Reply to 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 found 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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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.

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 Thaumarchaeota, or 3) release of isoGDGTs from the soil matrix as potential explanations.

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.

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.

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.

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.

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.

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.

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.