

## ***Interactive comment on “Probing the fate of soil-derived core and intact polar GDGTs in aquatic environments” by F. Peterse et al.***

**Anonymous Referee #3**

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The manuscript by Peterse et al. aims to investigate the fate of branched and isoprenoid GDGTs in marine and freshwater aquatic environments by performing 6-month long soil incubation experiments. Due to the continuous increase in papers that rely on GDGT-based proxies as terrestrial markers and/or for paleoclimate reconstructions, such studies are topical and necessary. However, due to major shortcomings in the set-up and execution of the experiments, this study fails to provide a valuable contribution to the field. The only way I see publication of this article justified is if the authors change the main scope of the manuscript, including changing the title (as reviewer #1 suggested) and revising the bulk of the discussion.

Reviewer #1 and #2 have already addressed some of the main concerns regarding the experimental set-up. I would like to add the following:

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(1) If the goal of the study was to look at the degradation vs preservation of branched and isoprenoid GDGTs, the soil should have been sterilized before addition of river or ocean water. The way the experiment was set up, the authors cannot exclude in-situ production of GDGTs within the soil, which might explain why there was no degradation observed for IPL- or CL-branched GDGTs. This also accounts for the control sample, which should have also been sterilized before addition of the water inoculum.

(2) There was no control over the changes in microbial community composition over the time course of the experiment. Did the experiments really reflect natural conditions? If monitoring the microbial diversity with genetic methods was out of the scope of this study, some monitoring of basic parameters, such as nutrient, oxygen or sulfide concentrations would have helped to assess the conditions of the incubation experiments.

(3) The time frame of the experiment was too short to gain actual knowledge on the degradation vs preservation of branched and isoprenoid GDGTs. The authors acknowledge that very high turnover times of years to decades have been shown to exist for branched GDGTs (page 11579 line 1-4). There does not seem to be any novel insights gained from this study other than confirming previous findings.

(4) Lastly, I wonder why the authors only used water as microbial inoculum and not marine sediment? A lot of important and poorly understood diagenetic transformations actually occur within the sediments and not the water column.

Page 11572, line 21: They authors should also take into account more recent studies where it has been shown that degradation of ether lipids seems to occur on much slower timescales than acyl lipids, e.g. Logemann et al., 2011 and Xie et al., 2013 PNAS 110, 6010-6014.

Page 11574, line 10: According to Ingalls et al., 2011 and Close et al. 2014 information on the free living (IPL-containing) community is lost by just using 0.7 µm GF/F filters. This observation and the use of 0.7 µm GF/F filters should be included in the discussion.

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Page 11574, line 17: Was the initial microbial community and subsequent changes monitored? I highly doubt that the natural microbial community was preserved.

Page 11574, line 25: If the samples were frozen, how was the supernatant pipetted off?

Page 11575, line 20: How come C46 GDGT standard was added in this case before the polar-apolar separation? Also, why were different eluents used compared to the column separation of the soil samples?

Page 11577, lines 23-26: This sentence needs revision. As mentioned above, it has been recently established that degradation of ether lipids, such as GDGTs seem to appear on much slower time scales (Logemann et al., 2011; Xie et al., 2013). Secondly, if the authors would have wanted to look at degradation they should have sterilized the soil before incubation with water.

Page 11578, lines 4-7: I doubt that differences in head group are the explanation of the observed stability in branched GDGT abundance given the general concerns of the set up of the incubations (see general comments above). Since the authors did not look at the different types of head groups present in the IPL-branched GDGTs this paragraph is purely speculative. I strongly suggest revising or removing this section as no assured statements on stability or degradation of branched GDGTs can be made with the experimental set-up used in this study.

Page 11578, line 22- page 11579 line 4: If the authors were aware of turnover rates of years to decades, how come they designed their experiment for only 6 months?

Page 11579, line 15: Is this total isoGDGT or just CL or IPL-iso GDGT?

Page 11580, line 7: Just recently, based on circumstantial evidence, also Euryarchaea have been suggested as possible sources for crenarchaeol (Lincoln et al, 2014, PNAS 111, 9858-9863).

Page 11580, lines 8-13: This entire section is quite speculative as no ammonium was  
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measured.

Page 11580, lines 13-19: I would welcome a citation here, supporting the statement that Thaumarchaeota are the dominant archaea in soils (e.g., Leininger et al., 2006, Nature 442, 806-809).

Page 11580, line 27- p11581, line 5: Again, this is pure speculation, I suggest that such a discussion should only be included if IPLs are actually measured. It can easily be argued the other way as most IPL-GDGTs found in soils actually have glycosidic headgroups (e.g. Liu et al., 2010, OGC 41, 653-660, Peterse et al., 2011, OGC 42, 1007-1015).

P11582, lines 15-21: It is quite simplistic to argue that GDGT-0 is mainly derived from methanogens considering that GDGT-0 is one of the most abundant lipids in many archaeal cultures, including Thaumarchaeota (e.g., Schouten et al., 2008). Also didn't the authors argue earlier that most of the archaea in the soils are Thaumarchaea?

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