaded positively on PC1 and negatively on PC2. This indicates these two measurements are statistically different from one another. So, while the values in Fig. 4 don't appear to have the 1:1 relationship that would be expected with the proportional increase in hot spring activity, statistically these two are different enough from one another.



- 3) Is there more climate context for Lake Magadi over the study period? The African Humid Period was mentioned (and it needs to be cited in the main text) as occurring in interval 1, but were there any climate events of note beyond interval 1 that could've contributed to our understanding of the biomarker records at Lake Magadi? Currently, the manuscript formulation implies much of the biomarker patterns observed are due to changes in hydrothermal inputs, but looking at Fig. 4, while hydrothermal inputs may explain some of the story, it doesn't seem to explain the whole story. If so, what are other drivers to the methane cycling indices?

- We recognize that we did not fully describe the climatic context of this work and note that the following three papers discuss the climate in this region of the East African Rift Valley in more detail:
 - Owen, R. B., Muiruri, V. M., Lowenstein, T. K., Renaut, R. W., Rabideaux, N., Luo, S., ... & Mbuthia, A. (2018). Progressive aridification in East Africa over the last half million years and implications for human evolution. *Proceedings of the National Academy of Sciences*, 115(44), 11174-11179.
 - Owen, R. B., Renaut, R. W., Muiruri, V. M., Rabideaux, N. M., Lowenstein, T. K., McNulty, E. P., ... & Stockhecke, M. (2019). Quaternary history of the Lake Magadi Basin, southern Kenya Rift: Tectonic and climatic controls. *Palaeogeography, palaeoclimatology, palaeoecology*, 518, 97-118.
 - Muiruri, V. M., Owen, R. B., Lowenstein, T. K., Renaut, R. W., Marchant, R., Rucina, S. M., ... & Wang, C. (2021). A million year vegetation history and palaeoenvironmental record from the Lake Magadi Basin, Kenya Rift Valley. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 567, 110247.
- Briefly, in the region around Lake Magadi in the time ranges of this study (ca. 456 to 14.9 ka) there was a gradual aridification noted by *proportionally* larger inputs of hydrothermal flow, rather than an increase in hydrothermal activity, compared to other meteoric sources (i.e. rainfall and outwash from riverine inputs). That is, during drier periods when there was less rainfall, the higher proportions of hydrothermal flow maintained a perennially wet lake and there is not necessarily more activity from hydrothermal springs, we are seeing more evidence of the springs from a reduced dilution factor of freshwater inputs. Additionally, there are no noted mud cracks, erosion, or other evidence of the lakebed completely drying. These proportions are noted by increases in rare earth elements (REEs) and a gradual decrease in the proportion of Ca/Na. A noted exception is Interval 5, which we will reassess as there is a large spike in the Ca/Na and a dearth of REEs, lower than even intervals where proportions of hydrothermal input should be lower. One possible explanation is that Interval 5 is actually much wetter and more well-mixed such that an increase in other meteoric sources are having an impact on both the lake and the archaeal communities.
 - Additionally, after discussing it with other co-authors, the tephra seen in Interval 5 is likely much too altered (zeolitized) and no longer considered to be tephra. This is because tephra tends to rapidly alter under the conditions in Lake Magadi (i.e., high pH).
 - The notation of tephra will be changed on Figs. 3 and 4 to that of a perennial lake rather than as tephra ashfall.

Specific comments

Section 2.1: Were there any age estimates for paleolake shorelines? What about any lake level history records?

- Ages have been added from Renaut and Owen (2023).

L 221-223: A more thorough explanation of the [2]/[3] index would be useful. What is this index an indicator of? Is it for distinguishing mesophilic from high/low MI environments?

- We have taken this into account and added more information about the [2]/[3] index from Rattanasriampaipong et al. (2022)

Section 3.1, paragraph 1-2: The first few sentences of paragraph two are the same content as the Fig. 3 caption and can be deleted. I suggest then combining paragraph 1 and 2, specifying that the oscillations in GDGT indices correspond to shifts between intervals.

- **The redundant text has been removed and the paragraphs have been combined for clarity as suggested**

L 255-257: Were any samples taken and measured for biomarkers from presumed low-TOC sections? Is it possible samples presented here are not properly representative of the whole-core record due to selectively sampling only the dark, silty sections of the core?

- **One reason that the strategy appears to not be optimal is a result of the relatively poor recovery of the core at ca. 55.4% (Line 126). As for the large spatial differences of 3-15 m between single intervals these are a result of areas with poorer core recovery where there was either no sample or the skipped intervals were too mineral rich or simply a brecciated material that could not be effectively sampled.**

Section 3.1, paragraph 3: Alongside the average index values, I suggest including the standard deviations.

- **These have been added to the manuscript text.**

Section 3.3: As mentioned, CPI is more a metric of degradation/diagenesis (something acknowledged later in the main text) rather than terrestrial sourcing. Broadly suggesting FAMES to be terrestrially sourced (L 294) counters the point of the last paragraph in the section, which is that short-chain FAMES are diagnostic of SRB in sediments and, in the context of the manuscript, presumably living in the lake. The evidence for SRB presence is also somewhat lacking. Four compounds were listed as possible indicators of SRB, C15:0, C15:0-iso, C17:0, and C17:0-iso FAMES. It sounds like only 1 of the 4 compounds (C17:0 FAME) were identified in the 15 samples measured for FAMES. Were there attempts to identify C15:0-iso and C17:0-iso in these samples? C17:0 FAMES are not exclusively produced by SRB so the presence of these compounds cannot absolutely be attributed to SRB. Since pyrite presence did not always overlap with C17:0 FAME presence, are there other lines of evidence suggesting SRB presence?

- **The FAMES and their interpretations have been removed from the manuscript.**

L 347-352: The phrasing in these lines is confusing. It seems to imply there is AOM therefore we expect to see high methane cycling indices rather than the other way around. It also seems to imply AOM should exist because there is SRB which isn't always true.

- **This has been removed and modified based on the suggestions of both reviewers regarding the FAMES and their interpretations in the core.**

L 387-388: This sentence should be moved earlier to the beginning of the paragraph as it does a much better job explaining how you know interval 2 is more influenced by methanogens than by AOM.

- **This has been rearranged**

Section 4.1.2: The first half of the second paragraph repeats the info from the first paragraph (e.g., increase in crenarchaeol, low methane cycling indices) and should be consolidated to avoid redundancy.

- **This has been consolidated and the repeated text has been removed**

Section 4.2: The majority of this section discusses hydrothermal input data cited in other papers with little mention of the links to this manuscript's findings until the very end. While interesting, it doesn't seem to merit a full section. The most relevant point is the last sentence so the rest of the section could reasonably be condensed and incorporated into another section (perhaps 4.1.2 MI-off periods).

- **We have clarified and condensed parts of this section per the reviewer's request, but we do feel that this section is important and distinct enough to remain separate from 4.1.**

Table 1: This table is more appropriate as an appendix rather than in the main text, or even excluded entirely and left as a submission to a data repository. I suggest condensing this table down to only feature average index values (along with their standard deviations) for each of the 6 intervals. Leave out the fractional GDGT abundances, pyrite presence, and C17:0 ng g⁻¹ sed extracted columns. Also, I suggest formatting the table using the table function in MS Word rather than copying directly from Excel. You may also consider turning some of the info from table 1 into a figure, perhaps a box-and-whisker plot showing each index for each interval.

- **The table has been shrunk per the author's suggestions and the more detailed remaining data will be uploaded as a supplementary table to Biogeosciences.**

Figure order: The figures are not ordered in the sequence they appear in the main text. The current order of figure appearance is 1, 3, 2, 5, 6, then 4, and should be renumbered in the order they appear.

- **The Figure numbers have been corrected. The figure which had GDGT structures has been removed (Formerly Fig. 2).**

Technical corrections

These will be addressed once editor approval has been granted for submitting the manuscript.

L 15-16: Since the biomarker data for this manuscript spans 456 ka to 15 ka, it's more accurate to say <500 ka rather than <700 ka.

L 29-30: References needed for "modern studies of both prokaryotic and eukaryotic organisms"

L 30-31: Extra space before period.

L 48: Replace "CH₄" with "methane" for consistency.

L 67-68: This sentence can be condensed into the end of the previous paragraph by writing "Nitrososphaerota (formerly Thaumarchaeota) and Thermoproteota (formerly Crenarchaeota)" with their corresponding references.

L 71-73: Edit sentence for consistency. Something like "... representative of not only the Group 1 ANME consortium (ANME-1) that produce GDGTs, but also of Group 2 and Group 3 consortia (ANME-2 and ANME-3 respectively)."

L 76-78: References needed for "previous studies have used GDGT-0 and GDGT-2 ratioed to the GDGT crenarchaeol value..."

L 105: Duplicated the word "season". Should just be "rainy season".

L 117: Missing space before "Although Lake Magadi..."

L 125: Replace "partend" with "end". Replace semicolon separating the latitude and longitude with a comma.

L 128: Change "dated at ~ 1 Ma" to "dated to ~ 1 Ma"

L 130-132: Rearrange sentence from "... were subsampled and freeze-dried from dark brown to black silty clay intervals in the core" to "... were subsampled from dark brown to black silty clay intervals in the core"

then freeze-dried.”

L 132: Replace “samples” with “intervals”

L 132-133: Specify reasons for high TOC assumption. Is it just the dark coloration of the sediment? Also, specify what is meant by “best results”. Is it just higher yield of biomarkers?

L 135-137: Edit sentence for flow and consistency. From "... a large subhumid lake, when the freshwater lake was fed by rivers and groundwater continuously, to the small, tectonically restricted, saline alkaline pan partly fed by hot springs" to "... a large, freshwater, subhumid lake, fed continuously by rivers and groundwater, to a small, tectonically restricted, saline alkaline pan, partly fed by hot springs"

L 168: Change "step" to "rate"

L 201: Change "differ from" to "differ in"

L 201-202: Edit last part of sentence for clarification. Change "... even those that are saline and alkaline" to something like "particularly those in saline, alkaline environments."

L 210, 219, and 225: For consistency with eq. 1, use fractions when formatting eq. 2, 3, and 4. Change the "x" to a multiplication symbol and delete the percent symbol after 100.

L 226: Blank line labeled as eq. 5. Delete and renumber the equations that follow.

Formatting GDGT index names: Consider eliminating spaces within index names. For instance, writing "%GDGT-2/cren" instead of "% GDGT-2 / cren".

L 244-246: For depth values, use the same number of decimal places for consistency. I suggest using 2 decimal places for all values. Also, replace semicolons with commas.

L 250: In "cren'", change the apostrophe to the actual prime symbol. This should be the case for all subsequent instances of cren'.

L 274: Leave out mention of Interval 6 and just leave Interval 2 considering Interval 6 has not yet been discussed at this point in the results section.

L 279: Can remove first sentence and start paragraph with second sentence.

L 279-280: Specify what is meant by "similar pattern". Is it that bulk OM d13C oscillates between high and low values between intervals? If yes, say it.

L 341-342: Change " $0.3 < MI < 0.5$ " to " $MI < 0.5$ " as 0.3 doesn't seem to be a relevant value.

L 417: Replace "these intervals are" with "this interval is"

L 440: Remove "(Table 1)"

L 455-457: Change "green checkered patterns" to "blue regions". Also Fig. 3 was cited in parentheses twice.

Fig. 3 caption: Give the full names of the indices. Replace "%0/Cren" and "%2/Cren" with "%GDGT- 0/Cren" and "%GDGT-2/Cren" respectively.