Anonymous Referee #3

(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.

A: The original aim of the experiment was to determine the fate (or rather behavior) of soil GDGTs upon entering an aquatic environment and its corresponding microbial community. The fraction which is most likely most sensitive to changes/degradation is the one containing the IPLs. In modern sediments, this fraction is generally assumed to be primarily derived from living biomass (regardless of the discussion on preferential preservation of certain types of IPLs in sedimentary archives). In order to monitor this living fraction, we did not treat the soil to keep the IPLs as they were in the field. Autoclaving, for example, would have transformed the IPL-GDGTs present in the soil into CLs due to the high temperature and pressure during this procedure, which would have inhibited us from studying the living fraction.
Nevertheless, we do agree with the reviewer that with this experimental setup we can not exclude in situ production of brGDGTs during the incubation based on the stable IPL-derived brGDGT concentrations. However, we explicitly mention this in section 3.1 of our manuscript.

(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.

A: We have now analyzed the TOC and TN content in the incubated soils, which we have added to the revised manuscript. In short, the TOC content shows a weak, but significant decrease in the river and control setups, where it varies from 1.7-1.4% ($R2=0.37$, $p=0.01$) and from 1.7-1.6% ($R2=0.56$, $p=0.024$), respectively. The TN content shows no significant trends. Normalization of GDGT concentrations on TOC content does not change the trends described in the initial manuscript, and therefore does not affect our interpretation.

(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 of 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.

A; The turnover rates of brGDGTs in a soil determined so far vary from 'a few months' (Huguet et al., 2013, GCA) to 'about 20 years' (Weijers et al., 2010, BGS) to 'less than 45 years' (Peterse et al., 2010, OG). However, these turnover rates are based on the total pool of brGDGTs in a soil, i.e. CLs and IPLs, and our understanding of the (rates of) behavior of IPL(-derived) brGDGTs in soils is actually very poor.
Our timeframe was determined based on the general assumption that the majority of the headgroups is lost within a few days upon cell death. We thus anticipated that 152 days of incubation should be enough to capture this IPL degradation process and to identify the potential contribution of these degraded IPL-brGDGTs on the CL fraction. That this is not the case was not in the line of our expectations and should thus be considered as a novel insight. Besides, our results indicate that IPL-brGDGTs in rivers may in fact be soil-derived, as they do not necessarily degrade in an aquatic environment. In recent studies however, the detection of IPL-brGDGTs in rivers are interpreted as evidence for in situ production.

(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.

A; We agree with the reviewer that the processes that take place in marine and river sediments are important. However, before soil material reaches the sediment, it will have to pass through the water column. Also, the water itself plays a major role in the actual transport of the soil material as part of the SPM. It is thus important and logical to first evaluate and understand the potential transformations of the soil material in an aquatic environment before studying any processes that may take place in the sediment.

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.

A; The study of Logemann et al. is already discussed in section 3.1 of the manuscript for exactly this finding. However, as also suggested by reviewers 1 and 2 we now introduce this study, and the study of Xie et al. in the introduction of the revised manuscript.

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 um GF/F filters. This observation and the use of 0.7 um GF/F filters should be included in the discussion.

A; We are aware of these findings. Note that all the IPL(-derived) data presented in our study are based on the incubated soils and not the SPM. The only sample for

which a GF/F has been used to determine the (CL-)GDGT composition is the ocean water. The river water has been filtered through a 0.2um PES membrane filter and should thus represent the complete GDGT community in this water. We have clarified this in the revised manuscript.

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

A: These changes have not been monitored, so no statements can be made on the preservation of the natural microbial community.

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

A: The samples were briefly thawed upon arrival at ETH, so that the water could be pipetted off, and the soil material was immediately refrozen and freeze dried after that.

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?

A: In contrast to the soils, the SPM samples have not been analyzed for IPLs as they were microwave extracted. This procedure results in a (partial) loss of the headgroups due to the high temperature and pressure conditions during extraction. Thus, there is no need to separate these extracts into a CL and an IPL fraction. Instead, the GDGT fraction was obtained following the common CL-GDGT procedure using a polar-apolar separation.

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.

A: Logemann et al. and Xie et al. have both used marine sediment for their experiments, which is considerably different from the modern soils that we have studied here. For example, the oxygen conditions are lower in marine sediments than in surface soils, which likely results in a larger fraction of preserved 'fossil' lipids in marine sediments than in surface soils. Furthermore, or possibly as a result of this, the fastest turnover rates reported for (archaeal iso)GDGTs in marine sediments are at least one order of magnitude higher than the slowest rates for (bacterial br)GDGTs in soils. The differences suggest that the results of these studies should maybe not be directly compared, but rather be used as an indication of the processes that may take place during the incubation.
Concerning the sterilization of the soils before incubation we would like to direct the

reviewer to the reply on comment (1).

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.

A: We agree with the reviewer that this paragraph is quite speculative without knowledge on the types of headgroup, so we have not included this part in the revised version of the manuscript.

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?

A: As replied above to comment (3), we anticipated on seeing changes in the IPL fraction within this time. We have added a section with recommendations for future experiments to the revised manuscript, in which we discuss the longer incubation time, as well as a more natural soil:water ratio and an improved control setup.

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

A: This amount corresponds to the total pool (CL + IPL) of isoGDGTs. We have clarified this in the revised version.

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).

A: This is also mentioned by reviewer 1 and we have added this reference, but like to note that 'the jury is still out' on the confirmation of Euryarchaeota as producers of crenarchaeol (Schouten et al., 2014, PNAS).

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

A: We agree with the reviewer that we can only hypothesize that the potentially higher NH4 release in the ocean water setup has caused the highest increase in isoGDGTs. However, this is one of the plausible explanations that we can draw from our data. We have now also included the suggestion of reviewer 2 that additional release from the soil matrix may take place as an alternative explanation in the revised manuscript. Nevertheless, this explanation is just as speculative as the availability of NH4, as it does not explain why only the abundance of isoGDGTs increases while that of brGDGTs remains constant.

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).

A: This part of the paragraph has slightly changed in the revised version of the manuscript so that it is not really relevant to cite this study here, but we do refer to this paper at the start of this paragraph, when Thaumarchaeota are introduced for the first time.

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).

A: Both other reviewers also pointed out that our data does not provide direct evidence that isoGDGTs contain mostly phospho-headgroups. We therefore deleted this sentence from the manuscript.

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?

A: We agree with the reviewer and decided (also based on comments by reviewer 2) not to include this paragraph in the revised version.