Dear Prof. Seidenkrantz,

Please find attached the 2nd revised version of our manuscript "Evaluation of the distributions of hydroxylated isoprenoidal GDGTs in Holocene Baltic Sea sediments for reconstruction of sea surface temperature: The effect of changing salinity." submitted to Climate of the Past (p- 2022-19). We have adjusted the manuscript according to remaining comment of Referee #3 as specified in our detailed rebuttal.
We hope that the revised manuscript is now acceptable for publication in CP.

Rebuttal
In this rebuttal we have listed all the comments/remarks of the referee in italic font and our response in normal font.

Referee #3

In the new version the authors sufficiently addressed most criticisms.

We thank the referee for this positive assessment.

However, one major point still stands. The authors maintain that OH-GDGTs from base hydrolysis of culture biomass are representative of the sedimentary OH-GDGT pool. I find this to be very unlikely due to their biological sources in the environment and find the argumentation by the authors not convincing. This is for two reasons, 1) that most (i.e., close to 90%) of lipids in Thaumarchaeota are glycolipids and not phospholipids 2) that the composition of the lipid pool in the water column and surface sediments is similarly dominated by glycolipids. The authors state that their results are representatives because phospholipids are degraded faster than glycolipids. Even though that is true, this differential degradation cannot account for sedimentary signals. If that were the case, there should be more glyco-GDGTs in sediments than core GDGTs (if the latter are predominantly derived from phospholipids) because phospholipids are so rare in planktonic and benthic archaea. This is clearly not the case given the many studies on IPL-GDGTs in sediments and water columns that found the opposite to be true, including many works by the lead author. Therefore, base hydrolysis cannot be representative of the average lipid composition (or OH-GDGT composition) of the culture or of sediments. I recommend the authors include a more extensive, critical and honest assessment of the caveats of their approach. As a minor point, the citation of the theoretical study of Schouten et al. (2010) could be accompanied by additional references to environmental studies such as Lengger et al. (2012, GCA).

We thank the referee for sharing once more his/her thoughts on this topic. However, an important topic that the referee fails to see is the fact that all calibration studies based on OH-GDGTs have only taken the core OH-GDGTs in the surface sediments (typically the first 1 cm) into account. So, for these calibrations it is not really relevant in what form the OH-GDGTs reside in the water column or surface sediment (glycolipid or phospholipid fraction) since only the OH-GDGTs in the free (core) form have been analyzed and used for SST calibrations. Of course, this is of concern since during subsequent diagenesis glycolipids may release core OH-GDGTs, which, if they would have a different distribution, may alter the distribution of the core OH-GDGTs.

The comments of Referee #3 on the earlier version of this manuscript made us aware that it would be nice to present also data on the distribution of the IPL of the two enrichment cultures grown at different temperature. After a detailed search of old LC-MS files, which were run in 2011, we came across the analyses of the IPLs of these two cultures, the results of which we have now included in Table 2. Although quantitative data (i.e., distributional data) should be cautiously interpreted because of the strongly varying response factors, these data confirm the trends we see in the acid (see below) hydrolysates and we have included this data in the manuscript (with a clear description of the caveats). We feel that this solves the last issue raised by referee #3 because it basically shows that the OH-GDGT indices have similar values in both fractions.

This search for the original data also revealed that there was an error in the experimental description of the work-up procedure of the two cultures in the PhD thesis of Lisa Warden (which formed the basis for this manuscript); we checked the original notebook of the technician who performed this experimental work and it became clear that the Bligh Dyer fractions were *acid* and not *base* hydrolysed! (In line with all our other research on lipids in archaea from that time). The acid hydrolysis method results in the release of all polar groups from IPL-GDGTs, although partial dehydration of OH-GDGTs occurs. This is now clearly described in the revised manuscript. This, together with the new results on the distribution of the intact IPL-GDGTs, now hopefully will satisfy Referee #3. Because of this, discussion of the different forms of OH-GDGTs (glycol- vs. phospholipids) is not relevant anymore and we have omitted these descriptions.

We thank this referee for her/his persistence on this topic because it prevented us from making a significant error.