Interactive comment on “Benthic Archaea as potential sources of tetraether membrane lipids in sediments across an oxygen minimum zone” by Marc A. Besseling et al.

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We thank the reviewer again for the additional comments. Here are our replies.

1) I agree with many of the modifications proposed by the authors. Most importantly, since they use an unpublished method, they will need to demonstrate in detail the separation of the different IPL-GDGT cyclized GDGT-headgroup combinations, and exclude, e.g. the co-elution of unsaturated GDGTs with regular GDGTs.

As stated in our previous reply to comments regarding the use of a modifications to the original normal phase IPL method, we do not agree with the reviewer that this repre-
resents an “unpublished method”. The basis of the method is unchanged and thus we do not feel the functioning of the method deserves a lengthy discussion in a paper that focusses on different issues. As indicated before, we will illustrate the improvements made in the method by adding illustrative figures in the supplemental information. We cannot fully exclude the co-elution of unsaturated GDGTs-IPLs with regular GDGT-IPLs. Per suggestion of the reviewer we re-analyzed the anoxic sediment samples (surface at 885 mbsl, and subsurface of all the stations) using a reversed phase LC/MS method to estimate the contribution of unsaturated core GDGTs to the GDGT-CL pool. As expected after a couple of years of storage the IPLs were partially decayed and could therefore not be reinterpreted. We did observe a trace of potentially unsaturated CL-GDGTs (e.g. GDGT-0:1) but these represented <0.25% of the total CL pool.

2) I appreciate that determining response factors for GDGTs is very challenging due to the lack of commercial standards but I think that this would have been perfectly feasible for the small number of compounds the authors have analyzed. I urge the authors to clearly state that they have not used response factors and how this affects interpretation of the results. It is not acceptable to cite the response factors from Van Mooy & Fredricks (2010) since they were measured on a different chromatographic method and different instruments and on compounds that are not comparable to GDGT-IPLs. GDGT-IPLs are twice the size of the standards measured in that paper and thus the relative ionization efficiencies will be dramatically different (this will also change the relative effect of one versus two glycosidic moieties). Further, ionization of GDGT-IPLs will heavily depend on elution time, since the method chosen by the authors uses a strong polarity gradient. This will strongly affect the efficiency of ionization of GDGTs relative to their retention time and will likely lead to a systematic underestimation of more apolar GDGT-IPLs (e.g. MH) that elute earlier when the mobile phase consists mostly of hexane (which is not beneficial for ionization).

We do not agree with the referee that isolation of standards to the purity required for use as standards (i.e. >95%) to determine response factors is “perfectly feasible”. In
fact, it is a large project in itself that requires a large amount of source material (hard to culture archaea), many man months of experimental work and large amounts of solvents to do the purification as well as proofing of the material by NMR and mass spectrometry. Given the fact that isolated IPLs are also unstable, and the fact that absolute quantitation does not contribute to the discussion (as we focus on relative distributions in comparisons with the genetic fingerprint of the sediments), we do not feel the effort was or is justified. We have already indicated in our response to the reviewer’s earlier comments that we will clarify in our materials and methods that we do not use response factors to correct peak areas. We therefore also do not plan to cite the Van Mooy & Fredricks (2010), or apply their published response factors, but merely used this work to illustrate what the impact of the difference in response factors might be.

We have assessed the difference between the HPLC-MS method using a diol-column used for the study here and the one that uses a reversed phase LC column (Wörmer et al., 2013) using a fresh sample of North Atlantic SPM. Looking at the ratio of MH:DH:HPH for cren and GDGT-0, the NP IPL method may underestimate the MH-IPLs by a factor of 10. However, even when taking this into account, this does not change the conclusion of this study. We have focused our discussions on the HPH variety of the IPLs as this is the best life marker among the IPLs. MH-GDGTs are certainly produced directly by archaea but can also have a sizable fossil contribution and are decay products of DH- and HPH-GDGTs, especially in stored extracts.

3) Lastly, the authors should to assess why the fractional abundances of IPLs are so different between the current study and their earlier study on the same samples (Lengger et al., 2012)

Lengger et al. (2012) studied only IPLs with a crenarchaeol core using an SRM-MS method, therefore in fact quantifying the fragments and not the parent molecules. Lengger et al. (2014) isolated various IPLs and quantified core GDGTs after hydrolysis. These methods are very different from the more direct detection method used in the
present study where signals are assessed in the MS1 signal. It is, therefore, not unexpected that results may vary. However, the distribution of IPLs described in our manuscript, when excluding the MH IPLS (for the reasons discussed above), actually resembles the data shown in the 2014 paper very closely. We will add some sentences concerning this topic to the manuscript.

References


