Associate editor decision: Publish subject to minor revisions (review by editor)
by Cindy De Jonge

Public justification (visible to the public if the article is accepted and published): **Dear Allix Baxter and co-authors, thank you for responding to the comments of the co-authors in a timely fashion, and for accepting the suggestion to shorten and streamline the manuscript. Following this major revision I have read through the resubmitted version, and only have a few small editorial comments, as well as a suggestion to make the relevance of this study for globally distributed lake systems even more clear. I invite the authors to correct these small typo's and address my suggestion below.**

We kindly thank the editor for their work on reviewing the resubmitted manuscript and providing further suggestions for improvement.

L 132: "subapine zone" seems to be a typo
L 144, check punctuation around the Sepulchre reference.
L 193, check punctuation around Moernaut reference.
L 479, Table number is missing
L 492: %isoGDGT2: the ecological relevance of this ratio is not introduced.
L 564: Rewrite as: 'niches of GDGT producers'.
L 784: check punctuation around Baxter et al. reference.

We have incorporated all minor changes suggested above.

Suggestion for making global relevance more clear:
Throughout the discussion there is not many comparisons made with other lakes. Is this because similar water column and/or sediment studies in the framework of the lake system evolution are uncommon? Or because Challa behaves differently than other lakes?
Certainly, studies of lake sediment records that both discuss the effects of basin evolution on GDGT climate proxies and are supported by adequate water-column investigation are still very uncommon. The limited water column studies that are currently available indicate that oxycline depth is a major controlling factor on the abundance and spatio-temporal distribution of GDGTs in stratifying lakes. However, how exactly GDGTs respond to changes in oxygen availability may differ between lakes globally, as we have addressed in our earlier work (e.g., van Bree et al., 2020 BGS on brGDGTs and Baxter et al., 2021 QSR on isoGDGTs) and has also been addressed elsewhere (e.g., Sinninghe Damsté et al., 2022, QSR). This is also the reason why we have not advocated here or previously (Baxter et al., 2021, QSR; Baxter et al., 2023, Nature) for applying GDGT proxies validated for use in Lake Chala (e.g., the BIT index) to other stratifying lakes without first conducting thorough modern-system studies. Instead, we argue that it is key to establish whether lake sedimentary archives reflect stable water column conditions throughout lake history prior to downcore application and interpretation of GDGT proxies as climate records.

We emphasized this issue in the final sentences of section 5.2 of the discussion in relation to the 5- and 6-me brGDGT distribution in Lake Chala with the following text (updated slightly from previous version):

"Certainly, the exact relationship between 5Me- and 6Me-brGDGTs and the particular water column zones which allows their relative proportion to be used as a qualitative proxy for lake depth in Lake Chala is not likely universal across different lake systems. Even in the similarly meromictic Lake Lugano (Switzerland) markedly different brGDGT distributions with depth occur (Weber et al., 2018) implying that comprehensive local water-column profiling of GDGT distributions is necessary prior to interpretation of down-core BIT index or IR_{6me} *records".*

To address the 'uniqueness' of Lake Challa, could the authors include a statement in their conclusion whether the interpretations on GDGT niches and derived temperature ratios based on low latitude meromictic Lake Challa are fully/in part/not at all applicable to shallow (seasonally stratified or fully mixed) lakes or lakes at higher latitudes (seasonally variable temperature and a colder hypolimnion)? Would a shallow lake be comparable with a Lake Challa Zone 1-3 for instance.

We refer the editor to sections 4.5 and 4.6 of Baxter et al., 2021, QSR where we included a comparison of the isoGDGT SPM data set to other studies of isoGDGTs in diverse lake systems and additionally speculated about the functioning of our presented upper water-column stratification proxies (e.g., BIT, f[CREN'], isoGDGT-0/cren) in lakes shallower and deeper than Lake Chala. We have added the following statement to our conclusion in order to stress that our results are likely not applicable to lakes in other regions / mixing regimes:

"We stress that the niche partitioning of GDGT producers within the distinct water-column mixing zones of Lake Chala underpinning the temperature proxies as presented here is potentially unique (as discussed previously in Baxter et al., 2021, QSR). Dimictic and monomictic lakes, as are common in cold- and warm-temperate regions, may be equivalent to zones 1-2 or zones 1-3 of Lake Chala depending on their trophic status (i.e., level of primary productivity), as this would control the occurrence and seasonal persistence of hypolimnetic anoxia. Unstratifying (truly shallow) lakes enjoy year-round oxygen injection and nutrient upcycling, and thus present GDGT producers with entirely different niches. At the other extreme of the depth gradient, permanent stratification of the lower water column (meromixis) can be created and maintained by different processes, and with different prevalence in temperate and tropical lakes (Gulati et al. 2017), consequently the occurrence of GDGT niches as in zones 4-6 of Lake Chala is more likely site-specific (and as documented here, may change over long time scales). Thorough modern-system studies should ideally be conducted before applying such proxies to the sedimentary record of any type of lake system.".

Gulati, R. D., Zadereev, E. S., & Degermendzhi, A. G. (Eds.). (2017). Ecology of meromictic lakes (Vol. 228). Cham: Springer.

Furthermore, the authors might want to 'philosophize' how we interpret the fact that the reconstructed sedimentary temperatures (based on brGDGTs) rely on the contribution of two different water layers and potentially different communities

of producers (zone 3 vs zone 5 and 6)? How does this for instance relate back to the mechanism that a single brGDGT producer modulates the lipid composition of their membrane to regulate the membrane fluidity?

It is possible that 5- and 6-Me brGDGTs are produced by different communities, as we have discussed in van Bree et al. (2020, BGS), and that changes in community structure may affect the temperature signal recorded by the MST proxy. Work on Lake Lugano by Weber et al (2018, PNAS) likewise suggested that brGDGTs likely have distinct bacterial producers in different parts of the water column. However, given the fundamental work showing that methylation in a wide range of membrane lipids is adjusted according to temperature (e.g. Suutari & Laakse, 1994; Chen et al., 2022) and the fact that studying only the core lipids (e.g., without the polar head groups) misses critical information about the full structure of the original membrane, we do not believe it is appropriate to assume that community structure, and not membrane adaptation is the prime driver of these relationships. Considering this, and that we have already speculated about the producers of brGDGTs and the consequences for temperature proxies in van Bree et al. (2020, QSR) we chose to not discuss this further in the present manuscript which focusses on assessing the application of paleotemperature proxies to the Lake Chala sediments.

Chen, Y., Zheng, F., Yang, H., Yang, W., Wu, R., Liu, X., ... & Zeng, Z. (2022). The production of diverse brGDGTs by an Acidobacterium providing a physiological basis for paleoclimate proxies. Geochimica et Cosmochimica Acta, 337, 155-165.

Suutari, M., & Laakso, S. (1994). Microbial fatty acids and thermal adaptation. Critical reviews in microbiology, 20(4), 285-328.

We hope with these minor changes our manuscript is now ready for publication in Biogeosciences.

Sincerely,
Dr. Allix Baxter
On Behalf of all co-authors