Rebuttal to the comments of referee #3

Sinninghe Damsté et al. describe the distribution of OH-GDGT lipids in Baltic Sea surface sediments, sediment cores and a thaumarchaeal culture grown at different temperatures. The manuscript is thorough and well-written and many significant, open questions on OH-GDGT proxies are addressed by the authors.

We thank the referee for this positive assessment of our manuscript and the useful comments.

However, there are some issues with the methodology that could complicate interpretation of the results. First, the authors used base hydrolysis to degrade intact polar OH-GDGTs into core lipids, presumably to facilitate analysis. While this method preserves the hydroxyl groups compared to acid hydrolysis, it does not quantitatively remove the headgroups of thaumarchaeal IPLs (Schouten et al., 2008, AEM) and may selectively degrade IPLs according to their headgroup composition. This will lead to biases in the resulting OH-GDGT quantifications and indices because it is known that OH-GDGTs mostly occur in the form of glycosidic IPLs rather than phosphatidic IPLs in Thaumarchaeota (Schouten et al., 2008, AEM; Pitcher et al. 2011, AEM; Elling et al., 2014, GCA), which are not significantly degraded to core lipids by base hydrolysis. Further, the proportion of core structures is different for different IPL types and markedly different between core OH-GDGTs and OH-GDGTs (predominance of OH-GDGT-0) and IPL GDGTs (predominance of OH-GDGT-2) in thaumarchaeal cultures including the strain most closely related to the organism used in this study, N. limnia (Elling et al., 2014 GCA; Elling et al. 2017 GCA; Pitcher et al., 2011 AEM). It is unclear why the lipids were not analyzed as IPLs, which would have circumvented these issues. If aliquots of the lipid extracts are still available, I would recommend re-analyzing the samples using a method appropriate for detection of IPLs.

The referee is right that the distribution of OH-GDGTs in different GDGT classes (core lipids, phospholipids, glycolipids) may be different. In our analytical approach, we applied base hydrolysis which results in the hydrolysis of phospholipids but leaves the glycolipids intact. This approach was chosen for two reasons. (i) Upon base hydrolysis the OH-GDGTs remain intact, whereas acid hydrolysis (which would also hydrolyze glycolipids) would result in (partial) removal of the hydroxy group of the OH-GDGT, rendering the assessment of their distribution impossible. (ii) This approach results in the "summed" distribution of core and phospholipids GDGTs, which likely comes close to the distribution observed in surface sediments since phospholipids are much more prone to hydrolysis in the environment than glycolipids (Schouten et al., 2010). After all, for this paper, we are interested to compare our culture results with those of the distributions of the core OH-GDGTs in sediments reported in earlier studies which used OH-GDGTs as a paleotemperature proxy. The referee advises to analyze the IPLs as such. Unfortunately, this is not possible as these experiments were performed >10 years ago and no cell material is available anymore. Furthermore, the quantitative extraction of distributions of OH-GDGTs from such data will be extremely complicated since response factors for GDGT IPLs with different head groups and containing OH-GDGTs as cores are unknown and likely to vary substantially, which would make such an exercise difficult and the results would be hard to compare with OH-GDGT distributions in sediments, the aim of these experiments.

Second, the authors quantified the fragmentation products of OH-GDGTs at m/z 1300, 1298, 1296 and did not include the 1318,1316, 1314 ions, which could explain some of the variation compared to previous studies and during replicate analysis due to variations in ionization efficiencies due to. This approach was used by some previous studies (e.g., Lü et al., 2015, OG), i.e., in contradiction to what is stated in lines 159-161 and 321-322.

With the APCI method we applied, the OH-GDGTs hardly (<1 %) generate MH^+ ions (m/z 1318,1316, 1314) and the MH^+-18 ions predominate, so it does not really make sense to include them in the integration. The referee is right that in the studies of the Bremen group the MH^+ ions were taken into account in the integration and we will modify lines 159-161 and 321-322 accordingly. In other studies, only the MH^+-18 ions were taken into consideration. In our opinion, these slightly different methods in quantification, will not make a significant difference. In fact, these different methods of quantification have not prevented the combination of data acquired with both quantification methods in earlier important studies for the development of OH-GDGT-based palaeoproxies (see Lü et al., 2015; Fietz et al., 2020).

Third, the authors do not provide sufficient information on the growth of the cultures such as detailed growth conditions (e.g., volume of medium, volume and type of container and closure, substrate concentration, medium composition) or growth data (e.g. growth curves) to allow assessment of the influence culture conditions on lipid profiles. For instance, it is probable that growth at 4 °C was much slower than at 22 °C. It is known that growth limitation and growth rate influence GDGT cyclization (Qin et al., 2015, PNAS; Hurley et al., 2016, PNAS) and thus it is probable that these factors influenced the presented OH-GDGT data in addition to the influence of temperature. These issues need to be discussed in the manuscript and their potential impact on the results from the culture sample needs to be quantified or acknowledged.

Details on the experimental conditions to maintain and grow the enrichment culture were highly similar to those reported in Berg et al. (2015). We will more explicitly refer to this in the revised text but in our view, there is no need to repeat all experimental details. At both growth temperatures the enrichment cultures were grown in batch cultures until reaching the stationary phase as monitored by FISH/DAPI staining and microscopical observations. In our exploratory experiments, the influence of growth rate has not been studied. Considering the slow growth of the enrichment culture (>40 days until reaching stationary phase at 22°C; Berg et al. 2015), and an even lower growth rate at 4°C, it would constitute an enormous effort to precisely assess growth rate and its impact on GDGT data. Thus, this was entirely beyond the scope of the present study and would reflect a full research project on its own. As requested by the referee, however, we will acknowledge in the revised paper that differences in growth rate may affect the distribution of OH-GDGTs in addition to temperature.

Line comments:

Line 18: Missing word? OH-GDGT [indices] for SST reconstruction?

It will be corrected to "OH-GDGT proxies".

Line 145: Please add the original citation of the extraction method, not just a derivative.

We agree with the referee that preferably the original reference should be quoted. However, "Bligh Dyer extraction" is now such an established term that a quote to their 1959 paper (with over 60,000 citations) is in our opinion no longer required. The appreciation of their work comes with its name after the two authors of this paper (Bligh and Dyer, 1959). We refer to Pitcher et al. (2011) because we performed the extractions exactly according to that protocol.

Line 225-227: This is confusing. If the concentrations of OH-GDGTs could not be determined, then how was %OH-GDGT calculated?

Absolute concentrations of both iso-GDGTs and OH-GDGTs could not be determined because an internal standard was not used for the sediments from this section. However, using the integrated peak areas of iso-GDGTs and OH-GDGTs, %OH-GDGT could still be calculated.

Figure 1: There are several issues with the legend and caption. The caption states that surface sediments are green squares, but the map and legend show blue circles. The caption further states that sediment cores are blue circles but they are red squares in the map and legend. The red text on brown background in the map are hard to read. Consider coloring land areas grey.

Figures 2-5 are very low resolution and hard to read. Adjust?

Figure 4: The colors for the "Baltic Sea (Kaiser and Arz, 2016)" and "global set" should be changed. They are indistinguishable for red/green colorblind people.

Figure 5b: This panel needs a legend displayed on the figure. The colors of the triangles are indistinguishable for red/green colorblind people.

In the revised version we will adjust the figures and their captions/legends according to the useful comments of the referee.

References

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