Reply to reviewer #1

1) I do not think that the design of the experiment is appropriate to investigate the fate of soil-derived GDGTs in aquatic environments. The authors incubated soil samples in water using a soil:water ratio of 1:10. This corresponds to the incubation of soil in a water-saturated environment and just allows monitoring the growth of Archaea and branched GDGT source microorganisms in such conditions. Therefore, the aim and title of this paper should be modified to accurately reflect the design of the experiment. I would say something like: "Abundance and distribution of GDGTs in soils incubated under water-saturated conditions". The investigation of the fate of soil-derived GDGTs in aquatic environments should take into account the fact that soil-derived organic matter is present at low concentrations in such environments and also that natural conditions are complex.

A: We agree with the reviewer that natural conditions are complex, which is exactly why we decided on a laboratory approach and used an incubation experiment to study the fate, or behavior, of soil-derived GDGTs in aquatic environments. The consequences of using a soil:water ratio of 1:10 are already discussed in the manuscript (section 3.4), but based on the comments of the other two reviewers we will also add a recommendation section to the manuscript in which we will propose to use a lower ratio for future experiments. Nevertheless, the 1:10 ratio used in this experiment still reflects 'soil in an aquatic environment', of which the water saturation of the soil is a logical consequence. We therefore propose to keep this aspect in the title of the paper. However, since all reviewers indicate that we monitor the behavior of soil-derived GDGTs rather than their fate, we will follow the suggestion to change the title and the focus of the revised manuscript into 'A laboratory experiment on the behavior of soil-derived core and intact polar GDGTs in aquatic environments'.

2) I would have used a different control setup, where microbial activity is inhibited. I would have added some chemical agent such as zinc chloride to the mixture of soil and river/ocean water to stop all microbial activity. In contrast, the authors simply mixed distilled water and soil sample, leading to the growth of GDGT source microorganisms (especially Archaea) during the incubation in distilled water. Consequently, similar results were roughly obtained in terms of GDGT abundance and distribution, whatever the type of water used for the soil incubation (distilled, river or ocean water). This point is never discussed in the manuscript. In any case, the incubation in distilled water cannot be considered as a control one.

A: The rationale for using distilled water as a control was that, in contrast to river and ocean water, no(t much) allochthonous microorganisms would be added to the soil community when using distilled water, so that the fate/behavior of soil-derived GDGTs in an aquatic environment could be monitored under conditions that were similar among all setups. We do see the advantage of adding a chemical agent to the control setup to ascertain the inhibition of microbial activity. As also replied to reviewer 3, we have added a section to the revised manuscript with an evaluation of our experimental setup, and recommendations for future experiments. The design of

the control setup will be one of the recommendations.

Abstract
Line 12. As commented above, the authors can only say that the soil signature remains unaltered during the incubation under water-saturated conditions.

A: We agree with the reviewer and will adjust the phrasing in both the abstract and later in the manuscript.

Line 21. The authors should take into account the fact that substantial amounts of brGDGTs can be produced in situ, thus overprinting the signature of soil-derived brGDGTs.

A: The occurrence of in situ brGDGT production in aquatic environments is addressed in the introduction, as well as in the discussion. The stable brGDGT abundances and distributions in our experiment is likely a result of the relatively high soil:water ratio used in this study, which has probably caused an overprint of the soil-derived brGDGT signature on potential aquatic production during the time of incubation. This issue is well discussed in the initial version of the manuscript.

Page 11571, line 19. Please also refer to papers where branched GDGTs were investigated in peats.

A: We have added references to Weijers et al., 2009, Geomicrobiology Journal, and Liu et al., 2010, Organic Geochemistry.

Page 11573, lines 3-7. It would have been interesting to determine the origin of branched GDGTs in the Rakaia River by comparing the abundance and distribution of these compounds in soil and water samples collected along the river.

A: We of course agree with the reviewer. In fact, SPM and bank sediments of the Rakaia River, as well as three other rivers on the South Island of New Zealand, are being analyzed on a.o. brGDGTs as part of a larger study on the fluvial transport of terrestrial organic carbon. The results will be published at a later stage.

Page 11573, lines 21-24. Please add a map showing where the soil and water samples were collected.

*A: We will add a map with the sample locations to the revised version of the manuscrip*t.

Page 11575, line 13. Please specify the average carryover of CLs into the IPL fraction.

A: The carry over was <8% for brGDGTs, and <1% for the isoGDGTs. We have added this information to the manuscript.

Page 11576. Please specify the average analytical uncertainty of the CBT, MBT', BIT and TEX86 indices.

A: Regular reruns of selected samples on the HPLC-MS at ETH show that the analytical error on the indices is <0.01. We have added this information to the manuscript.

Page 11577, line 10. IPL-derived brGDGTs are less abundant than CL brGDGTs in all samples.

A: This is indeed true and we did not mean to state otherwise. We will rephrase this in the revised manuscript.

Page 11577, lines 11-13. Please be less assertive: these experiments suggest that soil-derived brGDGTs might not be sensitive to photodegradation.

A: We will change this accordingly.

Page 11577, lines 15-20. Please refer to Fig. 3.

A: We will add this reference.

Page 11578, lines 25-30. In the peat study by Huguet et al. (2013), there were no changes in brGDGT concentration, only in brGDGT distribution. This shows that the brGDGT distribution may be affected without any change in brGDGT abundance.

A: We agree with the reviewer. We do not find a sentence in the manuscript where we claim otherwise, however, since reviewer 2 also mentions this, we will address the findings of Huguet et al. more explicitly in this section of the manuscript.

Page 11579, line 6. Remove the "but" and change "affect" by "effect".

A: OK.

Page 11579, lines 15-17. Please specify if the amounts reported correspond to total concentrations (i.e. CLs + IPLs) or not.

A: The amounts indeed correspond to CL+IPL GDGTs, which will be clarified in the revised version.

Page 11579, lines 22-23. The increase in total isoGDGT concentration is not so clear in Fig. 2, taking into account the analytical error on GDGT measurements. In addition, the fact that CL and IPL concentrations are reported separately does not help to visualize such an increase. Is this increase statistically significant? Please provide p-value.

A: The increasing trend of CL+IPL crenarchaeol is significant in all setups, although with variable r2 due to the variation in duplicate samples (0.74 in river water, p=0.000; 0.30 in ocean water, p=0.014; 0.52 in distilled water, p=0.034). The trends in CL+IPL isoGDGT1-3 is only significant in the river and ocean water setups (0.56, p=0.000 in river water; 0.40, p=0.014 in ocean water; 0.24, p=0.215 in distilled water). We will add R2 and p-values to the manuscript where appropriate and try to change Figure 2 in such a way that these trends become better visible.

Page 11579, line 26. Please refer to Supplementary Material.

A: Done.

Page 11580, lines 1-19. Please refer to the paper by Lincoln et al. (2014, PNAS) showing that crenarchaeol may also be produced by planktonic Euryarchaeota.

A: As also suggested by reviewer 3, we will add a reference to this paper. Note, however, that 'the jury is still out' on the confirmation of Euryarchaeota as producers of crenarchaeol (Schouten et al., 2014, PNAS).

The authors should discuss the fact that the isoGDGT increase was also observed in the incubation with distilled water (control setup). This implies that isoGDGTs are produced during the incubation, independently of the type of water used for the experiment. For example, IPL isoGDGT concentrations are higher in the incubations with distilled water than with river water. This partly questions the argument of nutrient availability affecting isoGDGT concentrations, since there are no nutrients in distilled water.

A: We share the opinion of the reviewer that isoGDGTs are produced in all setups/water types, but the absolute abundance of IPL-isoGDGTs is highest in the ocean water setup. Besides, an actual (weak) increase in IPL-isoGDGT concentration is only significant in the ocean water setup (r2=0.24, p=0.031); the other setups thus have relatively constant concentrations of IPL-isoGDGTs. We will mention this more explicitly in the revised version.
We would like to note that the IPL-isoGDGT concentration is never (significantly) higher in the setup with distilled water than in that with river water. We think that the reviewer may have misread Figure 2, in which the connection of the sample intervals may have caused the suggestion that IPL-isoGDGT concentrations are higher in the soil incubated with distilled water.

Page 11580, line 20. Once again, please specify if the increase in CL isoGDGT concentration is statistically significant.

A: The increase in total (CL +IPL) cren and isoGDGTs is significant in all setups, except for total isoGDGTs in the control. We will add R2 and p-values to the manuscript where appropriate.

Page 11580, lines 23-26. The authors never compare the results of the incubations in

river and ocean water with those of the control incubations. Nevertheless, the proportional decrease of IPL-isoGDGTs with time is observed in all the incubations, even the control ones. This point should clearly be discussed in detail in a revised manuscript.

A: As replied earlier, we will mention that this is the case in most setups in the revised version.

Page 11581, lines 1-5. This paragraph is not convincing. Indeed, the authors previously argued that IPL brGDGTs, which very likely possess phospho head groups, are stable because their side chains are ether-bound. Therefore, the same argument should be used for IPL isoGDGTs. Nevertheless, the authors use the opposite argument here, saying that IPL isoGDGTs very likely contain a phospho head group and are therefore rapidly degraded. Please be consistent in the discussion, using the same argument for the two types of GDGTs (isoprenoid and branched).

A: We agree with the reviewer that this explanation may be confusing and not entirely consistent with the explanation used earlier for brGDGTs. As also mentioned by reviewer 3, we have no data that directly indicates the headgroup composition of the isoGDGTs. We will therefore just focus on the observations and refrain from further speculation when revising the manuscript.

Page 11581, line 6. I would modify this sentence: “To evaluate if and how soil GDGT signatures are modified during the incubations (: : :)”.

A: OK, we will change this.

Page 11581, lines 10-11. I disagree with this sentence. The authors showed that there was no obvious increase or decrease in brGDGT concentration, but changes in brGDGT distribution could have occurred. Changes in concentration/distribution can occur independently.

A: We have taken the decoupling between production and distribution changes (cf. Huguet et al., 2013) into account and have revised the manuscript accordingly.

Page 11581, line 12. Surprisingly, the CBT was observed to increase at the end of the control incubation.

A: Since the distribution changes in the IPL-derived brGDGTs in the control setup are reflected in both the CBT and the MBT’ indices, we prefer to keep the sentence at it is. Note that the changes are relatively small (e.g. the maximum change in MBT’ of 0.04 corresponds with only 0.3 C in reconstructed temperature), and that only the MBT’ index value at t=152 days is significantly different.

Page 11581, line 17. Please also refer to the Supplementary Table.

A: OK.

Page 11581, lines 26-30. What is the limit of detection of the brGDGTs with cyclopentane moieties? These compounds may be present in the IPL fraction, but at such a low concentration that they are not detected. In the present study, 10 g of soil were incubated. The extraction of larger amounts of soil (30-50 g dry weight) may be sufficient to detect IPL brGDGTs. In any case, it seems difficult to conclude that brGDGTs with one or more cyclopentyl moieties are produced at a lower rate than those without just because they are not detected.

A: We have used the following criteria to determine the detection limit: i) the peak area needs to be >10000, and ii) peak height needs to be 3x the baseline. Although no traces of brGDGTs with cyclopentane moieties could be observed in the chromatograms of these samples, we will change 'absence of brGDGTs with cyclopentane moieties' into 'below detection limit'.

Page 11582, line 15. Please be more moderate: "Our incubation results suggest that (: : :)".

A; We have changed this.

Page 11582, lines 23-26. Tracing the absolute amount of brGDGTs in rivers may not be a reliable tracer of soil OC in all aquatic systems. It will depend on the proportion of soil derived and in situ produced brGDGTs in the aquatic system investigated. If brGDGTs are mainly produced in situ (in the water column and/or sediment), measuring the concentration of brGDGTs will not help in tracing soil OC.

A: We agree with the reviewer that measuring the absolute amount of brGDGTs in rivers may not work in all systems, but this is also not something we claim in our manuscript. Our suggestion to in this river system use absolute amounts of brGDGTs is merely a result of the findings from our incubation experiment, in which crenarchaeol concentrations increase and brGDGT concentrations remain constant. We do not deny that brGDGTs may be produced in the water column and/or sediment of a river, however, our experiment provides no direct evidence for in situ production of brGDGTs that may alter the initial soil signature. Since this is already clearly stated in the manuscript, we choose not to change this section.

Page 11583, lines 5-6. What do the authors mean by "growth of specific microorganisms"? Please specify.

A: By preferential growth of specific microorganisms we mean to say that there are likely several producers of GDGTs in soils. As a consequence, distributional changes can then either be explained by membrane adaptation, or by the preferential growth of specific GDGT-producers (assuming different producers synthesize GDGTs in different distributions). We will clarify this section in the revised version.

Page 11584, lines 1-5. In order to investigate aquatic branched GDGT production, I would have incubated river/ocean water with the corresponding sediment sample.

This would be more relevant than the incubation with soil sample.

A: This seems indeed a legitimate setup to study in situ production of brGDGTs. However, this was not the scope of our experiment. We aimed to investigate the fate(/behavior) of soil GDGTs in river/ocean water, simulating the exposure to aquatic conditions and corresponding microbial communities during land-sea transport. To us it thus seems more logical to use soil rather than river/marine sediment for this experiment.

Page 11584, line 13. The difference in branched GDGT distribution between the river SPM and catchment soil sample suggests that at least some branched GDGTs are produced in situ in the river (in the water column and /or sediment), even though this result is based on only one soil and one SPM sample. The hypothesis of riverine in situ production cannot be excluded and should be taken into account.

A: The distribution differences between brGDGTs in river SPM and the one soil sample may be explained by several factors, of which in situ production is indeed one. Although we do not observe direct evidence for in situ production in our experiment, for which we give multiple explanations, we do nowhere in our manuscript exclude in situ production in rivers from taking place. Nevertheless, we will carefully reread this section in order to clarify this.

Page 11584, lines 21-22. The distribution of brGDGTs differ between the river and ocean SPM, and between the river SPM and catchment soil (Fig. 4). Therefore, I would not say that “soil brGDGT signatures delivered to the oceans will echo those entering the corresponding fluvial network”.

A: This is a valid argument and we have taken this into account in the revised version. We have also included a reference to the recent publication of Zell et al., 2014, GCA, who show a similar trend, i.e. that brGDGTs in marine SPM close to the mouth of the Amazon River have a different distribution than those in river SPM, which they contribute to marine brGDGT production, even further complicating the interpretation of brGDGTs in coastal margin sedimentary archives.