Utrecht, 22 December 2014

Dear editor,

I hereby provide you the requested response letter in which we have listed our replies to the reviewers' comments in one document. Since we already responded in great part to all the individual comments in the online replies (posted 15 November), we have sometimes only added the page and line numbers of the corresponding adjustments in the revised manuscript (part of the response file submitted 16 November, and resubmitted with this letter). I hope that this letter meets the expected degree of detail, and that you find the revised manuscript suitable for publication in *Biogeosciences*.

On behalf of all co-authors I whish you a merry Christmas,

Yours sincerely,
Francien Peterse

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'.

We have added section 3.5, in which the consequences of our setup are addressed

(soil:water ratio used, duration of the experiment, recommendations for future research). Note that this reviewer may have slightly misunderstood the aim of our study, which was to study the fate (and expected degradation, based on previous studies discussed on p. 10, line 2-19) of soil-derived GDGTs rather than their growth or production. Also this point is highlighted in section 3.5 (p. 16, lines 6-13). The reviewer does have a point when stating that we have monitored the behavior rather than the fate of soil-derived GDGTs, so that we have adjusted the title of the ms, as mentioned above and in the online reply letter.

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.
These issues are now addressed in section 3.5.

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.
We have done this throughout the ms, e.g. on p13, line 4.

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.
See e.g. p4, line 17 - p5, line 2, or p15, lines 4-10.

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.
P3, line 28.

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 manuscript.
See figure 2.

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.
P 8, lines 11-13.

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.
P 8, lines 27-28.

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.
P 9, lines 15-16.

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.
P9, lines 17-18.

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

A: We will add this reference.
This is now Figure 5, p9, line 20.

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.
P10 line 32 – p10 line 6.

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

A: OK.
P11, line 8.

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.
P 11, line 18.

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 r^2 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 R^2 and p-values to the manuscript where appropriate and try to change Figure 2 in such a way that these trends become better visible.
p-values added on multiple places in the ms, e.g. p11 line 27, p12, lines 22-25. Figure2 is now figure 3.

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

A: Done.
P11, line 30.

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).
P12, line 12

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. P12, lines 22-25, and p12 line 31-p13 line 2.
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.
P12, line 22-25.

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.
P12 line 31 – p13, line 2.

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.
We have deleted this paragraph.

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.
P13, line 4.

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.
P13, line 7.

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.
P13, line 6.

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'.
P13, line 23-24.

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

A; We have changed this.
P14, line 5.

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.
P14, lines 23-26.

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.
Section 3.5

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.
Section 3.5, specifically p15, line 10-26.

Referee #2

Abstract Line 4-7 as already pointed out by rev. 1 your experiment does not allow you to asses suitability of brGDGTs as terrestrial tracers, it only allows you to determine degradation (or lack of thereof) in waterlocked soil conditions and then only for 152 days which is too short as shown by many previous studies (some of them unquoted, such as Kim et al. 2010, Huguet et al. 2008).

A: We are assuming that the reviewer is referring to 'Selective preservation of soil organic matter in oxidized marine sediments (Madeira Abyssal Plain)' by Huguet et al., 2008, GCA, and 'Contribution of river-borne soil organic carbon to the Gulf of Lions (NW Mediterranean), by Kim et al, 2010, L&O? Unfortunately, we could not find specific indications in these papers that degradation of soil-derived brGDGTs under water-saturated conditions will take longer than 152 days. We would like to refer to our reply on comment (3) of reviewer 3 where we provide further arguments for the time frame of our experiment. In addition, we have extended the discussion on the duration of our experiment to section 3.5 of the revised manuscript.

P15 line 27-p16 line 5.

Line 10 There is nothing in your setup that allows you to measure production, especially since it is too short compared to previously estimated decadal turning times (e.g. Weijers et al. 2010). I would rather say that the lack of changes indicates there is no significant changes in brGDGTs in the water column that may have settled in your soil, pointing to a lack of water column production and a cessation of soil production when water locked.

A: We have moderated this sentence in the abstract and also later in the manuscript. However, we believe that production during the incubation experiment should become visible in an increase in GDGT concentration with time. That our data does not show such an increase in brGDGTs to us suggests that brGDGT production (but also degradation or release from the soil matrix) has either not (yet) taken place, or takes place in such rates that any changes are leveled out (e.g. production = degradation).

Moreover, based on our data and the suggestion that brGDGTs are also produced in the water saturated, anoxic part of peat bogs (e.g. Weijers et al., 2009; Liu et al., 2010; Peterse et al., 2011), we do not think we can make the statement that brGDGT production is inhibited due to water saturated conditions in our experiment.

P2 line 20-21.

Lines 13-15. That you see an increase in isoGDGTs does not necessarily indicate production. As you did not filter incubation water you may be getting additional settling form your water to the soil. Which brings me to a crucial point you treat the soil as an inert part of your experiment when in fact it plays a major role in both signal storage/preservation and transport. While sandy loam is quite sandy it still contains clays that will certainly store lipids very efficiently and may provide a matrix preservation effect, which has already been shown by Huguet et al. 2008. Moreover when transporting your soil to the river the structure and grain size distribution will be crucial as bigger particles will be transported first and allow faster degradation

(but of course also settle first probably not reaching the coastal area).

A: As also mentioned in the reply to reviewer 1, as well as in the manuscript, due to the proportion of soil:water in our experiment, the contribution of aquatic GDGTs is likely negligible. Besides, the control setup with distilled water shows similar trends, indicating that the contribution of settling from river or ocean water is limited.
The release of lipids from the soil matrix is indeed an aspect that we have not considered, so we have included this explanation in the revised version. However, the absence of changes in brGDGT distribution or abundance in our incubation experiment may suggest that brGDGTs are not released from the soil matrix in large amounts. Although this does not automatically imply that isoGDGTs will behave in the exact same way, it may be an indication that the preserving role of the soil matrix is limited in this experiment.
P2, line 20, p12, lines 2-4, p14, lines 23-26.

Lines 20-21. It may also show that the archaea living in the water column have a different distribution or that those in soil and/or that some iso-GDGTs degrade faster than others or are released from soil matrix faster.

A: The different distributions produced by archaea living in the water column vs in the soil is exactly what we mean with 'a shift in source organism(s)' (abstract, line 20-21). We have also added the option of different production/degradation rates.
P2, line 29.

1. Introduction: Line 29-pg11572. Here you should introduce papers by Smith et al. and Fietz et al. that talk about the adequacy of the BIT.

A: The papers of Smith et al. and Fietz et al. both indicate that the BIT index in coastal marine sediments may be driven by the production of crenarchaeol rather than by the input of soil-derived brGDGTs. We believe that referring to this finding is more appropriate in the discussion part of the manuscript than in the introduction, so that we can me a direct link to the results from our experiment.
P14, line 16.

Lines 20-21-pg11572. Well not sure about that Harvey and white worked both bacterial lipids and presence of fossil intact lipids has already been shown...certainly needs to be toned down.

A: We have adjusted this sentence and have added the remark that recent studies (e.g. Logemann et al., 2011) have indicated that certain IPLs are less sensitive to degradation. We would like to note that this has not (yet) been shown for brGDGTs though, and are produced by bacteria after all.
P4, lines 25-27; P10, lines 12-13.

Line 5-7 –pg 11573 well the fact that there is little primary productions does not necessarily hinder heterotrophs growth so I would be careful here.

A: Thank you for the comment, we have revised this sentence in the manuscript.
P5, line 10-12.

2. Material and Methods:
Line 10- I fail to see how you obtained enough material filtering 10 L of water when in all studies much higher amounts need to be sampled. Also when filtering with a 0.7um filter you are really collecting SPM but not free living organisms which has been shown to grossly underestimate IPL presence (Ingalls et al. 2011). As you are comparing your values with the soil in the incubations (and not with 0.7 filters of the incubation water) in the soil samples will then contain all fractions that have reached the soil during your experiment and not only SPM. Meaning your experiments have two major flaws you did not estimate the abundance of lipids accurately in your water samples and you are not comparing equal fractions when analyzing water SPM and soil sample. Also on experimental setup you never say how many water samples you analyzed?

A: We are not sure what 'all studies' refers to. Nevertheless, the filters have just been used to determine the distribution and amount of CL GDGTs in SPM in river and ocean water, i.e. two samples. In case of ocean water, 10L yielded enough SPM for the analysis of GDGTs. As also clarified in the reply to a similar comment by reviewer 3, only ocean water has been filtered over a GF/F. The river water has been passed over a 0.2um PES membrane filter and should thus represent the 'free living' community in this water. We will clarify this in the revised manuscript.
The incubation water was filtered over a GF/F for a selection of samples at the end of the experiment, but GDGTs were only present below detection limit, probably due to the relatively small sample size of 100ml. Because of the lack of results, this exercise has not been included in the manuscript.
P6, line 9-11.

Lines 18-20 I wonder how well this mimics an in situ process? In a natural environment very small solid particles will be carried into a river and then down the river at a very high water/sediment ratio with constant movement (not sure the shaker mimics that) and with varying rates of exposure to sun and oxygen.

A: We agree with the reviewer that the conditions in the natural environment are different from those in the laboratory. However, this is inherent to taking an experiment to the laboratory. We tried our best to mimic the natural conditions where possible, for example by using a shaker table for constant movement, or using natural light conditions. Regardless, it would not have been possible to perform our incubation experiment in the natural environment.

Line 10-Pg 11576-You cannot calculate a deviation with 2 samples.

A: We agree with the reviewer. We have adjusted the text as well as the figures, which now plot all individual data points to display the spread in results instead of an error bar.
P8, line 10-11, and figures 3-5

3. Results and discussion
3.1.BrGDGTs
Lines 7-9 I think the large spread between the 2 replicates hints at a heterogeneous soil sample and this should be discussed further and taken into account when interpreting the results.

A: The soil sample has been homogenized before taking subsamples for the experiment, which should make up for soil heterogeneity. Indeed, the concentration of brGDGTs in the two subsamples representing t=0 are only ~3% apart (248 and 230 ng brGDGT/g incubated soil), suggesting that the soil was well homogenized. Also the second control point (t=91) shows a concentration offset of only ~6% (312 and 355ng). Only the last point (t=152) shows a larger offset (~23%). As mentioned in the manuscript, this last point results in a trend towards increased brGDGT concentrations with time. Instead of soil heterogeneity, this may indicate that brGDGT production has just started to take place in one of the incubation bottles. However, since this trend is weak, not significant, and not visible in the IPL-derived fraction, we have refrained from further speculation in the manuscript.
P9, lines 15-16.

Line 10-13-pg 11577. As they may be protected by a matrix and really a small fraction of the soil would be exposed to the light (in contrast to SPM floating in a river) I don’t think you can say they are not photo degraded.

A: As also suggested by reviewer 1, we have weakened this statement in the revised manuscript.
P9, lines 17-18.

Line 24-pg 11577. There has been a lot of debate on the liability of IPLs and this should be reflected here, but overall I think you cannot make the connection with higher production so easily.

A: This sentence in the manuscript is immediately followed by a discussion on the presumed lability of IPLs and the influence of the type of head group, but we will critically reread this paragraph and try to clarify or update the discussion where possible. In addition, based on the comments of reviewer 3 have add to the introduction that the turnover rates of ether lipids may be slower than previously anticipated based on the findings of e.g. Logemann et al 2011 and Xie et al 2013.
P10 line 33 - p11 line 6.

Lines 25-30-pg 11578 Your study is not comparable to that of Huguet et al. 2013, first they did not change the natural soil structure and conditions, secondly they used a much longer time span and third they did not see changes in abundance.

A: Because there are differences in experimental setup between our study and that of Huguet et al., we do not intend to make a direct comparison between the experiments, but rather focus on the turnover rate of brGDGTs that they determined. Since reviewer 1 also highlights this point, we have clarified this section in the

manuscript and mention that Huguet et al show that distributional changes can take place unrelated from the concentration of brGDGTs, so that no expectations can be drawn from their findings regarding the concentration of brGDGTs in our experiment.
P10, line 32-33.

Line 6-pg79-remove but

A: OK.

Lines 7-10. I think my main concerns here are a) not all nutrients in soil will be released by adding water (and certainly the type of water will be crucial in that process), b) the different masses will contain nutrients (yes the distilled water too), and c) if you have no alive brGDGT producing organisms (as you seem to hint from your no production conclusion) the nutrient concentrations are not relevant.

A: As long as the majority of the brGDGTs remains orphan and the exact environmental controls that influence their occurrence and distribution have not yet been validated, we do not dare to exclude the potential influence of nutrients, which is why they are at least mentioned here. We do agree that the type of water will likely influence the release of nutrients from the soil, as we also discuss in the section on isoGDGTs. However, the water type (and/or nutrient composition/release) seems to have no influence (yet) on brGDGTs, as no changes are observed. Finally, we do nowhere suggest that there are no living brGDGT-producing organisms in our soil. The only observation we can make based on our data is that the absence of distributional or concentration changes may be caused by equal production/degradation rates, or a very slow growing rate of the producing organism(s).

3.2-iso
Lines26-2 pg 80: I strongly disagree with this statement firstly as pointed out before 0.7 will only give you SPM lipids and not free living organisms. Secondly 100 mL is very low a sample and could easily not be representative. And finally it is not relevant what concentration you had in soils but rather how it increased over time.

A: We share the reviewer's concern of sample representativeness, so the water container was vigorously shaken every time before adding 100mL to each soil sample. We agree that 100mL is a relatively small sample, in which it is hard to determine the GDGT concentration. For future experiments, we therefore suggest to use a larger volume in the evaluation and recommendation section that we have added to the revised manuscript.
P15, line 21-26.

Lines 6-13 pg80: This is highly speculative as a) you cannot prove that the increase in crenarchaeol comes from production, b)It has been demonstrated that Thaumarchaeota favour low ammonia concentrations (e.g. Martens-Habbena et al. 2008), c)you did not measure nutrients in the water before or after.

A: a) Indeed, we have no direct evidence for crenarchaeol production as we have not monitored the microbial activity in the samples. Nevertheless, the concentration of crenarchaeol increases over the course of the experiment, which implies that crenarchaeol must be added from somewhere. To us, production seems a logical explanation, but based on earlier comments we will also mention preferential release of isoGDGTs from the soil matrix as an alternative source. b) There are also studies that have demonstrated the opposite and have demonstrated amoA expression at high levels of NH4 (e.g. Erguder et al., 2009, and references therein). c) We have now analyzed TOC and TN in the incubated soils and have added these data to the revised manuscript. As also replied to reviewer 3, the TOC and TN data do not change any of the trends described in he initial manuscript.

a) p12, line 2-4
c) p9, line 2-7

Lines 15-19: I don't think you can deduce that from your data.

A: We have adjusted this part of the discussion and now list 1) production of soil Thaumarchaeota, 2) production of marine Euryarcharchaeota, or 3) release of isoGDGTs from the soil matrix as potential explanations.

1) p11, line 20.
2) p11, line 11-12
3)p11, line 2-4.

Lines 24-30-pg80: Nothing new here, as you pointed out in your introduction intact lipids degrade faster than core lipids however in all cases lipids even with a phosphor head group will take long to degrade (see previous degradation experiments!) especially in a water logged soil where potential degrading organisms may not be doing too well.

A: We have changed this sentence in the revised manuscript and now better indicate that our observations support previous findings.

P12, line 31-33.

Lines 3-5 pg 81: You have no evidence to support this statement either measure intact lipids or remove sentence.

A: The other reviewers have also made this point. We agree with the reviewers and have not included this sentence in the revised version.

3.3-Distribution

Line 14-pg. 81: I would say worryingly, as if the biggest change is your control then there is a factor you are not considering or it is not the right control. I would certainly make a much bigger effort to explain this.

A: As also replied to reviewer 1, the observed distributional changes are relatively small, and only the MBT' index value at t=152 days in the control setup is significantly different from the other data points. However, note that the maximum change in

MBT' of 0.04 corresponds with only 0.3 C in reconstructed temperature. Monitoring over longer time scales may reveal how this deviation further develops. As mentioned earlier, we have added a recommendation section to the revised version in which we suggest the longer incubation time, as well as an improved control setup for future experiments.
Section 3.5

Line 26-81: I must agree with rev. 1 not detected does not mean not present.

A: We also agree and have changed this sentence accordingly.
P13, line 23-24.

Lines 1-4-pg82: with your data you can certainly not talk about rates of production.

A: This point has also been mentioned by reviewer 1. This statement is no longer included in the revised version.

Line 10 &15-pg 82: You cannot talk about production.

A: We have changed 'production' in line 10 into '…stems from the increase in crenarchaeol…', but the 'production' in line 15 refers to the study of Zell et al., 2013 and the interpretations therein, so we have left this unchanged.
P13, line 31-32.

Lines 4-6-pg 83: Yet I would argue that hardly any production takes place, especially in soils and that the differences steam from the IPLs and CLP present in the water.

A: As mentioned earlier, we have added the options of an aquatic contribution as well as a potential contribution from the release of GDGTs from the soil matrix to the list of explanations. Unfortunately, our data do not allow identifying the exact source of these GDGTs.
P14, line 23-26.

Lines 6-10-pg 83: highly speculative you have no data and there are no supporting publications on this.

A: We agree that this section is speculative since we have not determined the headgroup composition in our samples. However, the statement that soil Thaumarchaeota produce GDGTs with different headgroups than those in the marine environment is well referenced, and should thus count as a potential explanation for the observed distributional changes during our experiment.

Lines 14-21-pg 83: Well to have methanogens your soil should have been anoxic (which I doubt form a sandy loam), if your soil had been anoxic and water logged to start with your experimental setup would have been better and your result likely very different.

A: This section does not seem to contribute to the actual discussion and scope of the paper, so we have not included this paragraph in the revised version.

3.4. Comparing
Lines 4-11-Pg 84 To be honest with a river that is 150 km long there is no doubt that your soil and water samples are not representative, especially since you already have replicates that at heterogeneous so I think you should take this final section and use it to reinterpret your results in a more sensible and temperate manner.

A: We have done our best to avoid sample heterogeneity, both with subsampling soil as well as the addition of incubation water. In our reply to an earlier comment we have indicated that the initial conditions of the incubation bottles were similar and that any deviations have started during the incubation.
We have changed this section into an evaluation and recommendation section in which the soil-SPM offsets are now discussed in more detail.
Section 3.5

4.Conclusions I think this section needs to be modified fully after major changes in the rest of the manuscript.

A: The conclusions have been modified to take into account the revisions made to the manuscript.
P16, line 28-32.

Reviewer #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.
Now section 3.2, p9 line 26-29.

(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.
See sections 2.2 and 3.1

(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.
P16, line 26-32.

(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.
P4, line 25-27.

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.
P6, line 9-11; p15, line 21-26.

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.
P6, line 27.

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.
P11, line 3-6.

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.
Section 3.5

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.
P11, line 18.

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).
P12, line 11-12.

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.
P12, line 6.

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.