

Response to reviews for MS2-Control on **Controls on brGDGT distributions in the suspended particulate matter of the seasonally anoxic water column of Rotsee**

Anonymous reviewer 1:

The study of Ajalloeian et al. reports branched GDGT data and genetic data from SPM obtained from a large part of a single season in lake Rotsee, as well as comparisons with surface sediments and soils. The discussions follow the often-used strategy for interpretation of branched GDGT data, i.e. one correlates concentrations and indices with all environmental or genetic quantitative parameters which happened to be measured and discuss all significant, and some non-significant, correlations. As often happens, some of the correlations agree with previous studies and some do not leading to the often-made conclusion that multiple environmental parameters influence brGDGT distributions.

The data are useful and add to a growing collection of such data which are not easy to obtain. The unfortunate thing is that I do not see (yet) how this data improved our understanding of lacustrine branched GDGT except 'it is complicated'. There have been more than plenty of those kind of studies on lake branched GDGTs (the far majority perhaps) so this is not a new insight. This is not the authors fault but one would like to see studies where more clarity is obtained and solid conclusions can be drawn. Can we still apply branched GDGTs as a lake temperature proxy? Should we just stop altogether in applying it? Is this single seasonal study of SPM sufficient to say that a brGDGT record from Rotsee is not useful for paleoreconstructions?

We appreciate the detailed and constructive feedback offered by this review. As the reviewer acknowledges, single study systems across one year have limitations, of which the most pertinent one is the difficulty in making a conclusive statement what this variability means for the downcore record. On the other hand, this type of study allows the temporal dynamics of GDGTs in a lake system to be described, which is a necessary step for understanding why a global MBT'_{5ME} values are observed to depend on temperature. As such, this study reveals both mechanisms at play that determine the temperature dependency of the MBT'_{5ME} (sections 4.1 and 4.2) as well as a section that discusses initial implications for the sedimentary record (4.3).

Specifically, our findings indicate that while MBT'_{5ME} shows a response to temperature in the epilimnion, the onset of seasonal stratification plays a more significant role in the observed MBT'_{5ME} shifts. This represents a novel insight, highlighting that MBT'_{5ME} is not solely controlled by temperature but also by lake stratification dynamics.

Additionally, we discovered that in the hypolimnion, MBT'_{5ME} responds more to chemical conditions than to temperature. These findings suggest that while MBT'_{5ME} may not universally serve as a temperature proxy, its utility in paleoclimate reconstructions remains valid if the sedimentary brGDGT signal primarily reflects the epilimnion.

Importantly, this study shows for the first time that the Isomer Ratio (IR) shows a stronger correlation with mean annual temperature changes compared to MBT'_{5ME} and the observed correlation between MBT'_{5ME} and IR in epilimnion.

To address your concerns and clarify the role of stratification, we have updated sections 4 and 5, specifically adding and revising Lines 559-562 and 657-662. These revisions emphasize the novel contributions of our study, particularly regarding the stratification-related dynamics of MBT'_{5ME} and the potential of IR as a reliable temperature proxy. The new lines now read:

Line 559-562 in section 4.2.1: *“The observed changes in MBT'_{5ME} in the epilimnion are more indicative of stratification dynamics than direct temperature variability alone. During mixing events, the influx of hypolimnion-derived brGDGTs significantly alters the MBT'_{5ME} signal, reducing its responsiveness to cooling temperatures in the epilimnion.”*

Line 657-662 in section 5: *“In Rotsee, seasonal temperature changes drive stratification, identifying temperature, oxygen, and pH as the most influential environmental parameters affecting brGDGT distribution in the water column. MBT'_{5ME} values exhibit a muted response to water temperature in the epilimnion, however reflecting its sensitivity to stratification onset rather than a direct temperature dependency. In contrast, the IR demonstrates a stronger linear correlation with temperature changes, highlighting its potential as a reliable paleothermometer. While the application of IR for paleotemperature reconstructions shows promise, further calibration across diverse lake systems is necessary to establish its robustness.”*

My main criticism of the study (details below) is that it fully relies on correlations. For this to work, the quantitation, number of data points and representativeness of the samples must be sufficient. I am worried about these aspects. For example, as far as I can see there has not been a ‘true’ replicate analysis done (i.e. independent work-up of 2 parts of the same filter with addition of IS to the raw extract) so there is no way of knowing the real error in the quantitation. Some of the changes in concentration or indices are fairly small and we have no way of knowing if they are real changes or not. The number of data points is reasonable (12, but seems less for e.g. nutrients, i.e. 7 if I counted correctly) but I am unclear how representative is this season for Rotsee? Would another season have shown the same? Several studies have shown that biomarker lipid patterns can vary from season to season. I realize this is additional effort but it is a concern which needs to be addressed.

We appreciate the reviewer’s concerns regarding the reliance on correlations and the robustness of our quantification. brGDGTs were “semi-quantified” following well-established protocols commonly used in the GDGT research community (lines 207-211). We ensured comparability among samples by using the entire TLE amount for each analysis and maintaining consistent sample preparation methods.

To address concerns about quantification variability, six independent samples underwent separate extraction processes, showing a concentration variation of 15-20%.

We also acknowledge the issue of data completeness. Due to logistical constraints, cation and anion measurements were unavailable for the last four months. Since no significant correlations were found between nutrient levels and GDGT distributions, these nutrient data have been moved to the supplementary material, and Figure 2 now includes only parameters with full coverage: conductivity, alkalinity, dissolved oxygen, temperature, and pH.

We will address each of the reviewer’s concerns in detail in their comments below.

Detailed comments:

Line 40-41.has become a widely accepted tool for lacustrine paleothermometry.... Unsure about that wide acceptance for lakes but at least nothing is mentioned in the introduction on ‘success stories’ of this proxy in lake temperature reconstructions. Perhaps a few examples of apparently nice temperature records, as well as clearly wrong/biased records, would be useful in the introduction.

We appreciate the suggestion to provide context on the success and limitations of brGDGT-based temperature reconstructions in lakes. To address this, we have added references (Loomis et al., 2012; Watson et al., 2018; Ramos-Roman et al., 2022) to the introduction (lines 77-79), highlighting studies where brGDGTs have been successfully applied to lacustrine settings around the globe for temperature reconstruction. These examples illustrate both the potential and challenges of using GDGTs in different lake environments, providing a balanced perspective on their reliability and limitations. This addition clarifies the broader applicability of brGDGT proxies and sets the stage for our investigation into their specific responses in Rotsee.

New added/edited lines:

Lines 77-79: *“These calibrations have been successfully applied to generate high-resolution temperature reconstructions in lake sediment records, such as those from East Africa (Loomis et al., 2012), the Northern America (Watson et al., 2018), and the Mediterranean region (Ramos-Roman et al., 2022).”*

Lin 112. I would have expected some lake DNA (metagenome) studies as a reference.

Thank you for pointing this out. In Line 112, we reference studies that highlight the limited abundance of Acidobacteria in lake systems. Specifically, the studies by Weber et al. (2018), Van Bree et al. (2020), and Avila et al. (2017) demonstrate that Acidobacteria are generally less abundant in freshwater environments, including lake systems. For example, Weber et al. (2018) and Van Bree et al. (2020) explore European and African lake systems, where Acidobacteria are not major components of the microbial communities.

Additionally, on Line 117, we discuss studies by Dedysh and Sinninghe Damsté (2018) that suggest other microbial groups, beyond Acidobacteria, might be responsible for lacustrine GDGT production. These references, along with those by Parfenova et al. (2013), Weber et al. (2018), Van Bree et al. (2020), and Avila et al. (2017), support the argument that Acidobacteria are often not considered the primary producers of GDGTs in lakes.

The amplicon-based studies referenced, provide a robust basis for understanding microbial community composition in lake systems.

Line 124. Are these the only seasonal studies of lake brGDGTs? I thought there were more. If so, did they reach similar conclusions?

Thank you for your observation. You are correct that there are additional seasonal studies on lake brGDGTs. In response, I have included references to studies by Baxter et al. (2024), Zhang et al. (2016), and Buckles et al. (2014). These studies report varied findings, including a non-

homogeneous impact or lack of impact of seasonality, often with observed "cold" or "warm" biases on reconstructed MAT in their respective lakes.

Nevertheless, our findings align with the broader trends observed in these studies, which generally indicate that brGDGT distributions are influenced by multiple environmental factors beyond temperature alone. However, the specific responses can vary depending on the lake's characteristics and conditions. We have clarified this point and incorporated these references in the revised manuscript in lines 124-131:

“In contrast to soils, which show no variability in brGDGTs between seasons (Weijers et al., 2011; Naafs et al., 2017), brGDGT concentrations and distributions in lakes vary seasonally, with reported increases in brGDGT concentrations during spring and fall isothermal mixing (Loomis et al., 2014a; Miller et al., 2018). This seasonality can introduce biases in reconstructed mean annual temperatures (MAT), resulting in "cold" or "warm" biases or even inconsistent seasonality effects, depending on the specific conditions and brGDGT production dynamics of the lake (Buckles et al., 2014; Zhang et al., 2016; Baxter et al., 2024). These findings underscore that brGDGT variability in lakes is often influenced by multiple factors beyond temperature alone, which can vary depending on the lake's unique characteristics”

Line 206-209. This error is not the error in quantification as the ionization differences between IS and GDGTs were not corrected (I think? If so, the ng amounts calculated are just a complete guess) and the IS was added at the really final stage of fraction preparation (errors due to hydrolysis+workup are not included). At best this is a repeatability error of the instrument. I cannot therefore agree with the statement at these lines because this error really represents the (unrealistic) best case scenario and ignores the complete workup and sample inhomogeneity errors. I would recommend splitting a sediment sample, or even better a filter cut in two pieces, and work this up completely separately to obtain a better constraint of the overall quantification error.

Thank you for your detailed feedback on this aspect. We acknowledge the limitations in our current approach to error estimation, as the internal standard was indeed added at the final stage and does not account for errors from the entire workup process, including hydrolysis and extraction. We agree that this primarily reflects instrumental repeatability rather than a comprehensive quantification error. The variability in ionization efficiency is expected to be captured by the re-analysis of individual samples and thus by the instrument error as reported. In addition, we maintained consistent sample preparation protocols that were applied on very similar sample types (all filtered SPM).

As mentioned in our response to your first comment, we now also identified six independently processed samples where the entire extraction and workup steps were repeated. The observed variation in brGDGT concentrations between these samples was within 15-20%, i.e. the same as the previously reported instrument error. For example, in the July-epilimnion sample, brGDGT Ia concentrations ranged from 0.84 ng/L to 0.98 ng/L, while IIIa ranged from 1.17 ng/L to 1.33 ng/L. These details have been added to the methods section (lines 217-220). This data is now added to the supplementary Table 1 of the article as well.

Line 375-380 and line 483. IPLs are nearly always a small fraction of the total lipids. Since direct measurements of IPLs were not done, this could even be an overestimate, i.e. if some of the GDGTs

released by acid hydrolysis were not derived from IPLs but from matrix-bound material. Why are these IPL abundances so low, if the assumption is that all this material is derived from living biomass? Do branched GDGTs mostly occur as CL in the cell? Is cell lysis so quick or happening during filtration?

Thank you for raising these important points. Our data, along with observations from surface sediment records, indicate that GDGTs are indeed present in both core lipid (CL) and intact polar lipid (IPL) fractions, often displaying distinct distribution patterns. This suggests that GDGTs can be produced in both forms, potentially reflecting different stages of microbial activity or degradation processes.

We have highlighted these aspects in lines 491-498 referencing Raberg et al. (2022). Our data, along with observations from surface sediment records, indicate that GDGTs occur in both core lipid (CL) and intact polar lipid (IPL) fractions, potentially reflecting different stages of microbial activity or degradation processes. Raberg et al. (2022) observed that IPL brGDGTs constitute a small fraction of the total lipids generally. Particularly, IPL with phospholipid headgroups that are more abundant in water column, might degrade more readily into CLs compared to another group of IPLs.

We propose that degradation likely plays a role in the transition from IPLs to CLs, particularly for IPLs with phospholipid headgroups according to Raberg et al., (2022), but the relative contributions of cell lysis and in situ production to GDGT transformation remain unresolved.

New added/edited lines 491-498:

“Our data, along with observations from surface sediment records, indicate that brGDGTs occur in both core lipid (CL) and intact polar lipid (IPL) fractions, often displaying distinct distribution patterns. This suggests that brGDGTs can be produced in both forms, potentially reflecting different stages of microbial activity or degradation processes. Raberg et al. (2022) observed that IPL brGDGTs generally constitute a small fraction of the total lipids. Particularly, IPLs with phospholipid headgroups, which are more abundant in the water column, might degrade more readily into CLs compared to other IPL types. It is proposed that degradation likely plays a role in the transition from IPLs to CLs, especially for IPLs with phospholipid headgroups, but the relative contributions of cell lysis and in situ production to brGDGT transformation remain unresolved.”

Line 385 and further. Coming back to the 15% error in quantification. It is not indicated here what the errors in brGDGT indices are. Are the changes observed larger than the assumed errors? At least replicate analysis could be done but preferably replicate work up.

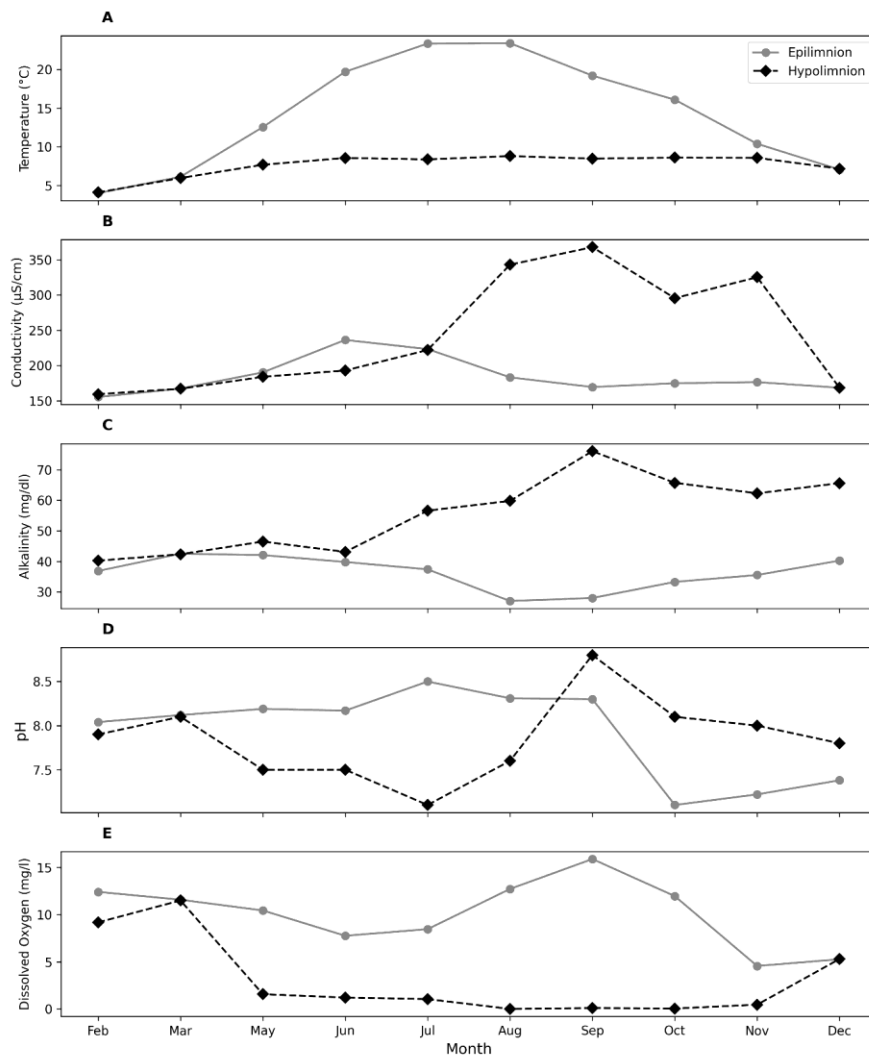
The error in brGDGTs indices is based on the duplicate measurements of 12 samples as mentioned in lines 212.

Fig. 2. There is a clear mismatch in the no. of data points with only 7 data points for the cat- and anion concentrations. Can we really make solid conclusions on so few datapoints?

We agree that drawing solid conclusions from a limited dataset may be problematic. To address this, we have updated Figure 2 to include only those inorganic parameters (conductivity, alkalinity, dissolved oxygen, temperature, and pH) for which data are available throughout the entire sampling period. Some nutrient values, which were available for a shorter duration, have been moved to the

supplementary data section. The manuscript has also been updated to reference the correct data in Figure 2 and the supplementary data for the nutrient values throughout.

Current figure 2:



Newly added references to support added information in the manuscript:

Avila, M. P., Staehr, P. A., Barbosa, F. A., Chartone-Souza, E., and Nascimento, A. M. (2017). Seasonality of freshwater bacterioplankton diversity in two tropical shallow lakes from the Brazilian Atlantic Forest. *FEMS microbiology ecology*, 93(1), fiw218.

Baxter, A. J., Peterse, F., Verschuren, D., Maitituerdi, A., Waldmann, N., and Sinninghe Damsté, J. S. (2024). Disentangling influences of climate variability and lake-system evolution on climate proxies derived from isoprenoid and branched glycerol dialkyl glycerol tetraethers (GDGTs): the 250 kyr Lake Chala record. *Biogeosciences*, 21(11), 2877-2908.

Buckles, L. K., Weijers, J. W. H., Tran, X. M., Waldron, S., and Sinninghe Damsté, J. S. (2014). Provenance of tetraether membrane lipids in a large temperate lake (Loch Lomond, UK): implications for glycerol dialkyl glycerol tetraether (GDGT)-based palaeothermometry. *Biogeosciences*, *11*(19), 5539-5563.

LibreTexts. (2024). *Temperature effects on solubility*. LibreTexts Chemistry. Retrieved from https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_%28Physical_and_Theoretical_Chemistry%29/Equilibria/Solubility/Temperature_Effects_on_Solubility

Loomis, S. E., Russell, J. M., Ladd, B., Street-Perrott, F. A., and Sinninghe Damsté, J. S. (2012). Calibration and application of the branched GDGT temperature proxy on East African lake sediments. *Earth and Planetary Science Letters*, *357*, 277-288.

McManus, J., Collier, R. W., Chen, C. T. A., and Dymond, J. (1992). Physical properties of Crater Lake, Oregon: A method for the determination of a conductivity- and temperature-dependent expression for salinity. *Limnology and Oceanography*, *37*(1), 41-53.

Pearman, J. K., Biessy, L., Thomson-Laing, G., Waters, S., Vandergoes, M. J., Howarth, J. D., Rees, A., Moy, C., Pochon, A., and Wood, S. A. (2020). Local factors drive bacterial and microeukaryotic community composition in lake surface sediment collected across an altitudinal gradient. *FEMS Microbiology Ecology*, *96*(6), fiae070.

Ramos-Roman, M. J., De Jonge, C., Magyari, E., Veres, D., Ilvonen, L., Develle, A. L., and Seppä, H. (2022). Lipid biomarker (brGDGT)- and pollen-based reconstruction of temperature change during the Middle to Late Holocene transition in the Carpathians. *Global and Planetary Change*, *215*, 103859.

Watson, B. I., Williams, J. W., Russell, J. M., Jackson, S. T., Shane, L., and Lowell, T. V. (2018). Temperature variations in the southern Great Lakes during the last deglaciation: Comparison between pollen and GDGT proxies. *Quaternary Science Reviews*, *182*, 78-92.

Wu, J., Yang, H., Pancost, R. D., Naafs, B. D. A., Qian, S., Dang, X., Sun, H., Pei, H., Wang, R., Zhao, S., and Xie, S. (2021). Variations in dissolved O₂ in a Chinese lake drive changes in microbial communities and impact sedimentary GDGT distributions. *Chemical Geology*, *579*, 120348.

Zhang, Z., Smittenberg, R. H., and Bradley, R. S. (2016). GDGT distribution in a stratified lake and implications for the application of TEX₈₆ in paleoenvironmental reconstructions. *Scientific reports*, *6*(1), 34465.