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

Anonymous Referee #1

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This manuscript discusses an incubation study of two biomasses, yeast and a halophilic archaea, in Wadden Sea sediments to determine the degradation of IPLs present in these organisms. The authors find that IPLs of yeast are substantially degraded while the ether-bound IPLs of the archaea are not decreasing in concentration. The results suggest different degradation rates of ester-bound versus ether-bound IPLs and thus that care has to be taken in interpreting and comparing IPLs.

The topic addressed by this study is highly timely. As indicated by the authors and previous studies the use of IPLs as tracers for living biomass is based on only a few fundamental studies and their study fills in a nice gap. Some interesting and unexpected findings are made but the main finding is that degradation rates of ester and ether IPLs are variable confirming the observation of Harvey et al. (1986). Although

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this study does not discount the use of IPLs, it does clearly show that comparison of IPLs with different structural features should be done with care. Clearly not all questions are answered and more research is needed to squeeze out the finer details of the degradation patterns and mechanisms but this study provides an important step forward.

I do, however, have a number of major and minor comments which can hopefully be used to improve the manuscript.

My first comment is that the manuscript is not always clearly written and is 'cluttered' with data which are not used. In the discussion it becomes clear why, the design of the experiment was different than now presented, but this does not mean that all data obtained need to be presented. Clearly, the IPL data need to be presented but both the volatile fatty acid as well as the DNA data are virtually not used in the discussion. I think the fatty acid data are not needed while only some essential DNA data can be more briefly reported (which would considerably shorten the experimental section) . Instead the IPL results can be described more fully. I missed a good inventory of the IPL composition of the yeast and H. volcanii. Presumably the authors have measured this separately. HPLC chromatograms showing the initial IPL composition would be useful. I would also present the data differently, i.e. as percentages with the starting amount at time 0 set at 100%. This is more interpretable than an arbitrary number on the Y-axis.

I also miss a thorough comparison with the data of White et al. and Harvey et al. Is it as fast ? Faster /slower? Do the rates compare well with the anoxic experiments using sand of Harvey et al. or not ? By normalizing on 100% at t=0 they can make a better comparison with these data. As mentioned by Axel Schippers, some rate calculation and comparison with those of Harvey would be useful, even though the concentration measurements are not as good as those of Harvey et al. Also, some discussion on the residual IPLs left after incubation is useful. Both White and Harvey still had substantial amounts of IPLs left after days of incubation. Do you have similar amounts left ?

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What does this mean for interpreting IPL concentrations and comparing them eg with DNA/RNA data which have different half-lives ? These types of discussions are missing in this paper and are important for the broader implications of this study.

I agree with the conclusion that the ether-bound IPLs are more stable than ester-bound IPLs. However, I do not think you can conclude that head groups are irrelevant for degradation rates. First of all, the head groups with the ether IPLs are different from those of the ester IPLs: strictly speaking you can only reach your conclusion if you have ester and ether lipids with identical head groups. Furthermore, I do not think you can conclude that glycolipids are as labile as phospholipids. In comparing with Harvey et al., I would note that they used a 'real' glycolipid, i.e. without any polar head group, while you have a glycolipid with a sulfono group. As discussed by Harvey et al., with polar charged head groups the solubility in water will increase and the IPL is potentially more accessible to degradation enzymes. Thus, degradation rates can be different from those without any charged head group (independent of esters vs ether). Note that the archaeal IPLs reported in the deep biosphere do not contain a polar group and only a neutral sugar moiety. Although your conclusion is still warranted you have to be careful in what you compare. It may as well be that ether or ester lipids with neutral sugar moieties as head groups have different (slower?) degradation rates than those with a polar, charged head group.

Minor comments: p. 3291, l. 2. I would add Lipp et al., 2008 here.

p. 3291, l. 4-7. I found this reference on ladderanes weird as it does not concern IPLs, at least not the studies quoted. If they want to refer to ladderane IPLs they better use Jaeschke et al., 2009 GCA.

p. 3291, l. 24. Would add Schippers et al., 2005, Nature here.

p. 3293, l. 27. What do you mean by 'partly used' ?

p. 3295. l. 22. Is this an 'injection' standard ? I though more an external standard .

p. 3295. l. 23. Not clear how you quantified the yeast IPLs here. From the figures I finally noticed that you also used relative amounts like the archaeal IPLs. Please indicate this. From which MS (1 or 3) were peak area's determined and were these from base peak chromatograms ? More details are needed. Strictly speaking the approach used here does not guarantee that quantification is accurate. It might be that the matrix is changing and thus matrix suppression is increasing or decreasing with certain IPLs. However, it is probably not likely.

p. 3296. l. 9. Delete MS details, already mentioned.

p. 3300. l. 4-9. I found this section a bit weird as it gives conclusions before discussing the results.

p. 3300, l. 11-25. A lot of things are mentioned here to what was done with the incubation vessels and which were not indicated in the experimental section, eg sulfate addition, pH stabilization, sulfide removal. Please add these things to the experimental section, not here.

p. 3301, l. 7-8. This is already a conclusion, perhaps the result section is not the right place to mention this yet.

p. 3301. Why is 3.3 not merged with 3.2 ? As mentioned above a fuller discussion on IPL composition would be useful. Also, introduce the acronyms for the head groups in the text.

p. 3302. As mentioned above section 3.4 does not seem essential to me for the topic of the paper.

p. 3302 and 3303. Shorten 3.5. This section is now longer than the crucial IPL section. It is a pity that you did not perform specific QPCR on the DNA of the halophilic archaea using specific primers. This would have enabled to follow degradation patterns of the DNA and compare this with that of its IPLs. Although I agree that the absence after t=0 is evidence of a rapid breakdown of RNA, the DNA might survive longer.

p. 3305 l. 2-5. Where can I see the data to support this conclusion ?

p. 3305. l. 8-10. It is in my view a bit weird to have conclusions already before the discussion is initiated.

p. 3305. l. 11-19. The title of the paragraph is a bit weird, I am not sure what you mean by this. Also the first part of this paragraph is not clearly written, I have a hard time understanding what you want to say. Would you not expect that the analytical noise will increase near detection limits ? Now you seem to suggest the opposite. It would help to indicate analytical error with each sample point. This will show that your scatter mostly has to do with the heterogeneity in the sampling. It is unfortunate in this sense that the experiments were not done in triplicate to minimize this type of scatter.

p. 3307. l. 1-14. See my comments above. The conclusions of Harvey et al. were not exclusively about chemical stability but also about accessibility of enzymes to degrade IPLs. Both Harvey and White et al also conclude that this process is microbially mediated. What your results sort of fail to explain is how archaeal ether core lipids are formed. If you state that the ester- and ether-bond of the core lipids are crucial in degradation, not the lysis of the head group's, than how can we explain the formation of archaeal ether core lipids which are more abundant than archaeal IPLs (see Lipp and Hinrichs, 2008; Pitcher et al., 2009, Org. Geochem.) ? Clearly these must have formed by the lysis of the head groups of archaeal IPLs ?

p. 3307-3308. I do not see the relevance for most of the discussion in section 4.3. for the main focus of the study. I suggest to shorten this.

p. 3308-3309. The conclusions makes some new comparisons and implications. Part of this should have been in the discussion which, as mentioned above, can be substantially extended.

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