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Biogeosciences Discussions

Interactive comment on "A laboratory experiment of intact polar lipid degradation in sandy sediments" *by* J. Logemann et al.

Anonymous Referee #3

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Logemann et al. investigated the degradation of ester and ether-bond intact polar lipids (IPLs) by adding dead cell material of yeast and halophilic archaea to active Wadden Sea sediments. The results indicate that phospholipids with fatty acid containing core lipids are degraded relatively quickly, reaching values close to the detection limit after ca. 3 to 4 weeks, while archaeal ether lipids with phospho and sulfoglyco head groups remain relatively constant over the course of the experiment (100 days). The main conclusions of the authors are that different types of head group do not influence the degradation of IPLs, but instead the type of core lipid bond, i.e. ester- or ether lipids, is the determining factor in IPL degradation. They consequently advise that studies where archaeal IPLs are used as markers for living microorganisms should be considered with care.

General comments:

While studies on lipid degradation are much needed in the field of IPL research, they are often problematic to conduct, as simulation of in situ conditions is not a simple task. In light of this, the authors did a very nice job in monitoring the experiment with a variety of different microbiological techniques, and the production and consumption of electron acceptors and degradation intermediates. Although I think the demonstration of this background data is important in order to understand and interpret the experiment, I also agree with reviewer #1 that the presentation of this data can be slimmed down. Perhaps Figures 6, 8 and 9 and parts of the method can be made available as supplementary material.

Overall, I have two general concerns with regard to the experimental set-up as well as the main conclusions that are being drawn by the authors, as well as several small comments.

First of all I was surprised by the selection of yeast and halophilic archaea as cultures to add to the sediment. Adding cell material that is not indigenous to the environment does not mimic real conditions and could cause quite a number of potential problems. However, I do understand that this was the only way to monitor the degradation of IPLs and not confuse them with the in situ IPL inventory. One of the first problems I see is that the authors cannot exclude that the in situ microbial community fails to crack the robust cell wall of the halophilic archaea. H. volcanii has a very rigid cell membrane, with a hyperstable protein structure (S-layer), which typically requires extremely harsh conditions for disintegration (cf. Engelhardt and Peters, 1998 J Struct. Biol. 124: 276-302). The authors infer from the lack of detection of RNA that the cells of H. volcanii must have disintegrated shortly after the start of the experiment (Page 3303, line 29-page 3304, line 2; Page 3306, lines 1-6). However, to my understanding the absence of RNA simply demonstrates that the cell is no longer active; it is no proof that the cell has actually disintegrated. If the authors do not know if they are comparing the stability of cell walls instead of lipids, then they should be more cautious with the interpretation

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of their results.

This leads me to my second main comment: the authors seem to see the main impact of their work on the deep biosphere community where a discrepancy of the detection of bacteria vs. archaea with gene-based and lipid-based techniques was observed. However, their experimental set-up (high sulfate concentrations and the presence of mainly methanogenic archaea) does not reflect the typical conditions that are present in deep biosphere sediments. Instead, the microbial community of deep biosphere sediments is usually dominated by bacteria of the Chloroflexi, Gammaproteobacteria or JS1 candidate group (cf. Inagaki et al., 2006 PNAS 103: 2815-2820, Webster et al., 2006 FEMS Microbiol Ecol 58: 65-85) and by archaea of the MCG, MBG or SAG-MEG group (cf. Inagaki et al., 2006, Webster et al., 2006, Teske and Sorensen, 2008 ISME J 2: 3-18). To what extend do the authors think does the microbial community composition influence the degradation of IPLs? In the discussion the authors mention that "...enzymatic processes are the driving force in IPL degradation..." (Page 3307, line 13). I think this is something that should be discussed in more detail. Although synthesis and degradation of archaeal lipids is far from being understood, the limited amount of studies point to the presence of archaeal enzymes that are related, but inherently different to the bacterial lipid synthases (cf. Daiyasu et al., 2005 Archaea 1: 399-410). Similarly, the degradation of archaeal ether lipids does not seem to occur via the typical bacterial (phospho)lipases (cf. Choquet et al., 1994 Appl. Microbiol. Biotechnol. 42: 375-384). It is therefore likely that archaea produce their own enzymes for lipid degradation. Consequently, could it be that the archaeal ether lipids are not degraded in this study because the experimental set-up favors the activity of bacteria over archaea? It would be nice to see at least a short discussion on this in a revised version of the manuscript. Perhaps this can also be linked to the recycling theory that is brought forward on page 3308, lines 10-22.

As pointed out by Axel Shippers it would be nice to add rates of IPL degradation in the revised manuscript. After all, to determine "...the degradation rates of IPLs..." (Page

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3292, line 3-4) was one of the declared goals in the introduction. If a large scatter in the data set might bias the degradation rates this should be discussed.

According to the method section, the error in IPL quantification is 0.5 to 7%. However, this does not explain all the variations in the plots (Figure 3 and 4). Is there an alternative explanation for some of the values that are up to 50% higher than t0?

Next to the detailed microbiological analyses it would be interesting to see the changes in IPL composition of the in situ microbial communities in both the degradation experiment and the untreated control. Do the relative abundance of archaeal vs. bacterial IPLs reflect the 16S rRNA clone copy numbers and/or the total cell counts? Is there a noticeable increase in the archaeal IPLs?

I agree with reviewer #1 that this study cannot discern if the polar head group types have an influence on the IPL degradation rate. Firstly, only ester lipids with phospho head groups were investigated and secondly, since basically no archaeal IPLs were degraded a comparison between the stability of glyco vs phospho head groups also cannot be made.

The authors should check for consistencies with their nomenclature for archaeal IPLs: For instance in Figure 1 archaetidylglycerol is abbreviated as PG-Ar, but archaetidylglycerol methylphosphate is abbreviated as PGP-Me, not as PGP-Me-Ar. Also, the sulfono diglycosicid archaeol is not a diacylglycerol but instead a dialkylglycerol lipid. Please correct this and watch out for consistency throughout the manuscript and figure captions.

In a revised version, I hope that the authors address the above-mentioned concerns and restructure parts of the discussion and conclusion, including also the specific comments below.

Specific comments:

Page 3290, Line 20: for water column citations, the authors might want to include

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Schubotz et al., 2009 EM 11: 2720-2734 or Van Mooy et al., 2009 Nature 458: 69-72.

Page 3290, Line 25: replace “unbranched” with “non-isoprenoidal”

Page 3291, Line 2: what is meant with “water samples”? Rossel et al., 2008 investigated IPLs in sediments. You might want to add Lipp et al., 2008 who estimated actual abundances of bacterial and archaeal IPLs in sediments and add an appropriate citation for IPLs in the water column.

Page 3291, lines 4-6: To mention ladderane lipids here seems to be rather random when talking about quantification of bacterial and archaeal IPLs. Ladderane producing bacteria usually comprise only a very small amount of the total microbial community. Furthermore, the citations given are studies that did not investigate the intact ladderane molecules, more appropriate citations would be Bouman et al., 2006 FEMS Microbiol Lett. 258: 297-304 or Jaeschke et al., 2009 GCA 73: 2077-2088.

Page 3291, line 9: replace “unpolar” with “non-polar”

Page 3291, line 10: The authors might want to acknowledge earlier studies for d13C analysis?

Page 3291, line 19: The authors might want to add a sentence on the outcome of these studies? For instance: “ These studies did not provide a conclusive result. . .” or “By using different variables different results are yielded, which shows that modeling approaches alone cannot resolve this issue and that experimental data is needed. . .”

Page 3291, lines 24-27: I agree that a fossil component of archaeal IPLs might likely be the reason for an overestimation of archaea in the deep biosphere. However, it is also noteworthy that improved protocols of slot-blot hybridization and Q-PCR increased the yields of archaeal 16S rRNA genes compared to previous studies (Lipp et al., 2008). It should be acknowledged that the answer might lie somewhere “in the middle”.

Page 3293, lines 1-4: What is the amount of cells added?

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Page 3293, line 16: The authors might want to include a discussion on the pros and cons on using organisms that do not occur naturally in the sediment, see also general comments above.

Page 3293, lines 19-21: Introduce the abbreviations of the head groups in the text.

Page 3293, line 22: delete the “s” in “growths”

Page 3300, lines 14-15: How much sulfate was added, i.e. what was the concentration in the natural environment?

Page 3303, line 29-page 3304, line 2: I don’t think the absence of RNA is proof that the cell has disintegrated, see general comments above.

Page 3304, line 24: replace “for” with “of”

Page 3304, line 27: replace “for” with “of”

Page 3305, line 1: replace “for” with “of”

Page 3305, line 8: change “faster degraded” to “degraded faster”

Page 3305, lines 12-19: I would expect the scatter to be larger with smaller values close to the detection limit. Shouldn’t the accuracy of measurements increase if the measured values are within the range of the injection standard? Having said this, the scatter in the IPLs is quite large, for instance in Fig. 3 values for PE double in abundance at the third sampling point, and for PS there is a decrease to ca. 1/5 of t0 at and then after 40 days again an increase to 1/3 of t0. The ester-bond phospholipids also show a marked increase at the end of the experiment after ca. 100 days. How can this be explained? Are these variations within the error of quantification or are they a real signal? I would consider moving this paragraph into the method section.

Page 3305, lines 20-25: I don’t agree. To my understanding the authors cannot differentiate if they are looking at the degradation of cells instead of the degradation of lipids (see also general comments).

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Page 3306, lines 1-6: I don't think the absence of RNA is proof that the cell has disintegrated, see general comments above.

Page 3306, lines 8-9: Could adsorption and desorption processes also explain some of the scattering observed for the yeast phospholipids (Figure 3)?

Page 3306, lines 20: A statement with regard to different head groups cannot be really made because only phospholipids were investigated. However, it can be mentioned here that since the degradation of acyl lipids also occurs via the cleavage of the fatty acid side chains and not only the head group, the type of head group likely does not matter (cf. Matos and Pham-Thi, 2009, Plant Physiol. Biochem 47:491-503).

Page 3307, lines 8-10: With the presented data this statement cannot be made, see comments above.

Page 3307, lines 11-14: It would be nice to extend the discussion on the importance of enzymes, see general comments above.

Page 3307, lines 16-21: Perhaps the original purpose of the experiment should be introduced earlier in the methods section as this has influenced the design of the experiment?

Page 3307, lines 22-23: It should be acknowledged that in fact the physiological roles on most of the sedimentary archaea is not known (cf. Teske and Sorensen, 2008). Wuchter et al., 2007 investigated nitrifying archaea in the ocean and not in sediments, therefore I find this citation misleading in this context and suggest deleting it. Why is the discovery of heterotrophic archaea (Biddle et al., 2006) not mentioned? I suggest linking this paragraph to the lipid-recycling paragraph below.

Page 3307, lines 27- page 3308 line 9: The discussion of chloroplast RNA seems to interrupt the discussion on archaeal lipid degradation, perhaps it can be either moved up or down a paragraph.

Conclusions: In the conclusions it should be acknowledged that one of the reasons

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that the archaeal IPLs are not degraded is due to the experimental set-up, see general comments above.

Figure 1: check for consistencies in figure labeling. Following the description for panel (A) panel B should read: isoprenoid ether-bound core lipids of Haloferax volcanii bound to: phosphatidylglycerol (PG), methylphosphate phosphatidylglycerol (PGP-Me) and sulfono diglyco (S-GL-1) or as mentioned above in minor comments. Also, change sulfono diglyco diacylglycerol to dialkylglycerol.

Figure 2: I suspect the sulfate consumption is mmol per incubation vessel? For some points of the untreated control it seems like sulfate is not consumed, but produced, this is not very clear. Perhaps it would be clearer if the values are not summed up but reported for each time point?

Figure 4 and 5: It might be helpful to add a regression line to overcome the scattering. Again, please check for consistency in the nomenclature, see comments above.

Figure 6: Are the provided values per mL pore water or per vessel? Should it read short-chain fatty acid on the y-xis?

Figure 7: either change to total cell counts (TCC) or total cell numbers (TCN)

Interactive comment on Biogeosciences Discuss., 8, 3289, 2011.