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 2:

Ajallooeian et al. presented a record of brGDGTs in suspended particulate matter (SPM) from a monomictic, eutrophic temperate lake (Rotsee, Switzerland) over a 10-month period, examining both core lipids and intact polar lipids, in addition to surface sediments and soils. The authors aimed to elucidate which environmental variables, such as water temperature, water chemistry (e.g., dissolved oxygen, pH, alkalinity, and conductivity), or bacterial community composition, best explain seasonal variations in brGDGT distributions, and thus examine the sensitivity of MBT’5ME to seasonal and short-term environmental changes in the water column. Overall, the study underscores the influences of temperature, pH, and oxygen on brGDGT distribution, raising important considerations for using MBT’5ME in temperature reconstructions from stratified lake sediments. The manuscript is well-organized, but several issues need to be addressed before acceptance.

As environmental factors influencing brGDGT production and distribution, temperature, conductivity, dissolved oxygen, pH, and alkalinity were considered in this study. Among these, the authors suggested that in the oxic epilimnion, MBT'5ME was associated with temperature, while in the seasonally anoxic hypolimnion, MBT'5ME correlated with water pH. However, considering Tables S2 and S5, it appears that in the epilimnion, MBT'5ME is related not only to temperature but also to conductivity. In the hypolimnion, MBT'5ME is influenced not only by pH but also by temperature. Does this imply that temperature, pH, and oxygen are not only complicating factors for the use of MBT'5ME, but also conductivity?

Thank you for your thoughtful feedback. In Rotsee, conductivity correlates with temperature in the epilimnion, likely due to the increased ion mobility at higher temperatures and potential evaporation effects during warmer months. This interrelation complicates the interpretation of MBT'_{5ME}, as temperature and conductivity may both influence brGDGT distributions. However, as shown in our data (Tables S2 and S5), the relationship between MBT'_{5ME} and these factors is not straightforward, highlighting the complex interplay of multiple environmental drivers in Rotsee.

Conductivity can impact brGDGT distributions by influencing microbial activity and ionic strength in the water column, factors that may affect brGDGT production or preservation. Therefore, while the IR proxy strengthens the temperature-related interpretation for epilimnion-sourced brGDGTs, it is essential to consider the confounding influence of conductivity. Our findings emphasize the complexity of isolating temperature effects in lake systems where multiple environmental parameters are interrelated.

We have added lines 540 to 545 to clarify this point and emphasize the need for caution when interpreting MBT'_{5ME} solely as a temperature proxy, particularly in stratified and eutrophic lakes where multiple environmental variables interact.

Added lines now read:

"*Additionally, MBT'$_{5ME}$ shows a significant correlation with stratification-dependent conductivity ($r = 0.71$, $p < 0.05$). This relationship likely reflects the interconnected effects of temperature on ion mobility and evaporation, which influence ionic strength in the water column. Conductivity may indirectly affect brGDGT production by altering microbial community structure or metabolic activity (Pearman et al., 2020) highlighting its role as a complicating factor for MBT'$_{5ME}$, especially in eutrophic and stratified lakes with tightly coupled environmental parameters.*"

The authors proposed that the IR represents a stronger dependency on temperature in the epilimnion, highlighting the potential of using this proxy to identify brGDGT distributions dominantly sourced from the epilimnion within the water column by comparing MBT'5ME and IR in parallel. According to Table S2, IR also correlates with conductivity, similar to MBT'5ME. Would this suggest that although brGDGTs sourced from the epilimnion can be identified by comparing MBT'5ME and IR, MBT'5ME still does not fully reflect the influence of temperature alone?

Yes, you are correct—our data in Table S2 show that IR, like MBT'$_{5ME}$, also correlates with conductivity in the epilimnion. This suggests that, although comparing MBT'$_{5ME}$ and IR helps identify brGDGT distributions primarily sourced from the epilimnion, MBT'$_{5ME}$ potentially does not solely reflect temperature influences. The interplay between temperature and conductivity is now summarized in the manuscript in lines 540-545 as mentioned in the above reply.

Sediments from seasonally anoxic areas reflected average epilimnion SPM values, suggesting the deposition of epilimnion brGDGTs into the sediments. Does this suggest that the seasonal contribution from the anoxic hypolimnion plays a minor role in the application of MBT'5ME in such a lake? What could be the reason that the brGDGTs produced in the hypolimnion were not deposited or well-preserved in the sediments?

Thank you for highlighting this important point. Our findings indicate that sediments from the seasonally anoxic areas primarily reflect the average brGDGT composition from the epilimnion SPM, suggesting a relatively minor contribution from the hypolimnion. This could be due to several factors. (i) organic matter and brGDGTs produced in the hypolimnion may not settle effectively, as sedimentation dynamics favor epilimnion-derived inputs. (ii) organic matter from the hypolimnion could degrade during oxygenated phases of water column mixing, reducing its preservation in sediments (iii) the epilimnion, with its larger water volume and longer production period, likely dominates sedimentary inputs. (iv) the hypolimnion remains anoxic only seasonally, further limiting its overall contribution to the sedimentary record.

It is important to note that while these mechanisms are consistent with the data, further studies would be needed to definitively quantify the relative contributions of hypolimnion versus epilimnion-derived brGDGTs to lake sediments and we are only hypothesizing.

Other comments:

51-53: *"The production of intact polar lipid (IPL) tetraethers was observed exclusively in the anoxic hypolimnion during stratification, confirming anoxia as a key trigger for IPL tetraether production."* – If so, how should we interpret the IPL brGDGTs data shown in Figure 3D for the epilimnion?

We believe a potential explanation for the presence of IPL brGDGTs in the epilimnion (Figure 3D) lies in their seasonal distribution. The majority of IPL brGDGTs in the epilimnion are observed in October and November, while their production in the hypolimnion peaks during August and September. This temporal pattern suggests that IPL brGDGTs produced in the hypolimnion might be transported upwards during seasonal mixing events or deepening of the thermocline. This is highlighted in lines 496-502:

“The production of IPL brGDGTs in the hypolimnion is limited to anoxic conditions. This finding highlights the role of anoxia as a key trigger for in-situ IPL brGDGT production. While IPLs are detected in the epilimnion following the breakdown of stratification, i.e. during the mixing season (Fig. 3D), their distribution mirrors the hypolimnion pattern (Fig. 3C, D). This suggests that IPLs observed in the epilimnion are transported from the hypolimnion during the deepening of thermocline (October-November) and full water column mixing (November-December), therefore not supporting the production of IPL brGDGTs in the oxic epilimnion.”

494-495: “*The production of IPL brGDGTs in the hypolimnion is limited to anoxic conditions. This finding unequivocally highlights the role of anoxia as a key trigger for in-situ IPL brGDGT production.*” – However, in Figure 1, bottom water anoxic conditions also occurred in June and November, yet IPL brGDGTs were not produced. What is the reason for this?

Thank you for your observation. It is important to distinguish between suboxic and anoxic conditions when interpreting our findings. Suboxic conditions refer to low but detectable levels of dissolved oxygen (e.g., periods like June or November in our water column), whereas anoxic conditions indicate the complete absence of oxygen e.g., July-October. These distinctions are critical, as the production of IPL brGDGTs appears to require fully anoxic environments, rather than suboxic ones. Please also refer to the answer in question above.

499: “*… brGDGT IIIa’’, a compound which was not observed in Rotsee)*” – The compound brGDGT IIIa’’ is mentioned here but is not shown in Figure S1, nor is there any explanation for it. Is it necessary to mention it here at all?

We have removed the mention of brGDGT IIIa’’ from the manuscript as it is not observed in our data and does not contribute to our conclusions.

531: “*During the epilimnion mixing season, a decrease in brGDGT Ia and an increase in brGDGT IIIa are observed, reflecting the GDGT distribution found in the hypolimnion (Fig. 3C)*”. – When is the mixing season in Figure 3? It would be helpful to add this information to Figure 3, as mentioned in Figure 1: (i) isothermal mixing (December and February), (ii) stratification onset (June), (iii) stratified water column (August and September), and (iv) post-stratification conditions (November).

We have added the seasonal phases (mixing, stratification onset, stratified, and post-stratification) to the caption of Figure 3 to clarify the corresponding periods and enhance interpretability.

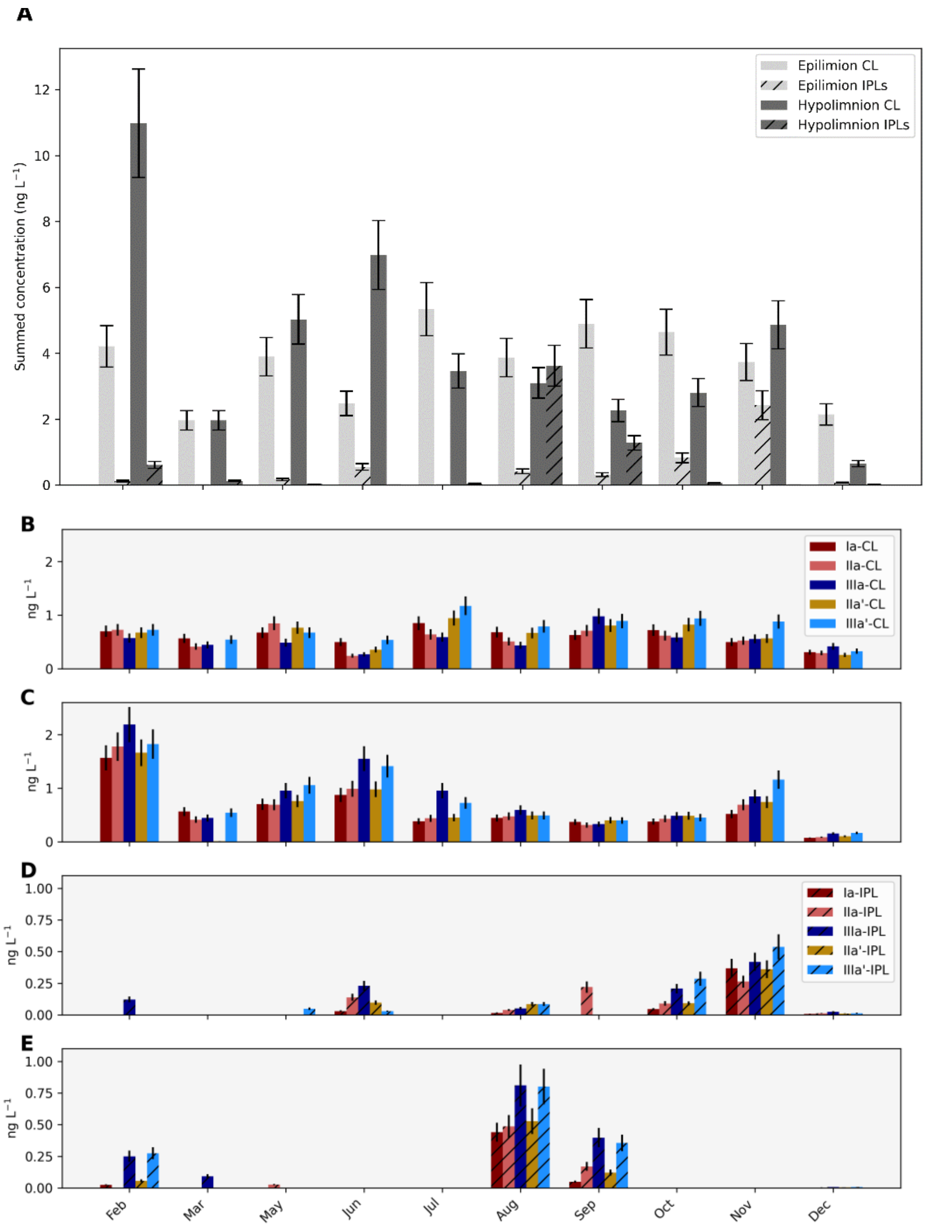

Fig. 3. A) Summed concentrations (in ng L^{-1}) of brGDGTs through the year in Rotsee. Light grey bars represent epilimnion, while dark grey display hypolimnion concentrations. Subplots B-E display concentrations of the five most abundant brGDGTs, with B-D representing epilimnion values, while panels C and E represent hypolimnion concentrations. CL and IPL BrGDGTs refer to core and intact polar lipids, respectively. Error bars reflect the estimated instrumental error (15%). The water column dynamics of Rotsee can be categorized into four distinct phases: (i) isothermal mixing during December and February, (ii) the onset of stratification in June, (iii) a fully stratified water column in August and September, and (iv) post-stratification conditions in November.

537-539" "*However, although the increase in concentration of Ia is observed in warm stratified months in the epilimnion, the absence of a correlation between Ia and temperature during colder months, contributes to the non-significant dependency between MBT'_{5ME} and temperature (r= 0.59, p= 0.10).*" – I guess in this text, Ia is referring to Ia-CL in Figure 3A. This should be clarified first.

Nonetheless, in my view, it is not clear in Figure 3A how the concentration of Ia increases during the warm, stratified months in the epilimnion.

We have clarified in the text that "Ia" refers specifically to **Ia-CL** shown in Figure 3A. We have slightly changed lines 537-539 to reflect the fractional abundance of Ia where the change in abundance is much more visible for the reader in figure 4.

Lines 537-540: "*However, although the increase in fractional abundance of CL-brGDGT Ia is observed in warm stratified months in the epilimnion (Fig. 4), the absence of a correlation between Ia and temperature during colder months, contributes to the non-significant dependency between MBT'5ME and temperature (r= 0.59, p= 0.10).*"

573-574: *"however, dissolved oxygen content (and conductivity and alkalinity to a lesser extent) seems to drive increases in cyclopentane-containing and 6-methyl brGDGTs (Supp. Table S2)."* – However, there was no relationship between IR and conductivity in Table S2.

Thank you for pointing out this inconsistency. We've updated the text to reflect that there is no **significant correlation** between IR and conductivity, aligning with the data in Supplementary Table S2.

577: *"… as well as the IR, CBT' and DC' (r= -0.65 to -0.78, p< 0.05)."* – delete IR in this sentence.

Noted and deleted.

577-588: *"This correlation is also observed in the IR (r=-0.64, r= 0.66, p< 0.05, for dissolved oxygen and alkalinity respectively)."* – r=-0.64 should be r=-0.64 in Table S2.

Noted and revised.

637-638: *"While CL brGDGTs are produced throughout the water column, the production of IPL brGDGTs seems confined to the anoxic hypolimnion."* Does this mean that IPL brGDGTs are produced specifically in the anoxic hypolimnion, implying that they are not produced in the epilimnion or other oxygenated parts of the water column? If so, how should we interpret the IPL brGDGTs data shown in Figure 3D for the epilimnion? This point should be clarified to avoid any confusion.

See reply above to the question about the delivery of IPLs produced in the anoxic hypolimnion to the oxic epilimnion during full water column mixing.

In many places in the text, "GDGT" was used instead of "brGDGTs." It would be better to use "brGDGTs" consistently.

Noted and revised to brGDGTs throughout the text.

For "Rotsee" or "Lake Rot," it would be best to use one term consistently throughout the text and figure captions.

Noted and revised to Rotsee throughout the text.

Fig. 8. The layer legend (i.e., 1-Surface, 2-Bottom) can be replaced with 1-Epilimnion and 2-Hypolimnion to ensure consistency.

Noted and revised.

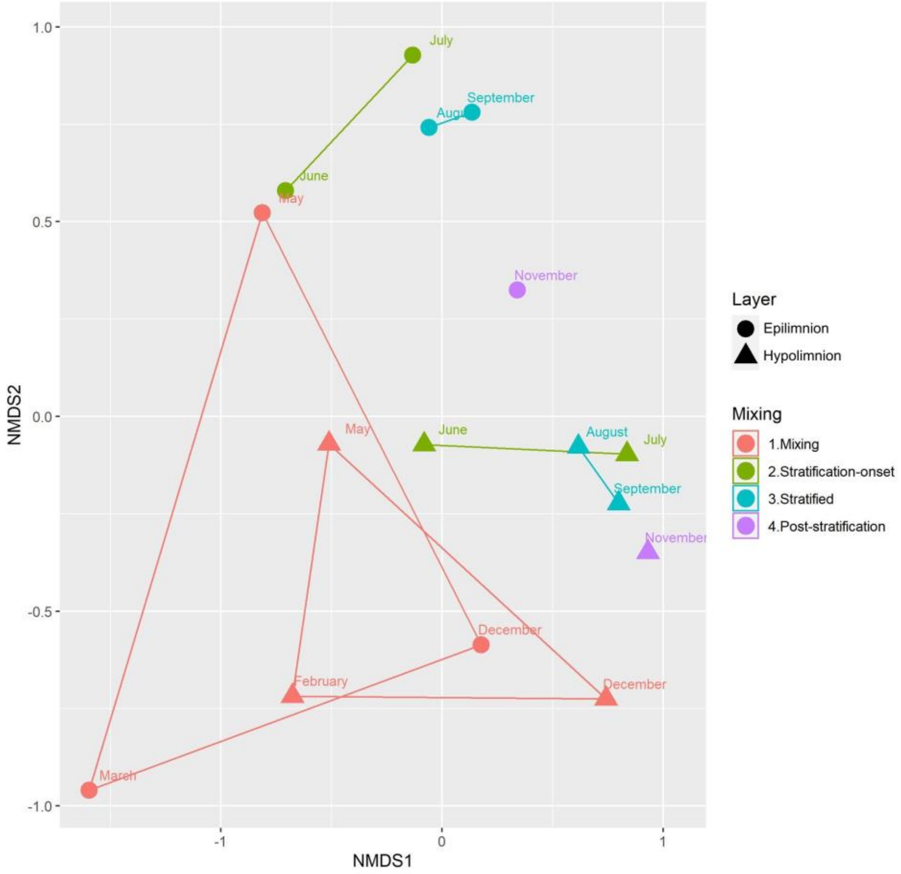

Fig. 8

Fig. 9: For figure, using different symbols for soil and surface sediment would be helpful.

Noted and revised.

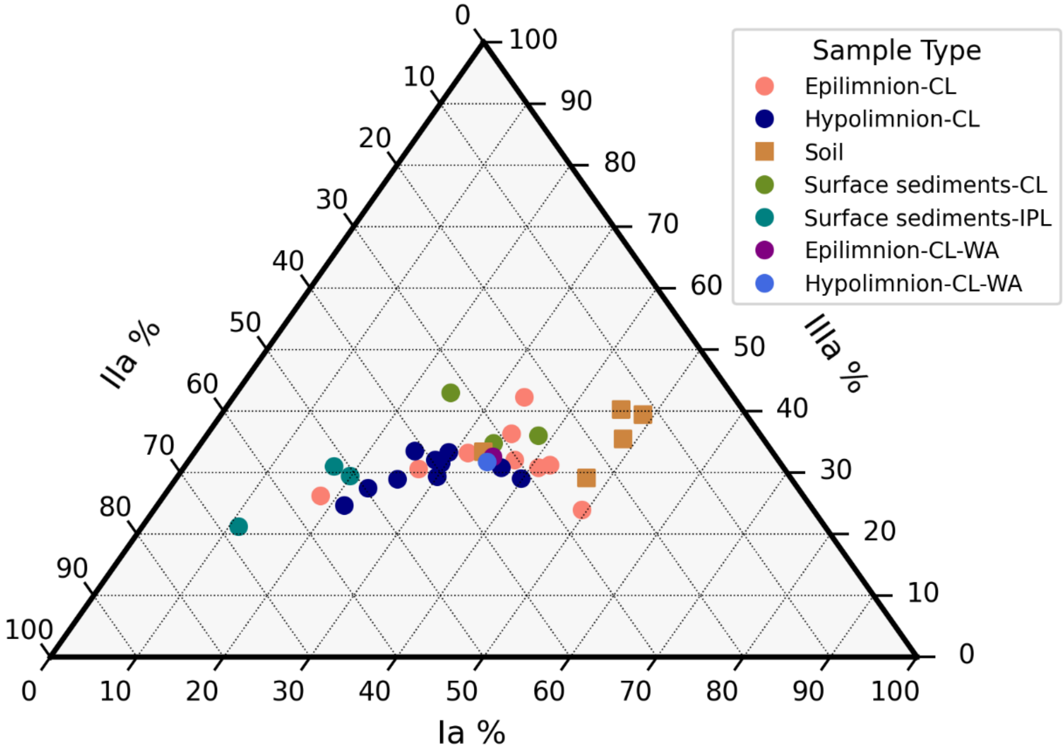

Fig. 9

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.

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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/Solubilty/Temperature_Effects_on_Solubility

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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.