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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.

Marc A. Besseling et al.

marc.besseling@nioz.nl

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We thank Dr. Lipp for his comments. We have responded to these below. Many comments are a reiteration of those made by reviewer 1. In those cases, we will refer back to our earlier replies made to reviewer 1.

1) The method has not been described before (with this choice of column) and it is not completely clear how the new results compare to previous installments. Most importantly, it is not clear which compounds are captured in the analytical window as according to the authors some important GDGT-0-based lipid which can be found during APCI analysis of hydrolysate cannot be found during IPL analysis. Also, the comparison of

relative proportions of lipids between samples in the current way is misleading. The ionization behavior of lipids varies strongly according to their structure and accordingly using the sum of peak areas for "total lipid abundance" and relative abundance calculated from this value is problematic. Since the authors have not used standards (and do not plan to) for correction of response factors, its perhaps best to compare absolute peak areas and report them in a table. The methodological shortcomings need to be addressed in a revised version (see also comments below).

As stated in the replies to reviewer 1, we do not agree with the reviewer that we used an undescribed method. All compounds captured with the previous type of diol column (standard analytical HPLC size) are also captured using the UHPLC version of the diol column. We also would like to add that no analytical method is fully comprehensive. There will always be compounds that will fall out of the analytical method due to polarity, molecular weight or other properties. The notion that there may be a source of GDGT-0 that is perhaps outside of the current analytical window is, therefore, not a sign that the method does not work, but simply an acknowledgement of the fact that, despite the efforts of the IPL community, we might not know everything yet. We, therefore, feel it is important to retain a discussion on this observation in the manuscript but will remove the statements regarding this "missing" GDGT-0 from the conclusion and abstract. With regards to the comparison of IPLs in relative abundances, we feel it is a fair representation of the observed peak areas and their differing contributions to the overall lipid profile to create a "summed area" and then calculate the relative contribution (expressed as a percentage) of a peak to this summed area. We don't see the difference with what the referee proposes (absolute peak areas) except that the numbers obtained are more easy to handle. As this normalization is done per depth (necessary to compare the lipid profile to the DNA profile), we agree that this way the differences in abundance between depths is unclear. We will therefore add a table to supplementary showing the total IPL peak area per sediment depth. However, we stress that although response factors may vary for different IPLs, this does not undermine the comparison of IPL occurrence and archaeal diversity (which is the main

thrust of the paper).

2) The data only compare poorly to previous results reported in Lengger et al. 2014 where absolute quantities and relative proportions of MH, DH, and HPH-GDGTs were reported. In this study, the hydrolysis method with subsequent APCI quantification was used and problematic response factors are avoided. However, the numbers just do not add up (see detailed comments). I am very surprised that this study is not included in the discussion at all. This also needs to be addressed thoroughly in a revised version.

As we state in our previous reply to reviewer 1, we acknowledge that MH has a reduced response in the NP IPL method compared to the RP IPL method. However when compared with the study of Lengger et al. (2014), we do see a good correspondence between the datasets when it comes to the DH and HPH IPLs. We will address this in more detail in the discussion of our revised manuscript.

3) Comment to author's reply to response factors raised by reviewer 1: The study by Van Mooy and Fredricks (2010, GCA) explicitly states that ". . .these RFs are not applicable for use in any future quantitative analyses of IP-DAGs using a ThermoFinnigan LCQ Deca XP ion-trap mass spectrometer or any other mass spectrometer. . .". I urge that no conclusions should be drawn from these data. It is also speculative that a 40-fold different is unlikely, as the two ion sources are different (heated vs unheated ESI) and the technology of the two mass spectrometers is also different (ion trap vs orbitrap). We probably all can agree that quantification with standards analyzed on the same machine as the environmental samples would be more appropriate here. I am more worried that the quantitative data here does not match previous data from the same samples (Lengger et al., 2014, see comments below). This hints to some methodological bias and supports suspicions from reviewer 1. These issues should be discussed.

As we state in our reply to the second comment of reviewer 1 we merely used this reference to discuss the possible differences in response factors between IPLs. We do

not intend to use it in the revised manuscript. The ion source used in this experiment is basically the same ion source that we have used for all our previous IPL work with the difference that the ESI probe now has a heating option. The heater in this experiment was set to a minimal T of 50 °C. Without active heating the source already reaches a temperature of ca. 40 C due to passive heating from the MS. However, this heating is dependent on ambient temperature and therefore fluctuates. The applied temperature of 50 °C is merely applied to stabilize this temperature and any effect it could have on ionization to make the method more reproducible but does not affect response or ionization behavior.

Detailed comments:

Line 70: typo "repertoire ".

We will modify this in our manuscript.

Line 86: was the PAF standard used somehow for quantification?

The response of the PAF standard was used to normalize for matrix effects and shifts in performance of the MS. The reported peak areas are after this correction. We will clarify this in the material and methods section of the revised manuscript.

Lines 93-98: this is a novel LCMS method that has not been published before. As reviewer 1 noted more information would be desirable in this case (the best way would be to report the new method in another peer-reviewed manuscript). It seems that the individual GDGTs with rings can be separated (judging from the supplemental figure), here some more mass chromatograms as supplemental figures would indeed be good for illustration. What other compounds can be separated, are the BDGTs/other methylated GDGTs, unsaturated GDGTs, hydroxylated GDGTs in the analytical window? Especially OMZ sediments should have abundant unsaturated GDGTs (Zhu et al., 2014, RCM). How do quantitative results compare to other published methods? Is the method suitable to comprehensively capture the archaeal lipidome?

This comment is merely a repeat of earlier comments by reviewer 1 and we therefore refer to our rebuttal to reviewer 1.

Line 101: the unit of resolution is not ppm. I also suggest to use "resolving power" instead of "resolution" as it is a better term.

We will modify this in our revised manuscript.

Line 166: according to Tab 2 it is 44.7% - I am sure this is due to rounding. However it would be great to have consistent numbers in the table and the text. Other values are also different in text and table, please check.

We will carefully check this and make the numbers consistent throughout the revised manuscript.

Lines 156-196: the "relative abundance" is given as proportion of peak area. This should at least be reported and the shortcomings of not using authentic standards need to be discussed thoroughly (see also major comments and line 381-397). Perhaps add absolute peak area values to the table?

As discussed above in our reply to Dr. Lipp's general comment, we will modify this in our manuscript. We will clearly state in the revised manuscript that no absolute quantifications were made and that the data cannot be interpreted as such.

Lines 194-196: Consistency with Lengger et al. 2012 study: please show the data of the comparison (table?). The Greek letter rho should be used for Pearson correlation coefficients - I assume that is what has been calculated here. Please add information on what is compared and how it is calculated. The letter "p" is usually used for statistical significance, p=1 would be really bad.

We do not agree with the reviewer that this would really add additional information to the manuscript. We analyzed the anoxic surface sediment sample at 885 mbsl with the same method as Lengger et al., 2012, where they studied IPL-derived GDGTs in similar sediment samples. We described this in our material and method section, lines

108-110. We separated the IPL fraction from the core lipids with the use of a silica column and flushing with MeOH. This IPL fraction was hydrolyzed for 3 hours. This to determine if the IPL-derived CL-GDGT distribution was altered due to degradation during storage. We compared the distribution of IPL derived GDGTs published by Lengger et al., 2012 to our data and found a significant correlation (= < 0.001) between the two analyses. It was a single measurement and minor test in our study. We will clarify this more clearly in our manuscript.

Line 360: add Elling et al. 2014, 2015, 2017 and Schouten et al. 2008 references for more complete IPL inventory of Thaumarchaeota.

We will add some of the suggested references to the manuscript. It is undoable to list for every statement a complete list of references. We will select the ones that are most original or appropriate.

Line 362-363 and 367-369: the cited studies have not looked at stability of HPH-GDGTs. DH-GDGT stability also has not been experimentally assessed by the cited Lengger et al. 2012 and 2014 studies. Please discuss the stability of phosho vs gly-colipids (and possibly ester vs ether lipids as no study has compared purely phospho vs glyco ETHER lipids, cf. Logemann et al. 2010) in a more balanced way and refrain from speculation without evidence; (additional) useful references in this context are Lipp and Hinrichs 2009, Logemann et al. 2010, Schouten et al. 2010, Xie et al 2013.

We acknowledge that Harvey et al. (1986) and Schouten et al. (2010) did not study the degradation of HPH-GDGT but sedimentary phospholipids in general. We will clarify this in the manuscript. Lengger et al. (2012) and (2014) did observe that the abundance of HPH-GDGTs decreased with increasing sediment depth in contrast to the MH- and the DH-GDGTs that remained equal or even increased in abundance with increasing sediment depth. This was interpreted to reveal the less stable nature of HPH-GDGTs. In the revised version of our manuscript, we will more carefully phrase this and expand the discussion with the references mentioned.

Line 371: What about the possibility of other archaeal sources for crenarchaeol, e.g. Lincoln et al. 2014?

Lincoln et al. (2014) describes the possibility of the (archaeal) Marine Group II as a potential source for crenarchaeol. We did not detect members of the Marine Group II in our sequencing data, therefore, we do not find it relevant to discuss this here.

Line 381-397: regarding the unknown IPL type for GDGT-0, what other compounds might have been missed? It seems like potentially a major proportion! There is a lot of speculation regarding the source of an undetected GDGT-0, but how sure can you be that there is no methodological problem with the method, especially as it seems to be used for the first time? Lengger et al. 2014 have done semi-preparative IPL separation into head group classes and found abundant GDGT-0 connected to MH, DH and HPH headgroups (Fig. 5 and Table A3 in the supplemental material, here station P900 0-2 cm). Comparison of these values and Tab. 2 for the 885 mbsl surface sample shows major differences and multiple values do not match: e.g. DH-GDGT-0 and DH-GDGTcren are 63.7 and (144+86.5=230.5; incl. regioisomer) ng/g sed, respectively, a ratio of âĹij1:3. According to Tab 2 in the current manuscript the corresponding values are "ND" and 43.1 %. Why is the DH-GDGT-0 not detected here, assuming a ratio of 1:3 it should have roughly 10% contribution? Another example is found in Tab 3, it seems that the 885 mbsl surface sample is dominated by 98.1% of DH lipids. However, Lengger et al. 2014 report much higher MH abundances than DH (roughly 800 ng/g vs 400 ng/g). What is the authors' explanation for this major difference? Please discuss all data in comparison to Lengger et al. 2014. Is the diol column method not sensitive enough to capture what had been seen with the prep-hydrolysis-APCI method before? Are some compounds not detected with similar efficiency as suspected by reviewer 1? Can the discrepancies be due to that what the authors report as "relative abundance" is in fact the relative proportion of total peak area for compounds which are known to behave dramatically different during ionization and cannot be simply summed together? A meaningful comparison is only possible with

standards and correction of response factors. Again, this comparison is important and needs to be discussed, especially before invoking unidentified and undetected IPL headgroup types for GDGT-0 which are somehow not in the analytical window of a new analytical method which has not been previously published.

We feel we have sufficiently replied to the analytical issues and will not repeat our answers here. We would like to point out that Lengger et al. (2012) and (2014) performed an indirect quantification of the GDGT-IPLs. Since this previous work used different approaches than we applied in the current study, it is not unexpected to find some difference. MH-IPL is also a degradation product of other IPLs and a portion of the differences may simply be due to differences in sample handling, which we now keep to an absolute minimum. Additionally, and as stated earlier, we acknowledge the underestimation of MH-GDGTs but as stated above, we would like to retain a paragraph of discussion on this. We had a detailed look into our analyses with regards to the discrepancy between the DH-GDGT-0 abundance between our study and Lengger et al. (2014). However, we truly did not detect any DH-GDGT-0 within our sample, the surface sediment at 885 mbsl, whereas our method is clearly able in doing so. This discrepancy may perhaps be explained by resolution differences in these two studies. Lengger et al., 2014 analyzed the top 0-2 cm, while we analyzed the surface with a resolution of 0-1 cm.

Line 384: is the reference Lengger et al. 2014 correct? Or do the authors want to cite the 2012 study? As explained above, the 2014 manuscript is probably as important as the 2012 study.

Lengger et al. (2014) is a valid study on the same suite of samples but uses an entirely different method. Also the sampling resolution in the 2014 paper is different compared to the current study. Nevertheless, we see the point of the referee and we will compare the Lengger et al (2014) data more closely with our results in the revised discussion.

Line 403: A known source are the Sulfolobales. Add to discussion, see also line 414-

417 comment.

This is mentioned further on in the manuscript as indicated by the reviewer (lines 414-416): “GDGT-0 with a cyclopentanetetraol head group has been previously detected in cultures of the hyperthermophilic crenarchaeal Sulfolobales (Langworthy et al., 1974; Sturt et al., 2004).” We did not find any evidence in our sequencing data for the presence of these hyperthermophilic archaea.

Line 409: is there evidence for selective preservation of water column GDGTs at this station? This would require a dramatic degradation of GDGT-0 which is abundant in SPM within the OMZ (Line 404). It seems more likely that the lipids are produced within the sediments. Rearrange.

As stated before, the lack of IPL-GDGT-0 could be partially explained by the underestimation of the MH IPLs. We will clarify this in the manuscript.

Line 410-412: the DH isomers have been reported previously in Elling et al. 2014 and 2017. Elling et al. 2017 studied the lipidome of several thaumarchaeal cultures and found isomers not only for DH-GDGTs but also for hydroxylated DH-GDGTs. Also, they found these structures not only for the acyclic structure (GDGT-0) but also for core lipids with more rings. Please add references to prior studies and discuss why only GDGT-0 has been found in this study.

We will add the references to our manuscript and clarify the text accordingly.

Line 413-414: which moieties? And what cannot be compared? Please clarify.

We are refereeing to the DH moieties, as mentioned in the previous sentences. We could not compare this because of the missing archaeal community compositions in these depths. We will clarify this in the manuscript.

Line 414-417: the GDGT-0 with a cyclopentanetetraol (formerly called GDNT) has not only been found in pure cultures of Sulfolobales but was also detected in sediments (Sturt et al., 2014; Lipp and Hinrichs, 2009). The sources in the present study are

likely the same as in these two studies. I assume the authors mean "microbial sources" and not "sources" (Line 416). Please rephrase. Have Sulfolobales been found in the molecular biological data?

See reply to earlier comment on line 403. Line 416, we will modify the text of the manuscript accordingly.

Lines 418-437: I do not think there should be references in the conclusions section. If you want to have references here, add all relevant ones (see below and reviewer 1 comments).

We agree with the reviewer, we will remove the references from the conclusion.

Line 420: what does "specially" mean here? I would rephrase to ". . .we have unraveled the high diversity of benthic archaea harbored in anoxic sediments of the Arabian Sea, as well as. . .". Remove "specially" and add "Arabian Sea".

With specially we referred to the high diversity of Archaeal groups in the anoxic sediments compared to the oxygenated sediments. We will change this in the manuscript.

Line 421: "increasing the repertoire of archaeal intact polar lipids detected" sounds as if many new archaeal IPLs were found. However, all the described lipids (and many many more that have been found in similar environmental samples) have already been described in the literature (see also reviewer 1 comments). Please rephrase or remove statement.

We agree with the reviewer on the ambiguity of the sentence. We will clarify in the manuscript that we did not detect any unknown IPLs. The focus of our manuscript is not the identification of new archaeal IPLs but to provide more information on their potential sources by a comparison with genetic data.

Line 424: add Elling et al. 2014 reference.

We will add the reference to our manuscript.

Line 426: change "important" to "abundant".

We will modify this into “relatively abundant”.

Line 429: rephrase to ". . .which could either be attributed to a fossil signal. . . or being IPLs synthesized. . ." (add "either").

We will modify this in our manuscript.

Line 431: why are these GDGT-0 derivatives "unusual"? They have been described in the literature before (e.g. Elling et al. 2014, 2017 for DH-GDGT isomers, HCP (then labelled as GDNT) in Sturt et al. 2004 and Lipp and Hinrichs, 2009).

Fair enough, we will mention this in the revised version.

Line 437: this implies the authors have only assumed a very low diversity before they analyzed the samples. Why is that? It is known that there is a large variety of IPLs in the environment (see all the details that reviewer 1 has posted in his/her comments), what was the rationale for a low diversity in these samples? I suggest removing this statement as it is not relevant what the authors have assumed.

We will remove the statement

Table 2/3: use consistent names "subsurface/deep".

We will alter this in our manuscript.

Fig 1: add "10-12 cm" to "subsurface", also in tables and other figures.

We will add this in our manuscript.

References Lengger, S. K., Hopmans, E. C., Reichart, G.-J., Nierop, K. G. J., Sinninghe Damsté, J. S. and Schouten, S.: Intact polar and core glycerol dibiphytanyl glycerol tetraether lipids in the Arabian Sea oxygen minimum zone. Part II: Selective preservation and degradation in sediments and consequences for the TEX86, Geochim. Cosmochim. Acta, 98, 244–258, doi:10.1016/j.gca.2012.05.003, 2012. Lengger, S. K.,

Hopmans, E. C., Sinninghe Damsté, J. S. and Schouten, S.: Impact of sedimentary degradation and deep water column production on GDGT abundance and distribution in surface sediments in the Arabian Sea: Implications for the TEX86 paleothermometer, Geochim. Cosmochim. Acta, 142, 386–399, doi:10.1016/j.gca.2014.07.013, 2014.

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