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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.

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Applications of molecular proxies for live biomass such as DNA, RNA, and microbial lipids have influenced our view on biomass concentration and taxonomic composition in sub-seafloor sediments. Yet there remains substantial uncertainty and controversy regarding the fate of these molecular markers on geologic time scales because their specificity to select for live biomass is in question. One important issue is the potentially higher stability of archaeal relative to bacterial intact polar lipids, which could result in overestimation of archaeal biomass in sediments; this is particularly important when the turnover of these proxy lipids is substantially longer than the average cellular life spans (cf. Lipp et al., 2008; Lipp & Hinrichs, 2009; Schouten et al., 2010). Existing studies (e.g., Harvey et al., 1986) have not yet provided a conclusive picture and there

remains a great need for further studies that result in a more quantitative understanding of lipid turnover under representative conditions. The current study seeks to fill this gap and is therefore timely. The authors conducted a 100-day-long degradation experiment with two types of inactive cells, the yeast S. cerevisiae and the archaeon H. volcanii. The former contains lipids that structurally resemble those of bacteria and therefore serve as a reasonable model for the latter. Two main conclusions are drawn: (1) Phosphoester lipids show similar degradation rates as glycolipids; this conclusion is based on minimal degradation of both lipid types in H. volcanii. (2) Ester-bound IPLs degrade faster than ether-bound lipids; this result is based on the relatively rapid decline in concentrations of phospholipids in the yeast.

The most important question guiding my evaluation is whether this study does advance the current state of the art. It possibly does, but the current presentation of data is not sufficient to unequivocally arrive at this conclusion. There are a number of important questions that need to be addressed before this manuscript can be recommended for publication in BG; I sincerely hope that the authors will be able to address these points. I will start with my most general comments before pointing to more detailed issues of technical nature.

General comments

Study design (1): As the authors state, the experiment was originally designed as "enrichment culture for lipid-degrading bacteria" and I wonder whether these conditions are fully adequate for obtaining conclusive results regarding lipid degradation rates. For example, I wondered whether the amounts of cellular biomass added to the sediment are not too high for testing the effects of degradation. Assuming that (i) the sediment has an average cell concentration of 109 cells per mL and (ii) this cell concentration corresponds to approximately 43 to 96 μg of dry cellular biomass per mL (cf. Simon and Azam, 1989; for microbes with a cellular volume between 0.1 and 0.4 μm3), the amount of 1.5 mg biomass added to 1 mL of sediment suspensions appears high, in particular if we assume that the indigenous microbial population, incl. their

enzymes, are largely responsible for the lipid degradation (that's what the experiments show). The authors need to discuss whether the degradation capacity of the microbial population is sufficient to cope with the biomass spike that exceeds the biomass of the natural population by probably at least an order of magnitude.

Study design (2): The authors wish to draw conclusions that relate to the structural properties of the lipids. However, the degradation experiment is based on the addition of killed cells, and their rate of disintegration may complicate the interpretation of the experimental results, given that the lipid membranes are probably protected by the respective cell walls of the two cell types (S-layer in the archaeal cells, polysaccharides, chitin, and glycoproteins in yeast cells). The authors suggest that these are rapidly disintegrated based on the non-detection of their RNA (and cells by cell counting?) but I wonder whether RNA analysis can tell us much about the fate of these cells in the incubations. The possibility should be discussed whether protection of lipids by cell wall material may affect the results and interpretation if, for example, the cellular disintegration limits enzymatic access to IPLs. To what degree may structural properties of the respective cell walls influence the observations, especially given the fact that all lipids from one organism appear to behave differently than those from the other organism? Consequently, the authors should be more specific in their description of their experiment and what they actually monitor. One could argue that the experiment is mimicking conditions in nature after cell death but it is not necessarily suitable to provide information on the fate of extracellular IPLs.

Manuscript organization: The initial study objective is still strongly reflected in the current organization of the manuscript. Much of the information on the community composition, biogeochemical parameters, etc. is rather of supplementary nature and appears out of proportion with respect to the amount of text and display items. Isn't the most important point of this material a documentation of the geochemical conditions and demonstration that the community hasn't been drastically disturbed and remains vital during the course of the experiment? This material should be toned down substantially;

instead the discussion of lipid degradation needs to be more strongly developed and balanced (see points above and below).

Relevance for the deep biosphere: The authors cite the deep biosphere research as motivation for this study. Therefore the manuscript should critically discuss how relevant the findings are for this system, especially also in terms of time scales of the experiment, the presumed time scales of cellular life cycles in the deep biosphere and the apparent scatter in the data that does unfortunately prevent extrapolation of the data to longer times.

Detailed technical comments, in order of appearance in text

P 3290: unbranched should read non-isoprenoidal

P 3291: replace ref. to Lipp et al. (2009) abstract by Lipp and Hinrichs (2009, GCA)

P 3291, L 24: reasons for contradictory results – Lipp et al. (2008) have demonstrated that previous DNA-based studies had underestimated archaeal biomass, please clarify

Objectives, P 3292, L 10: The description of untreated control does not make clear that no cells were added – rephrase. Independent of that point, the objectives should be specified, focused and adequately match the study design.

P 3392: TOC content – two different values are provided in text (0.22 and 0.23%)

P 3295: Injection standard – should be diether with two ALKYL (not acyl) moieties, what quantity was added (final concentration per mL of sediment)

Chapter 2.7: comment on detectability of added cells

Methods: Comment on addition of sulfate, when, what final concentration. How were sediments homogenized after addition of cells?

Results and discussion should be separated more strictly.

P 3300, L 18-21, explanation is not relevant here

P 3301, L 14-19, not clear if referring to biotic or untreated control because information in Fig. 5 is unclear.

P 3302, L 12-14, Sentence unclear, contradictory – rephrase: During the final phase of the experiment, the fermentation products were utilized at the same rate as they were produced or the consumption was faster than the formation.

P 3304, L 1-2, does a lack of RNA detection from H. volcanii really indicate disintegration of its cells? Provide more arguments supporting this conclusion.

Discussion, P 3305, L 12-17, this is information relevant for method or results section

P 3305, L 20-25, agree with statement related to compounds but what about potential effects of cell wall structure, please discuss.

P 3306, L 4, see previous comment referring to P 3304, L 1-2

P 3307, L 22: General statement on Archaea in sediments is not true given the apparent predominance of benthic archaeal lineages in many environments (e.g., Teske and Sorensen, 2008, ISME J). Aerobic ammonium oxidizers (Wuchter et al., 2007) are also not a major archaeal group to be expected in anoxic sediments. Either specify to uppermost Wadden Sea sediment or rephrase.

P 3308, discussion on diatoms is too long and distracts from main theme

Conclusions: I fully agree that we need to be cautious when applying molecular proxies and also with the potentially higher stability of archaeal lipids. However, in order to provide a balanced view you should state that DNA-based evidence is controversial as well. For example, Lipp et al. (2008) had revisited samples analyzed earlier by Inagaki et al. (2006) and showed that modified protocols resulted in substantially higher yields of DNA. Biddle et al. (2008; PNAS) arrived at a similar conclusion in their metagenomic study.

Fig. 2: Y2-axis – if this is sulfate consumption then it probably should be presented

as rate with unit (mol per volume and time). But this is inconsistent with these very high values and the absence of information on rate determination in methods section. Alternatively, these values are intended to represent concentrations but these seem to high as well – clarify and correct.

Fig. 3: Please comment on increase of concentrations of all IPLs shown at around day 5. Interestingly, this is not observed among the archaeal lipids. Any idea? Also between day 20 and the end of the experiment is little change in concentrations of the phosphor-IPLs. Please discuss.

General comment related to all IPL time series presentations: It would be a useful addition if you could also plot the ratio of your relative lipid response in relation to that of the pure culture after pasteurization (similar to Fig. 5 which shows the ratio over day 0; in this case you would use the expected aliquot equivalent based on the sample size and corresponding amount of culture). This would provide a deeper understanding of the processes in the experiment.

Fig. 4, caption, I doubt that the glycolipid is a diacylglycerol – should be dialkylglycerol

Fig. 5: what is the ether lipid in the untreated control. This is neither explained in the methods section nor in the caption. Presumably the lipids in the untreated control are being formed by the indigenous community and their concentration profile reflects balance of formation and destruction. Please discuss. Note that the caption does not allow the conclusion whether this is untreated control or abiotic control (see my earlier comment referring to P 3301, L 14-19; and check entire manuscript carefully for consistency). Please add information on which lipids are quantified in caption.

Fig. 7: Is the initial increase in cell concentration a result of the addition of cells or a stimulation of the indigenous community? Discuss.

Fig. 6, 8, and 9 mostly contain supporting information and could be transferred into a data supplement. Instead a HPLC-MS chromatogram with labeled peaks showing the

data quality at t0 and at the end of the experiment would be a useful addition.

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