Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2017-289-RC3, 2017

Biogeosciences
Discussions

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.

J.nbsp;S. Lipp (Referee)

jlipp@uni-bremen.de

Received and published: 11 December 2017

The manuscript by Besseling et al. describes a combination of molecular biological gene-based techniques with UHPLC/MS analysis of intact polar lipids to find potential archaeal sources of tetraether membrane lipids in sediments recovered from an oxygen minimum zone in the Arabian Sea. The authors compare archaeal lipid diversity and abundances with corresponding data gathered from sequencing of 16S-DNA/RNA and amoA functional genes and qPCR analysis. The results confirm previous observations of an active thaumarchaeotal community producing high quantities of HPH-GDGTs. Further, the authors speculate about the significance of members of the MCG

as sources for an intact polar lipid with a yet unknown polar head group. I received my review assignment after reviewer 1 had already posted his/her comments. As you can see below, I raise similar issues and support most of the reviewer's concerns.

The study is part of a series of manuscripts from the same group of authors where identical Arabian Sea samples have been analyzed. Naturally, the previous results are a point of reference. While the overall study design is state-of-the-art and the topic is of interest for the readers of Biogeosciences, I have some concerns regarding the applied analytical protocol for IPLs and the presentation of the quantitative data. Further, there are significant discrepancies with previous data gathered from the same samples. Finally, the reference list is not up to date - there are several studies concerning the thaumarchaeal lipidome which are relevant.

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).

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.

Best regards, Julius Lipp

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.

Detailed comments:

Line 70: typo "repertoire ".

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

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?

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.

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.

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?

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.

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

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 glycolipids (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.

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

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-GDGT-cren are 63.7 and (144+86.5=230.5; incl. regioisomer) ng/g sed, respectively, a ratio of $\sim$1:3. 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.

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.

Line 403: A known source are the Sulfolobales. Add to discussion, see also line 414-417 comment.

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.

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.

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

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?

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).

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".

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.

Line 424: add Elling et al. 2014 reference.

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

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

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).

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.

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

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

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2017-289, 2017.