Response to the review by Referee #2 on the manuscript cp-2021-66 "Biomarker proxy records of Arctic climate change during the Mid-Pleistocene Transition from Lake El'gygytgyn (Far East Russia)

We thank the reviewer for providing a careful review of our manuscript and will incorporate their suggestions, as detailed below.

Lindberg and co-authors report on the well-known and unique Lake E sediment record from the Russian Arctic. Their analysis of brGDGT and n-alkane biomarkers allow new and revised interpretations of past environmental change in terms of precipitation and vegetation across the mid-Pleistocene transition (MPT), a global and enigmatic period of climate change. Overall, the paper is very well written with clear accompanying figures that allow the reader to visualize the text appropriately. Although the authors do explore the utility of the brGDGT proxy using various calibrations and statistical analyses, I offer some suggestions for further data interrogation that I hope will benefit the paper. The fact that this paper provides a rare and continuous terrestrial Arctic paleoclimate record across the MPT naturally amplifies its impact. Collectively, with some revisions, this manuscript will be a highly valuable contribution to both the paleoclimate and brGDGT proxy community. I congratulate the authors on a very nice manuscript – it was a pleasure to read.

Specific Comments

Review: *L11: Please be consistent with ka or kyr throughout the paper. Same applies for Ma and Myr.*

Reply: Following the recommendations in "*Terminology of geological time: Establishment of a community standard*" by Aubrey et al. 2009 (Stratigraphy, vol. 6, no. 2), we use "ka" and "Ma" to represent geohistorical dates while "kyr" and "Myr" and used to represent geohistorical duration. We will carefully check that all uses of ka/kyr and Ma/Myr are correct throughout the manuscript.

Review: *L34-35: Maybe I'm just not familiar with ecology well enough, but it sounds oxymoronic to have cool forests indicative of a warm climate?*

Reply: Good point - we understand why this might be confusing. Presently, Lake El'gygytgyn is surrounded by tundra with the nearest trees ~150 km to the south. Thus, having any trees around the lake, even those representing cool forest species, indicates a significantly warmer climate. In our revision, we will re-word this for clarity.

Review: *L40: Might be worth briefly mentioning what proxies these T and P reconstructions are based on.*

Reply: The previous temperature and precipitation reconstructions, which only represent short segments of the overall Lake El'gygytgyn record, are based on pollen assemblages. We will add this to the revision.

Review: *L72: You say that MPT lake records are particularly rare. Are there any others? If so, would be worth adding some references, otherwise clarify that Lake E is the only known one!*

Reply: There are only a handful of lacustrine records continuously spanning the MPT, including Lake Baikal, Lake Malawi and Lake Ohrid, as only a relatively small number of lakes have been drilled to date.

We will revise this sentence to include appropriate citations and clarify that El'gygytgyn is the only such record from the Arctic.

Review: *L84: Can you provide a sentence or two on how the age model is constructed (i.e., what geochronological tools/proxies)? Do you have a sense of how the age model uncertainty (good/bad) effects the timing on your biomarker records? This seems particularly relevant for the spectral analyses.*

Reply: We utilize the age model of Nowaczyk et al. (2013), which is based on iterative tie-point identification using 1) paleomagnetic reversals, 2) comparison of biogenic silica to the LR04 oxygen isotope stack, and lastly 3) comparison of TOC and magnetic susceptibility to summer insolation. Given the resolution and uncertainty of the target curves (LR04 and insolation), the absolute age uncertainty could be off by 3-15 kyr, although the relative age assignments have a precision of 500 years (Nowaczyk et al. 2013). Since the Lake E age model is tuned to LR04, it may be circular to discuss leads and lags between datasets, and this is generally avoided in our discussion. Given the 3^{rd}-order tie points improve the relative precision to ~500 years, it remains useful to discuss the spectral results because the multitude of Lake El'gygytgyn proxies appear to exhibit different orbital-scale patterns. We will add a brief summary of these main points to the text about the age model.

Review: *L95: Please clarify which months you include in summer…JJA?*

Reply: Yes, we are referring to JJA for summer temperatures. We will specify this in the text.

Review: *L96: When do shallower regions reach 5-6 degC…summer? Please specify.*

Reply: Shallower regions within Lake El'gygytgyn reach 5-6 degrees C during the summer. We will clarify this in the text.

Review: *L100: Thermodynamics of the lake? Please specify.*

Reply: In this sentence, we are referring to atmospheric temperatures at Lake El'gygytgyn. Nolan et al. (2013) analyzed how atmospheric pressure patterns may or may not relate to the temperature at Lake El'gygytgyn. They found that recent warming signal at El'gygytgyn was not attributed to changes in the frequency of different weather patterns, implying that general warming of the atmosphere (rather than storm tracks) controls air temperature. We will revise this sentence for clarity.

Review: *L106: It seems like these 41 re-analyzed samples are a sub-set of the original (de Wet et al., 2016).*
Was there a strategy for why these ones were chosen rather than re-analyzing the entire dataset?

Reply: Yes, the re-analyzed samples are a subset of the original 174 samples that de Wet at al. (2016) analyzed. We did not re-run the full dataset due to the cost. Furthermore, as these samples do not contain significant amounts of the 6-methyl isomers, re-analyzing yields similar results. Every 3rd sample was re-analyzed. We will add some text clarifying how we selected samples for re-analysis and will do a better job emphasizing that the re-analyzed samples yielded similar results (we can add a figure to the supplement, also addressing the question raised by the other reviewer). A complete re-analysis of the de Wet samples is not needed, as the higher-resolution sampling is not necessary for the orbital-scale questions investigated here.

Review: *L109: This sentence is a little misleading because I do not believe de Wet et al. (2016) analyzed n-alkanes. Please rephrase. In addition, and similar to my prior comment, these do not represent all samples de Wet et al. (2016) analyzed. Was there reason behind how many and which samples were re-analyzed for brGDGTs and n-alkanes from the original sample set of de Wet et al. (2016)?*

Reply: The doctoral thesis of de Wet (2017) does include *n*-alkane analyses. This is cited correctly. The de Wet et al. (2016) EPSL publication only includes GDGT data. de wet (2017) ran 85 samples for *n*-alkanes, which we supplement with the 127 samples that we newly extracted for this study.

Review: *L141: I do not agree with the decision to only use 5-methyl indices. Micro/mesocosm experiments for lake brGDGTs (Martínez-Sosa and Tierney, 2019) in addition to the environmental samples from East Africa (Russell et al., 2018) demonstrate a positive correlation between the abundance of total 6-methyl brGDGT isomers and temperature. A lake record from Iceland also shows some 6-methyl isomers strongly correlated with T inferred from alkenones (Harning et al., 2020). Since we do not have culture experiments to better test which brGDGTs are produced across which environmental gradients, I think it is best to explore the full gamut rather than a subset.*

Reply: In this portion of the El’gygytgyn record, the 6-methyl isomers average 9% of the total brGDGTs. However, we note that many Lake El'gygytgyn samples do not contain any 6-methyl isomers, particularly in the Late Pleistocene. Thus, when analyzing the entire 3.6 Ma record (which we are doing in other publications), we need to use the 5-methyl indices, and so focus on those indices. We can explore use of some of the 6-methyl indices and the calibrations listed below in our revision.

Review: *L145: Like my previous comment, I suggest, especially since we do not yet have culture studies for brGDGTs and that there are not that many existing empirical lake calibrations that separate the 5 and 6-methyl isomers, all calibrations be tested. These would include:*
Feng et al. (2019)
Harning et al. (2020)
Raberg et al. (2021)
Since they are all also from or include high latitude/altitude locations, they should be just as applicable as the East African and Greenland calibrations and may offer some interesting insights (see later comments).

Reply: We will add these calibrations in our revisions.

Review: *L158: Is 211 the number of samples you analyzed (127) and those from de Wet (85)? If so, should this number read 212?*

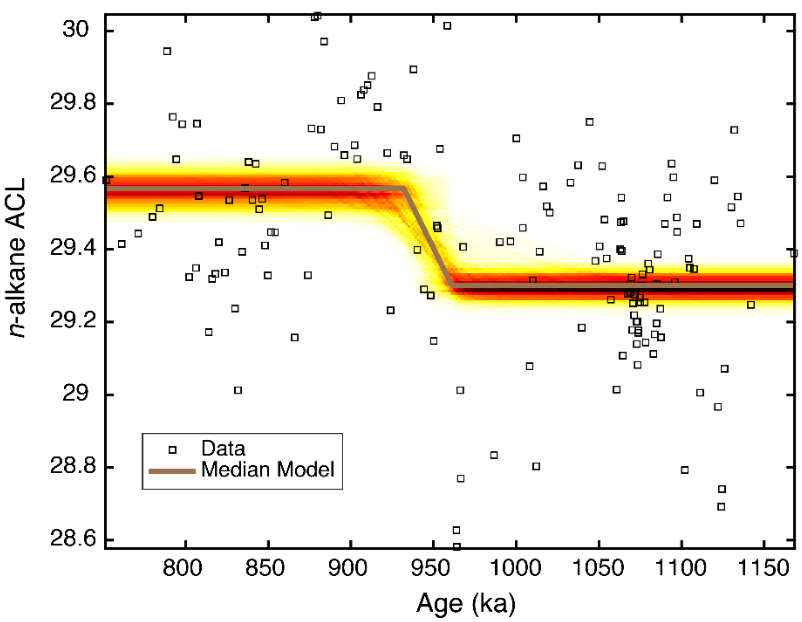
Reply: Yes, thank you for catching that. We will correct this in the revised text.

Review: *L164: CPI should be defined in the methods.*

Reply: The CPI equation is defined in the supplemental text. The reference in the methods will be adjusted to properly reflect that.

Review: *L166: Would it be possible to conduct a change point type analysis here? Otherwise, it seems*

rather arbitrary to select 900 ka as the boundary. I agree it looks like 900 ka reflects a regime shift, but our eyes can do weird things and I find that statistics help us be more objective.
Reply: We conducted a change point analysis on our *n*-alkane data and found the significant ACL shift to occur between 960 and 930ka (95% CI: 1019-895 ka). We retain the use of 900ka as a general partition because that is when numerous marine records in the North Pacific & North Atlantic observe a shift in ice sheet dynamics towards lower frequency variability (Head & Gibbard 2015). However, 900 ka is still only an estimated average for the timing, globally, therefore we believe that our analysis of ACL before and after 900 ka to still be valid. We will add a description of the change-point analysis and its results.



***Review:** L224: Other Arctic sites not mentioned suggest this assumption may be less robust (e.g., Dion-Kirschner et al., 2020), where the data show that terrestrial plants also produce a substantial amount of mid-chain plant waxes. I also skimmed the Wilkie paper, and it looks like they only reported on alkanoic acids, which do not necessarily correlate with alkanes as implied here. Might be worth briefly expanding on some of these limitations for your ACL interpretation.*

Reply: We have analyzed both *n*-alkanoic acids and *n*-alkanes on Lake El'gygytgyn samples (this work was conducted by a recent PhD graduate of UMass and in presently in-preparation for publication) and find similar results for both downcore concentrations and isotopic values for both compound classes. The C_{27} *n*-alkane and the C_{26} *n*-alkanoic acid are the most abundant. We still think, given the available evidence, that ACL is generally reflecting the large-scale vegetation changes occurring in the Lake El'gygytgyn catchment.

***Review:** L229: How do these previous studies link ACL and aridity? Modern instrumental calibrations or downcore proxy correlations? I'm not as familiar with using alkanes as a precipitation proxy, so it may help other readers in a similar boat. Is there a physiological mechanism that causes this response to precipitation variability?*

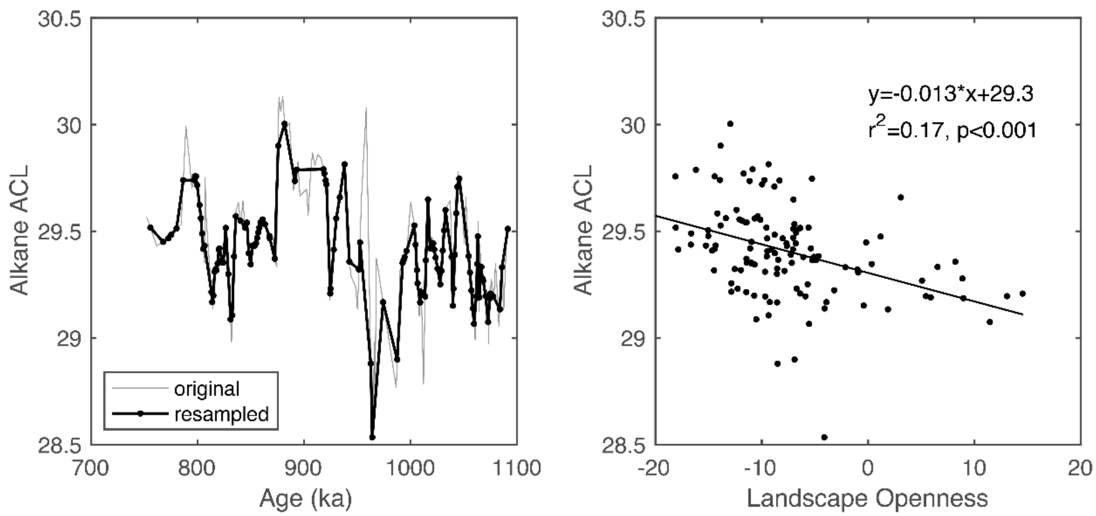
Reply: It has been widely reported in the literature that in samples from certain locations, ACL tracks either changes in temperature or aridity (with longer chain-lengths associated with increased temperature or increased aridity). However, to our knowledge, none of these studies were using ACL alone as the interpretation for aridity. Rather, typically another proxy is used (pollen, leaf wax isotopes) to provide the aridity interpretation and it was noted at ACL tracked changes in aridity. We will revise this section for additional clarity, including adding an additional citation to Bush & McInerney (2013), who discuss ACL in detail.

Review: *L233-234: Same comment as above. What proxies is this aridity condition based on? Please briefly mention.*

Reply: We will clarify that these prior studies based the aridity interpretations on proxies other than ACL.

Review: *L249: In re to the visual correlation between ACL and landscape openness index, are there any statistical regression techniques that could be used to support your observation?*

Reply: We have now performed regression analysis between ACL and landscape openness, confirming our qualitative description of how these two variables are negatively correlated. We recognize that a visual presentation of the correlation would be useful, and will include this regression in the supplement.



Review: *L251-252: Since you mention at the end of the previous paragraph that you refine the ACL interpretation for Lake E, does your interpretation differ from the prior studies you discussed? If so, in what way?*

Reply: While some prior studies have noted ACL tracks changes in temperature or aridity, we think most authors would agree that this is an oversimplification and multiple factors are responsible. We note that many prior studies do not have both pollen and leaf wax data. In this study, the addition of pollen data shows us that ACL broadly tracks the landscape openness index.

Review: *L257-259: I don't agree with the argument that dominance of 5-methyls suggests MBT'5Me is most suitable. Transfer functions for other non-biomarker proxies (e.g., foraminifera, chironomids, etc.) can include non-dominant taxa that are important for interpreting changes in T, or whatever the variable of interest is. Therefore, I'd suggest exploring all the available calibrations that separate the isomers, even those that may include 6-methyls, and those that include other calibration approaches, such as stepwise forward selection, as I suggest earlier. I'll also note that Russell et al. (2018) present a SFS calibration that does feature a lower RMSE than* *their MBT'5Me index calibration's.*

Reply: We cannot apply the 6-methyl brGDGT indices to some parts of the Lake El'gygytgyn record as only 5-methyl brGDGTs are present. In order to examine the full record (we are exploring this in other publications) and to get a continuous temperature reconstruction, we need to use 5-methyl brGDGTs. We note that the SFS calibration of Russell et al. (2018) includes brGDGT IIb' but as this brGDGT is typically not present in Lake El'gygytgyn samples, we did not apply this calibration.

Review: *L261: Fair to exclude Dang et al. (2018). And if the other calibrations I suggest are also not optimal*
is some similar way, it could be mentioned here, but each should be systematically evaluated.

Reply: We will evaluate each additional calibration suggested and will include them if deemed valid for comparison.

Review: *L265: Raberg et al. (2021) provide a number of Arctic sites in their modern lake brGDGT calibration.*

Reply: We will include the Raberg et al (2021) calibration in our revision as well as the additional recommended calibrations.

Review: *L270: Yes, naturally because they are all use the same index. I think this is also one reason why it will be interesting to try some other non-MBT-index calibrations to test if these patterns are consistent or not and explore what brGDGTs are key drivers in the indices. In this sense, it may also be interesting to conduct a Pearson correlation matrix as Feng et al. (2019) did to explore the relationship between individual brGDGTs. You may find similarities between other calibration Pearson matrices that could support use (or not) of a certain calibration.*

Reply: We will investigate this further and, if found to be important, we can add it to the revised manuscript.

Review: *L276-293: This paragraph seems to imply that the pollen-inferred T record is more reliable. Both pollen and brGDGT T proxies have various assumptions and therefore only reflect approximations of past events. I think this paragraph could be rephrased to exclude statements such as "unreasonably large, L280" and "more realistic, L284" and perhaps more objectively compare the 2 T proxy records (pollen and brGDGTs). One of the motivations for this study seems to be producing a rare continuous T record through the MPT. However, if we already have that through pollen, what's the value of brGDGTs here, especially if you think that are not realistic? There are many brGDGT studies that link different distributions to various environmental parameters (e.g., pH, salinity, DO, etc.), which may be a more valuable, or at least supplemental, discussion topic to include.*

Reply: We can revise these sentences to include more objective wording. We note that that pollen data only exist for certain portions of the Lake El'gygytgyn record; for much of the Lake El'gygytgyn core, pollen analyses are still in progress. One advantage of brGDGTs, is that the method is faster compared to pollen counting. We note that the pollen data spanning the MPT provides a landscape openness index but the authors did not provide a pollen-based temperature reconstruction (although pollen-based temperature estimates are available for certain discrete parts of the record, such as MIS 5 and MIS 11 (Melles et al., 2012)). Therefore, brGDGTs provide the first continuous temperature reconstruction from

Lake El'gygytgyn spanning the MPT. While, for the reasons described in the text, we caution readers against interpreting the absolute temperature values yielded by brGDGTs, we believe the warming and cooling trends in these data are robust. Certainly, other environmental factors are likely influencing the brGDGT record. We can add some text on this to the supplement.

Review: *L286: In Figure S5, it would be interesting to plot up some additional environments as well. Just eyeballing, there appear to be some similarities between brGDGT distributions in Lake E and Svalbard fjord sediments (Dearing Crampton-Flood et al., 2019) and marine sediments (see plots in Xiao et al., 2020), as examples.*

Reply: As Lake El'gygytgyn is a freshwater lake and has been a freshwater lake throughout its entire history, we feel it is appropriate to limit our comparison to other lacustrine datasets.

Review: *L318: Do other qualitative or quantitative climate records from Lake E show a long-term cooling trend that could be compared here?*

Reply: One of the novel aspects of our study is that it is one of the first continuous temperature reconstructions from Lake El'gygytgyn. There are numerous other proxies from this core spanning the MPT. While none show a clear cooling during the MPT, we also note that none are direct temperature proxies. We can add some information on other Lake El'gygytgyn proxies in our revision.

Review: *L321-322: Yes, a lack of MPT cooling at Lake E may be odd, but in addition to climate-driven hypotheses, our still growing knowledge of the brGDGT proxy may also limit or obscure these observations.*

Reply: We agree and feel that we have clearly noted the limitations of brGDGT data throughout the manuscript.

Review: *L413: Or there is another environmental or microbial factor that contributes to brGDGT distribution changes (e.g., Weber et al., 2018; De Jonge et al., 2019, 2021) and Facies C is still indicative of T.*

Reply: It is certainly possible that another factor contributes to brGDGT distribution changes and we can mention this in the revised manuscript. One advantage of using the BAYMBT calibration is that it spans a wide range of environments.

Review: *L440: You explain ACL earlier as a proxy for aridity, so might be better to limit your interpretations of ACL to that here as well. Why would trees expand under increased aridity?*

Reply: Based on the Lake El’gygytgyn pollen record published in Zhao et al. (2018), the amount of tree and shrub pollen increases during MIS 25, which is paired with a decrease in *n*-alkane ACL at this time. Based on our interpretation of ACL increasing with aridity and cooler brGDGT-inferred temperatures, this decrease observed during MIS 25 suggest wetter conditions in the region that would support the expansion of trees and shrubs. We will revise this sentence to read indicate that the decline in ACL coincides with an expansion of trees and shrubs in the pollen record.

Review: *L492: Clarify that this is increased “oceanic” stratification. It took me a few reads to realize you weren’t referring to Lake E.*

Reply: We will make this change.

Review: *L449: Can you specify which SST proxies? This makes it easier for the reader to independently compare different proxies, as each have different assumptions and interpretations.*

Reply: Yes, we will specify which SST proxies are used in the N. Pacific paleoceanographic records. These include alkenones, diatom assemblages and opal mass accumulation rates, and nitrogen-isotope records of nutrient utilization.

Review: *Figure 1: Panel B shows 2 yellow dots but only one is mentioned in the caption. Please clarify.*

Reply: We will revise the figure to only include the location of the drill core 5011-1 analyzed in this study. This figure was previously re-used from a different study that also included a second core, piston core LZ1024, which spans the past ~300 ka. Thank you for catching this.

Review: *Figure 3: Please clarify in the caption what the bold line is for n-alkane ACL…also a 5-point moving*
Average?

Reply: We will clarify this in the caption. Yes, it is a 5-point running mean.

Review: *Figure S5: Would be good to include all the lake sediment samples I mention from the other calibration studies in the ternary diagram.*

Reply: We can include samples from other calibration studies that are publicly available.

Technical Comments

Thank you for catching these errors. We will make these corrections.

L22: Change "exhibits" to "exhibit". Data are plural

L46: Comma after "ka"

L60: Insert "may have" or something similar before "worked". Otherwise, it sounds like this is what happened

L75: Capitalize "arctic", and check if needed elsewhere throughout the ms

L106: I suggest phrasing as "46.77 to 31.06 m" so that it is consistent with the ages you mention just before (i.e., older/deeper to younger/shallower).

L161: Change "they" to "the peaks" or something. It's always easier to follow if pronouns are not used.

L167: Extra and/or missing words around "samples characterized". Please clarify.

L362: Journal should not be referenced in in-text citations.

L389: Does it need to be mentioned or clarified that the LR stack is annual rather than summer biased as you argue the brGDGTs are?

L393-394: Are there any statistical regression analyses that can be used to support this observed alignment between brGDGTs and obliquity?

Reply: We argue that brGDGTs at Lake El'gygytgyn are aligned with obliquity because the spectral analysis of our record shows a strong 41 kyr signal (Fig. 4a) in line with the period of obliquity.

L412: There are some missing words in the latter half of this sentence.

L539: A little wordy/awkward phrasing…perhaps just "Spectral analysis of the brGDGT record, in comparison with marine records, suggests…"

L506: Add "Sea" after "Bering"

References (Not included in original text)

We will consider these references in our revisions.

Dearing Crampton-Flood, E., Peterse, F., Sinninghe Damste, J.S., 2019. Production of branched tetraethers in the marine realm: Svalbard fjord sediments revisited. Org. Geochem. 138, 103907.

De Jonge, C., Radujkovic, R., Sigurdsson, B. D., Weedon, J. T., Janssens, I., Peterse, F., 2019. Lipid biomarker temperature proxy responds to abrupt shift in the bacterial community composition in geothermally heated soils. Org. Geochem., 137, 103897.

De Jonge, C., Kuramae, E.E., Radujkovic, D., Weedon, J.T., Janssens, I.A., Peterse, F., 2021. The influence of soil chemistry on branched tetraether lipids in mid- and high latitude soils: Implications for brGDGT-based paleothermometry. Geochim. Cosmochim. Ac. 310, 92-112.

Dion-Kirschner, H., McFarlin, J.M., Masterson, A.L., Axford, Y., Osburn, M.R., 2020. Modern constraints on the sources and climate signals recorded by sedimentary plant waxes in west Greenland. Geochim. Cosmochim. Ac. 286, 336-354.

Feng, X., Zhao, C., D'Andrea, W.J., Liang, J., Zhou, A., Shen, J., 2019. Temperature fluctuations during the Common Era in subtropical southwestern China inferred from brGDGTs in a remote alpine lake. Earth Planet. Sci. Lett. 510, 26–36.

Harning, D.J., Curtin, L., Geirsdóttir, Á., D'Andrea, W.J., Miller, G.H., and Sepúlveda, J., 2020. Lipid biomarkers quantify Holocene summer temperature and ice cap sensitivity in Icelandic lakes. Geophys. Res. Lett. 47, 1–11.

Martínez-Sosa, P., Tierney, J., 2019. Lacustrine brGDGT response to microcosm and mesocosm incubations. Org. Geochem. 127, 12–22.

Weber, Y., Sinninghe Damste, J.S., Zopfi, J., De Jonge, C., Gilli, A., Schubert, C.J., et al., 2018. Redox-dependent niche differentiation provides evidence for multiple bacterial sources of glycerol tetraether lipids in lakes. Proc. Natl Acad. Sci. 115, 10926–10931.

Xiao, W., Wang, Y., Liu, Y., Zhang, X., Shi, L., Xu, Y., 2020. Predominance of hexamethylated 6-methyl branched glycerol dialkyl glycerol tetraethers in the Mariana Trench: source and environmental implication. Biogeosci. 17, 2135-2148.