Consolidated response to referees #1 and #2 for submitted manuscript "Provenance of tetraether membrane lipids in a large temperate lake (Loch Lomond, UK): implications for GDGT-based palaeothermometry"

First of all we would like to thank the two referees for their time and useful comments and suggestions. Both referees are generally positive about our manuscript which they consider timely, important and of interest to the Biogeosciences community. Below we address the different points raised by the two referees. Our responses to the comments are in italics.

Response to Referee #1

1. General comments

By studying the GDGT distributions in Loch Lomond, a temperate lake with large catchment area, Buckles et al. demonstrated that along with allochthonous input of branched GDGT from adjacent soil and streams there was also evident in situ production in the lake water and sediment. The application of GDGT based proxies in such circumstance was then considered to be problematic. Intensive work of sampling and lipid analysis can be recognized in this study. The discussion paper is well written. Sufficient data were provided and then comprehensively discussed. I believe this paper fits well into the scope of Biogeoscience and will be interesting to the organic geochemistry and geobiology community.

However, I do have a little concern for the distribution pattern of brGDGT in soil, river and lake samples (section 4.1). Indeed this lipid distribution difference among sample groups and together with their changing proportion of IPL vs. CL could indicate in situ production in water column and sediment of both river and lake. But, as shown in Fig. 4 there is clearly a source-to-sink profile of steady decrease in MBT' and increase in DC from soil to lake. If this pattern only represents a distinct biological source in each setting, then why does it follow such a gradual change from soil to lake? Is it only a coincidence? The increasing degree of methylation and cyclization in brGDGT from soil to river and then lake seems like a response of the same organism community to certain environmental gradient. What could it be, redox condition, pH or other parameters?

This is unlikely to be coincidence; however, we do not believe that all the brGDGTs here are derived from the same organism through its adaptation to different environments. Some of us have shown previously that the bacteria producing brGDGTs in soil do indeed adapt their lipid composition to changing ambient conditions (Weijers et al. 2007, GCA; Peterse et al. 2012, GCA). From a larger soil dataset a relationship could be derived between the degree of change in brGDGT lipid composition and environmental parameters. However, the distributions of brGDGT in rivers and lakes do not fit this relationship found for soils. It therefore seems unlikely that the same species or community is adapting its GDGT lipid composition to these settings; instead, we believe that different species/communities are present in each setting that adjust their GDGT lipid composition according to a (slightly) different relationship with environmental parameters. Given that the same type of lipids are produced, i.e. brGDGTs, we believe that the general principles behind the adaptation remain similar, which could explain the observed trend in MBT and DC from soils to lake.

2. Specific comments

p4194, line 5, Is there any concern about the sampling efficiency of using GFF filter? As discussed in a previous study (Ingalls et al. 2012 AEM), considering the small diameter of archaeal cells, the application of GFF filter, 0.7_m pore size, might underestimate the abundance of isoprenoidal GDGTs.

While Ingalls et al. (2012) demonstrated that 0.7 µm GF/F filters do not capture all isoprenoidal GDGTs, the utility of the current study is closely linked to the comparability of Loch Lomond with other lake and river systems whose isoprenoid and branched GDGT distributions have been investigated in detail. To our knowledge, even the most recent of these studies also continue to use 0.7 µm GF/F filters when investigating GDGTs in lake and river SPM (e.g. Kim et al. 2012, GCA; Zell et al. 2013, Limnol. Oceanogr.; Schoon et al. 2013, Org. Geochem.; De Jonge et al. 2014, GCA; Naeher et al. 2014, Org. Geochem.). The use of a less established method may impair the comparability of this study, particularly with respect to the branched GDGTs, whose producers have not been wholly characterised (cf. Sinninghe Damsté et al., 2011, 2014, AEM) and therefore their size not determined. We additionally observed that silt and other material rapidly clogged these GF/F filters in use, effectively reducing the pore size of the filter, so a high proportion of archaeal cells are likely still captured while the practical difficulties of filtration in the field are reduced.

p4196, line 24, what solvent was used here to load the TLE onto your column?

The first eluent, hexane : ethyl acetate (1 : 1, v : v) was used to load the TLE onto the column. After the addition of the first eluent to the vial, it was put into an ultrasonic bath for 5 mins before loading the TLE onto the column. After the first fraction had been collected and prior to the elution of the second fraction, the original vial was put into an ultrasonic bath for 5 mins with the second eluent (methanol) and loaded onto the column, to ensure that IPL GDGTs did not remain adhered to the glassware. This will be clarified in the revised text.

p4197, line 2, it was refluxed at what temperature?

It was refluxed at 80^{o}C. The revised text will be modified to reflect this.

P4199, line 19-21, is this the reason why the statistical analysis was only performed with core lipids (CL)? As shown in Table A1, 'sample group comparison (CL)'.

It was assumed that analytical error would be higher in IPL GDGTs due to their lower abundance, but the use of a 90% confidence interval should appropriately compensate for that as stated. The MANOVA statistical analysis presented here considered core lipids in the different sample groups as this was deemed to be more relevant to the aims of this study, which is intended to inform the use of GDGT-based proxies that are universally applied to core lipids when generating palaeoenvironmental reconstructions based on lacustrine sediment records.

p4206, line 15-17, the higher proportion of cyclization in the lower north basin is hard to distinguish in Fig. 5, why not to compare them with directly their DC values?

Reference will be changed from Fig. 5 to Table 2 in the revised text.

p4206, line 20-21, higher CBT in lower north basin? This is opposite of the higher proportion of cyclized brGDGTs (line 15-17). Higher CBT means less cyclized brGDGTs.

Error will be corrected in the revised text: 'higher' will be changed to 'lower'.

p4208-4209, ok, in situ riverine production seems reasonable, but how is the hydrodynamics of these rivers? River Endrick and Falloch are main inflows of Loch Lomond

Endrick Water is a meandering river that flows through relatively low-lying land, the characteristics of which are detailed in section 2.1 under 'south basin'. From observation during filtration, it carries a relatively high burden of silt that is discharged into the south basin of Loch Lomond. The River Falloch enters Loch Lomond at its northern point and flows from higher altitude terrain into the lake. The characteristics of the area it drains is detailed in section 2.1 as 'north basin'. The Scottish Environment Protection Agency (2000; West Region Water Quality Review) provides this information on catchment and discharge:

River	Catchment area (km^2)	Discharge (m^3/s)		
		Min	Mean	Max
Endrick	220	0.29	7.3	142.4
Falloch	80	0.03	5.9	226.6

An abbreviated description of the above will be added to the revised text.

The brGDGT IPL abundance in river SPM is even one magnitude higher than lake SPM (Table 2), while crenarchaeol IPL in both settings are similar. It is hard to imagine, if planktonic microbe in river water contributed this high brGDGT concentration under a dynamic condition, then their turnover time must be really short.

The turnover time for attached and free-living bacteria in rivers has been previously reported at around two weeks (Edwards and Meyer, 1986, AEM), hence the assumption that IPL brGDGTs in rivers represent the living brGDGT-producing community. Previous studies of brGDGTs in rivers also rely on this assumption (e.g. Zell et al., 2014, Limnol. Oceanogr.; De Jong et al., 2014, GCA). A note to this effect will be added to the revised text.

p4218, line 17-19, since there is no access to Buckles et al. 2014 (submitted), I don't understand how a decrease in %IPL was attributed to preferential degradation on in situ produced brGDGT. I think, here with preferential degradation the author means not only cleavage of polar head group but also degradation of the core lipid. If the sedimentary brGDGT producer dwells in surface or shallow subsurface sediment, hydrolysis only on the labile polar head groups of in situ produced IPL, which then contribute to the CL pool will also cause a decrease in %IPL in deeper sediment.

The reference mentioned will be updated in the text (Buckles et al. 2014, GCA has now been published). By preferential degradation, it was meant that the polar head group was cleaved, resulting in the CL GDGT. This will be clarified in the revised text.

p4218, line 20-21, in Takano et al., 2010 I don't see selective uptake on GDGT produced in sediment. So Takano's recycling hypothesis cannot explain why in a general case sedimentary input has no strong impact to the preserved water column signal.

This is mentioned for completeness to describe potential limitations of core top/surface sediment studies with respect to the early diagenetic processes of sedimentary GDGTs. It does not seek to explain patterns observed here as only surface sediments were analysed in Loch Lomond.

3. Technical corrections

p4198, line 20, the nomenclatures used in all following equations should refer to Fig. A1

Will be changed in the revised text.

p4199, line 11, please provide source and access information of the R program, such as a link of website?

Will be changed in the revised text.

p4201, line 15, CL: 1.08 not 1.07, see Table 2.

Typographical error will be corrected in revised text.

p4206, line 2, the averages of 0.42 in CL TEX86 is lower than that of IPL, not 'relatively high'.

Will be corrected in revised text.

p4207, line 26, not similar DC, clearly the lake sediments give generally higher DC compared to soil.

Will be corrected in revised text.

p4208, line 26-28, as shown in Table A1 all statistical analysis was did only with CL, but here the author is discussing difference in IPL or CL.

IPL will be removed in revised text with respect to the Table A1 reference. However, statistical significance with respect to "the difference between IPL and CL CBT values in riverine samples" is determined by a paired t-test.

p4209, line 11, there is only one literature of Zell et al. 2013 in the reference list.

Additional reference will be added to list in revised text.

In all figures, the panel labels are in capital letters, but not in the main text. Please make it consistent.

Will be altered in revised text.

p4214, line 1, Tables 2 and 4 p4215, line 16, should it be 'bias towards annual air temperatures'? not 'summer'.

The lake calibration by Pearson et al. 2011 (GCA) is calibrated to mean summer air temperatures, rather than mean annual air temperature (see p4199, line 1). p4215, line 16, correctly reads 'summer air temperatures'. The tables correctly read 'MSAT', defined as mean summer air temperatures. I cannot find a reference to air temperatures on p4214.

p4215, line 17, Shanahan et al., 2013 is not in the reference list.

Will be added to reference list in revised text.

p4218, line 13, Weijers et al., 2011 (GCA) is not in the reference list.

Will be added to reference list in revised text.

p4219, line 6, 'isoprenoidal' or 'isoprenoid' ? check and keep consistent for the entire manuscript.

The two are synonymous and interchangeable in this context.

Table 3, concentration unite 'ngg-1 dry wt.' as used in the other tables.

Will be altered in revised text.

Fig. 1, the green circle between P7 and P8 is not labeled, and S8 can not be found in the figure. The labels of compass are too small. There is no display of river Falloch and Endrick. Why the lower north and south basin area is shaded?

It was not possible to better present this figure in the landscape format of the discussion paper. We have been assured that this will be clearer in the standard Biogeosciences format for accepted submissions. If this is not the case, it will be edited for further clarity as suggested. The label at P1 actually refers to the sediment collected from beneath the sediment trap/SPM sampling location (square) rather than the green circle on top of it. S8 will be added in the revised figure and as the legend states, the green circles represent lake SPM and sediment trap locations.

Fig. A1, delete the prime on the mass to charge ratio of crenarchaeol regioisomer, m/z 1292'

This prime is standard presentation to denote the regioisomer of crenarchaeol.

Response to Referee #2

1. General Comments

In this manuscript, Buckles and colleagues examine a suite of samples from Loch Lomond and surroundings to better understand distributions and provenance of tetraether membrane lipids in lake sediments. This is a timely and important study, in that branched and isoprenoidal GDGTs in lake sediment archives are increasingly being used to reconstruct mean annual soil and lake temperatures, respectively. The application of these proxies is confounded by complex allochthonous and autochthonous sources, including growing evidence for in-situ brGDGT production within lake systems. By collecting a diverse sample suite that includes soils, river suspended sediment, river surface sediments, lake suspended sediments, lake surface sediments and a time-series of lake sediment trap particulates, coupled with detailed statistical analysis of the distribution of brGDGTs in both intact polar lipids and cores lipids, Buckles et al. present an attempt to disentangle the sources, production and delivery of brGDGT to lake sediments and the temperature signals these lipid distributions may carry.

This is a complex story, and the authors have done a very good job in arguing their main conclusions from this study, showing how these are supported by, or at least are consistent with, their data. These conclusions include:
(i) substantial in-situ production of brGDTs within the Loch Lomond lake system, although whether in the water column or within lake sediments (or both) is less clear;
(ii) distinct patterns of brGDGT distributions among sample populations such as soils, rivers and lake sediments, suggesting that the straightforward rationale of brGDGT in soils followed by erosion, transport and deposition in lake sediments is incomplete or too simplistic;
(iii) a caution that without full understanding of brGDGT distributions among possible sources to lake sediments, especially assessment of in-situ production, application of MBT/CBT-based temperature reconstructions rests on flawed forcing of calibrations between these brGDGT indices and air temperature that does not account for varying allochthonous contributions relative to autochthnous production; and
(iv) a second caution that with in-situ production, the BIT index is not a robust indicator of soil OM contributions to sediments.

2. Specific comments

While overall sound, there are four aspects to this study that raise some questions. The first of these relates to the investigation of the soils as a source of brGDGTs. Clearly soils in this and many studies contain ample brGDGTs as both IPL and core lipids. However, the soil sampling design seems to emphasize vegetation and land-cover, and not the likelihood of a particular soil to actually contribute to downslope/downstream sediment archives. There is no way to know in this study that the sampled soils actually contribute to river or lake sediments; future studies may wish to better emphasize soils that are geomorphologically best primed to contribute sediments: adjacent to channels, gullies, and stream banks, along the steepest slopes, and downslope from any tilled/disturbed land-use. Thus, while this study shows that the soil brGDGT distributions are not identical to river SPM and sediment brGDGT distributions, this does not require that soils in general are not contributing brGDGTs to the rivers but rather only that the soils sampled in this study may not be the most important contributors to the rivers. The assertion that there may be in-situ production of brGDGTs in these rivers is potentially consistent with the data, but may also be explained by not sampling the soils/locations that best contribute particulates to the rivers. It is curious that the river sediments and river SPM samples show similar brGDGT distributions, as these cannot be the same populations of sediment. Coarse sand (river sediments) represents a different sediment source and flow regime than fine clays and silt in suspended sediments. It would be interesting to examine if the brGDGT distributions in the finest size fraction of the soil samples better matched these river sediment samples.

Loch Lomond's catchment is characterised by different vegetation regimes and has been sampled as such. Wherever possible the soils were collected next to rivers/streams, in peaty soils on steep slopes and in areas of localised run-off, as those soils were considered most likely to contribute to GDGTs found in lake sediments. The large amount of water percolating through the soil may well carry clay particles with associated GDGTs to these streams. It is difficult to see the proximity of the analysed soils to water sources based on the map in Fig. 1 as many of these were small streams, or even in the path of localised run-off as with S2-S4, so this will be emphasised in the revised text (methods section). River sediment was also collected at each of the sites of river SPM collection. This will be described in the revised text (methods section). We accept the possibility that despite this sampling regime, some soils may not be representative of direct sediment influxes to rivers/streams and Loch Lomond itself. As a result, analysis of full populations of these sampling groups was undertaken to avoid the over-emphasis of the significance of a single soil/SPM collection/river sediment analysis.

Secondly, the authors use intact polar lipids as reflection of "living" bacterial sources of brGDGTs, and core lipids as representing relict and more degraded material. However, little is known about the time scale for degradation of IPL. If it is slow relative to the timescale of sediment mobilization across the landscape, then even IPL may be integrating older sources and bacterial communities in soils. This can be important in regions where that has been substantial land-use change in recent decades or centuries, as brGDGT-producing soil communities may have changed yet the landscape may still export some relict IPL. The timescale of IPL degradation also is important for considering in-situ brGDGT production in lakes and especially rivers. In the Loch Lomond watershed, the timescale of storage of lipids in soils is much greater than the timescale of transport in rivers (many years versus a few days), thus it is difficult to imagine substantial IPL degradation during the short time that these compounds are transported in rivers. As the authors mention, the difference in %IPL between soil and river samples is not statistically significant, and thus in-river brGDGT is not required.

In soils, it has been suggested that IPL branched GDGTs have a turnover time of greater than a year (Weijers et al., 2011, GCA). However in more dynamic environments such as river and lake water, IPL GDGTs are regularly utilised to trace living biomass in SPM (e.g. Zell et al., 2013, Frontier. Microbiol.; Buckles et al., 2014, Environ. Microbiol.; De Jonge et al., 2014, GCA). In the case of the small rivers and streams analysed here, these are oxic and turbulent environments where (soil derived) IPL GDGTs would be expected to undergo relatively rapid oxic degradation, in comparison to the relatively stable and locally suboxic soil matrix. While not excluding the potential presence of IPL GDGTs from soils reaching rivers and streams intact, we consider that the likelihood of all these IPL GDGTs completely retaining their labile polar head groups in the river/stream water, in the absence of an in situ source, is distinctly unlikely.

With respect to the impact of substantial land-use change in recent decades or centuries, we draw your attention to Peterse et al., 2010, Org. Geochem., which states that the CL pool of branched GDGTs has a turnover time of <45 years in the Scottish (UK) soils analysed.

Third, it remains unclear if autochthonous production of GDGTs occurs in the lake water column as well as sediments. Clearly, the brGDGT distributions in lake sediments are distinct from those in lake suspended sediments and sediment trap samples, while lake suspended sediments and sediment trap samples are not very distinct from river samples. This supports brGDGT primarily within the lake sediments and not within the water column. However, the differences in lake SPM CBT ratios between core lipids and IPL, as well as the close capturing of temperature depth profiles by TEX86, supports production of branched and isoprenoidal GDGTs, respectively, within the water column.

This correctly summarises our discussion on the topic of in situ brGDGT production in the lake's water column. In the absence of another compelling explanation for the statistically significant differences between IPL and CL brGDGT distributions in SPM and noting that CL brGDGT distributions in SPM are shifted towards those found in catchment soils, we conclude that autochthonous production does indeed occur in the water column.

Fourth, it would be valuable to know how river discharge and suspended sediment fluxes leading into Loch Lomond changed over the course of this study. Differences in GDGT concentrations, and for the sediment traps, the fluxes, may be in part explained by changes in suspended sediment concentration and discharge. Thus, different river flow regimes, and the landscape's geomorphic response to precipitation, runoff, and temperature, might result in different soil sources to the rivers and lake, different populations of soil-derived GDGTs as deeper soil horizons are accessed for erosion, and differences in the mixture between allochthonous sources and autochthonous production in the lake. Showing the concentration of brGDGTs in the sediment trap samples, in addition to their fluxes, may begin to inform this.

The sediment traps were deployed and sedimenting material collected approximately 5 years prior to the collection of the other sample groups. As such, we are unable to comment on the variation of GDGT fluxes in sedimenting material over the course of this study. The interpretation of the GDGTs yielded by settling particles has therefore been limited to ensure that our conclusions are robust. The main goal of our study was to characterise the different brGDGT pools in and around Loch Lomond in an effort to determine the provenance of the brGDGTs in the lake's sediment record. Certainly, a complete integration of our results with a robust study of sedimentary budgets would be desirable (if possible). At this point, however, this lies beyond the scope of the present study.

3. Technical corrections

p4190, l5-6: The authors should define here what is meant by Tierney10, Zink10, etc. This is clear much later in the manuscript that these are shorthand for different calibrations, but is not clear here where first introduced.

Additional explanation will be included in revised text for clarification.

p4195, l1: The authors should provide more detail on the sediment trap deployment and recovery of sediment from it.

Additional explanation will be included in revised text. This refers to the position of the sediment traps on the map (Fig. 1) and the handling of the sediment recovery.

p4196, l16: The authors should describe the composition and concentration of the phosphate buffer used in this extraction

Brief additional explanation will be included in revised text, as this is a standard method that is appropriately referenced.

p4201, l4: The Methods section and Table 2 indicate that River Falloch SPM was sampled only once, in September 2011. The statement that brGDGTs in river SPM was constant throughout the year is not supported.

This will be altered in the revised text; the two SPM samples referred to were taken at different points of the river's flow rather than in different seasons.

p4207, l21: Because some of the watershed is urbanized, and the uplands have widespread immature stony soils, it may be overstated to say that the catchment is fully soil covered.

This will be altered in the revised text to reflect that the catchment is almost entirely soil covered.

Table A1: For clarity the authors should indicate which sample pairs in the MANOVA analysis are for IPL and which are for CL.

All sample pairs are for CL. In the revised version this will be indicated in the figure legend of the revised text.

Figures: The ordering of figures is cumbersome, as Figures 4 and 5 are refer red to before Figure 3 in the manuscript.

Figure ordering will be rechecked in revised text.