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Interactive comment on "Water availability determines branched glycerol dialkyl glycerol tetraether distributions in soils of the Iberian Peninsula" *by* J. Menges et al.

J. Menges et al.

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General This manuscript describes the brGDGT distributions in 23 soil samples from the Iberian Peninisula, a relatively arid region. This is a relatively small dataset compared to previous publications in this field. It also has been established before that brGDGT distributions in soils from arid regions respond differently to environmental parameters such as soil pH and MAT (Peterse et al., 2012; Dirghangi et al., Org. Geochem. 59, 49âĂŘ60, 2013, not cited) but the authors push this a little forward by showing that the deviation of calculated MAT (using the global soil dataset) is negatively correlated with mean annual precipitation (MAP) and the aridity index (AI) and

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this aspect makes the dataset of interest. However, there are many issues with this manuscript that need to be fixed (see specific comments below). The title claims that water availability is the only control on brGDGT distributions, whereas the authors clearly show (although not surprisingly) that pH clearly effect their distribution (Fig. 1a) and the title should thus be changed. Furthermore, it is often not exactly clear what the authors have done (and why) and the use of statistics is not always straightforward. One also wonders why the authors have not done multivariate analysis to try to correlate the brGDGTs relative abundances in their sample set with envrironmental parameters suchs as pH, MAT, MAP and AI.

*The aim of the study was to assess the effect of hydrological conditions on the MBT'/CBT. Though the sample set is smaller than the global calibrations, so it is the area of study. Thus we believe that the sample set is adequate for the objective of the study as we cover a wide moisture range (405 mm to 1455 mm. mean annual precipitation per year), and 23 datapoints are sufficient to address the questions posed in the paper. Moreover while the overall calibration of MBT/CBT displays a linear relationship at a global scale it is when we look at a more regional scale where additional factors, which may be more pronounced in certain regions (such as aridity in our case) can be identified. This is a typical procedure for proxy calibration, where data from regional studies and global studies are used. The relatively small sample set compared to previous spatially larger studies does not discount any of our findings, as statistical relationships identified are robust (i.e. significant). In fact, it is the regional focus (which goes with a more limited amount of samples) on an arid environment which led us to identify the observed relationships. Following reviewer suggestions we have updated the references. The title claims that Water availability determines branched glycerol dialkyl glycerol tetraether distributions in our sample set from the arid Iberian Peninsula (and the majority, i.e. 55% of the variability in MBT can be explained by MAP for example) we never say either on the title or text that water availability is the only factor. But in the present sample set, it clearly is the major factor, as opposed to temperature. Given the size of the dataset we feel that the employed basic statistics provide more

robust conclusions than the use of multivariate analysis.

Specific comments:

P 9051, line 15. "original"? Not clear what is meant here.

*This is sentence 15: Soils with the highest absolute brGDGT abundances were located in the northern Iberian Peninsula, the area with the highest rainfall and cooler temperatures.

A search for the word "original" in our ms gave the following results:

-9047, l.5. "The new calibration equation by Peterse et al. (2012) has a lower correlation coefficient than the original one by Weijers et al. (2007)". We have changed "original one" to "original calibration" in case this was unclear. -9047, l. 15: "Furthermore, the relatively large scatter in the original calibration datasets (Weijers et al., 2007; Peterse et al., 2012)" We cannot see what could be unclear here and, thus, we did not change it.

-9055, l. 20 "because the addition of temperate soil data to the global data set increased the scatter of the original MBT calibration (Weijers et al., 2007)." Again, we cannot see what is unclear here, and, thus, we did not change it.

P 9051, line 14. The concentrations of the brGDGTs are normalized on TOC but TOC was not measured. Instead TOC was "estimated" using a loss of ignition technique that has been tested for lake sediments but not for soils (Heiri et al., 2001). Since the authors claim that brGDGT concentrations are partially dependant on TOC abundance (p9052, line 3), MAP and AI (Fig. 4) they should just measure the TOC content of the 23 soil samples studies (not a big effort) or provide a calibration set for which they show that the LOI values of their soils shows a good correlation to the TOC content.

*This concern was also raised by reviewers 1 and 2. I copy here the answer given to them: LOI is one of the methods commonly used to quantify organic carbon contents in soils. This was mainly because that is what we could access to process the sam-

ples. However even though the EA is more accurate the amount of sample analyzed is very small. Thus even with 3 replicates you measure a very small portion of your soil sample. The LOI instead is done in much bigger samples and thus a higher portion of the sample can be analyzed. Soil structure is much more complex than sediments and even homogenized samples will still present certain level of heterogeneity. By measuring bigger samples we hoped to average out some of that microheterogenity and thus compensate for the lower accuracy of the technique.

Furthermore, the reported summed concentrations of brGDGTs (1âĂŘ45 ng gâĂŘ1 TOC) seem to be three order of magnitude lower than those reported in the literature (typically in the μg gâĂŘ1 TOC range; e.g. Weijers et al., 2007; Kim et al. Limnol. Oceanogr. 55, 2010, 507–518; Yang et al., 2012; note that this is 2011 in ref list).

*The units have been revised and corrected.

P 9052, line 13. Why is this sample considered to be an outlier? Its concentration is higher than that of the others but still low compared to literature data (see previous comment). There is no basis to consider it an outlier and it should be used in all regression analyses (e.g. Figs. 4a and 4b).

*Once the units are corrected this is not the case anymore. Actually this value is clearly an outlier when using statistical tests, such as estimating the interquartile range (IQR). We now mention our decision based on the IQR in the ms.

P 9052, line 5âĂŘ16. These sentences should be carefully rephrased. Weijers et al. (2006) showed by determining the stereoconfiguration of the glycerol units that brGDGTs are most likely of bacterial origin. Based on their higher abundance in the catotelm of peat bogs they were speculated to be derived from anaerobic bacteria (Weijers et al., 2006). Subsequently, the application of molecular ecological tools showed that Acidobacteria (Weijers et al., Geomicrobiol. J. 26, 402âĂŘ414, 2009) were omnipresent in these peats and were, thus, a potential source organisms of the brGDGTs. This was subsequently confirmed by the study of Sinninghe Damste et al. (2011) who

identified brGDGT Ia in two species of Acidobacteria and detected the presence of the building block iso diabolic acid in all of the analyzed subdivision 1 and 3 Acidobacterial species. As concluded by Sinninghe Damste et al. (2011) this does not exclude that other bacteria are also producing brGDGTs. The authors conclude based on an absence of a relationship of brGDGT concentration (?) with pH (which was reported by Weijers et al., 2007 (not cited) and Kim et al. 2010 (not cited), but not by Peterse et al. (2012) and Sinninghe Damsté et al. (2011)) that Acidobacteria are "not necessarily the main source of brGDGTs". It remains entirely unclear where they base this rather vague conclusion on.

*This was modified according to reviewer's specifications and the last sentence was removed: None the less brGDGTs have been found in a range of environments regardless of the origin or redox state (see Schouten et al. 2013). So far brGDGT were identified in only two aerobic Acidobacteria species suggesting that they are synthesized by different bacterial communities (e.g. anaerobic and aerobic; Sinninghe-Damsté et al., 2011). This was confirmed by our data as despite covering a pH range from 4.8 to 8.7, we did not observe an increase in brGDGTs with lower pH contradicting earlier findings (e.g Peterse et al., 2010; Sinnghe-Damsté et al., 2011; Yang et al., 2011).

P 9052, line 26. One can only conclude that they are below the level of detection not they are not present.

*We cannot agree more and had expressed this exactly this way in the original ms: In eight of the samples (35% of the total) none of these brGDGTs are present, or their concentration is below the detection limit (Table 2) agreeing with previous results for 278 globally distributed soils (Peterse et al., 2012).

P 9053, line 7. How can one say that "many CBT values in the Spanish dataset underestimate the pH"? I assume the authors use the Weijers et al. (2007) or Peterse et al. (2012) calibration to calculate "pHest"? The way to go would be to show that the CBTâĂŘpH relationship for the Spanish soil dataset (Fig. 2a) is statistically different

from the global soil correlation. Judging from Fig. 2a and knowing the substantial scatter in the global soil dataset I doubt this. If this cannot be demonstrated, there is no basis for an underestimation of the pH in the dataset ("CBT bias") and the data of the Spanish soil sample set just follows the global soil dataset (which contains an order of magnitude more samples).

*We are not discussing on the spread of dataset just noting that many pH estimated values are lower than the observed ones. We have now added a sentence that clarifies the issue: Many CBT values in the Spanish data set lead to underestimated pHim values, with pHest values from 0.2 to 1.5 pH units below measured values, however this falls within the previously observed scatter (Fig. 3a).

P 9053, line 16. This heading is completely misleading. One cannot calculate temperature from MBT'. The authors provide equation (5), from Peterse et al. (2012), that clearly contains MBT' and CBT. This is because both pH and temperature effect the degree of methylation of brGDGTs in soils (see Weijers et al., 2007 for an extended discussion). I guess that the authors have used equation (5) to calculate MATest; if not they should explain clearly explain this. Assuming that they used equation (5) section 3.3 has to be rewritten completely (i.e. phrases like "an MBT' value of X.XX relating to a MATest of Y.Y°C" should be avoided).

*The title is changed to MBT'/CBT index

P 9054, lines 2âĂŘ3. This is a really confusing sentence. First of all, I guess a correlation between MBT' and MATim (not MATest) is meant. Secondly, I would basically conclude that there is no correlation between MBT' and MATim (it is just a scatter plot and P is much higher than for all other correlations presented). Consequently, the sentences following this section (lines 6âĂŘ11) should be skipped.

*This was changed also according to reviewers 1 and 2 specifications: Interestingly, MBT' and MATim show a weak but significant negative correlation within the Spanish sample set (R2=0.21; P=0.02) in contrast to the positive correlation between MBT and

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MAT observed by Weijers et al. (2007) and Peterse et al. (2012) (Fig. 2e).

P 9054, lines 12âĂŘ22. Again (like for pHest) it should be explained how MATest values were obtained (probably through the use of the global soil correlation). The following sentences focus on just one of the 23 samples studied. I feel that the text should concentrate on general trends, not on individual cases. The authors also mention that they tried to perform a regional calibration but do not present any data. This paragraph ends with a very vague statement (first part of sentence) and some kind of general conclusion which should be at the end of the paper (and should be specified; other environments?).

*The first part of the paragraph shows an example to illustrate an overall pattern.

The conclusion has been changed as suggested by reviewers: These findings constrain the use of the MBT'/CBT for paleotemperature reconstructions in the Iberian Peninsula and indicate that the environmental parameters controlling the distribution of brGDGTs have to be investigated in other areas.

P 9054, lines 23âĂŘ29. This is an important section for the manuscript but it is not clear. The experimental section mentions that MATres = MATim – MATest. The clear observation from the data is that for the studied soil sample set MATim > MATest, except for one sample with a MATest of ca. 22°C (missing from Table 1?). However, when MATres is plotted in Fig. 3d both positive and negative values are observed. This is in contrast with Figs. 4c and 4d where MATres is always positive. Clearly, this issue should be solved. This issue comes back in line 4 of page 9055 (over or underestimated?).

*This was already corrected according to reviewer specifications: Furthermore, our results clearly indicate that MATest residuals are not randomly distributed but rather the obtained MATest values from MBT' are underestimated above 10°C (Fig. 3d). This deviation was observed previously in the global data set but it is more pronounced in the Iberian soils (Fig. 3d). In addition the global dataset shows a negative residual dis-

tribution below 10°C (Fig. 3d). We interpret this result to mean that the key parameters controlling the MBT are not necessarily only temperature and pH. Recent studies have attributed the lack of correlation between MATim and MBT/CBT to factors such as vegetation change, soil type and changes in hydrologic moisture regime (e.g. Dirghangi et al. 2013; Loomis et al. 2013; Weijers et al., 2011).

P 9055, lines 13âĂŘ14. Poor use of statistics. Here the correlation between MAPim and MATim is called weak, whereas R2 is substantially higher that for the relationship between between MBT' and MATim.

*We do not understand the issue. The correlation is weak and is negative like the one observed for MBT MATim no comparison is made between correlation strengths.

P 9055, line 21. I don't think one needs the insert. In fact, one can argue that plotting the data from the global soil data set does not really help to get the message across.

*We kindly disagree with this. We prefer to keep the plot as we feel it makes it clearer that the effect of soil moisture is a global feature and thus of concern in other study areas. This is not only clear from our own results but from other studies such as Peterse et al. 2012 and Dirghangi et al., 2013.

P 9055, line 26 âĂŘ P 9056, line 3. See earlier comments on concentration: the reported concentrations of brGDGTs are very low in comparison with other soils and one wonders if this relates to the deviation from the global soil dataset.

*This was corrected.

P 9055, lines 15âĂŘ20. I believe one cannot make any conclusion based on soil type. The sample set (23) is too small and the number of type of soils (14) is too high for that.

*We believe Dr. Sinninghe-Damste refers to pg 9056

We did not make any conclusions based on soil type we just stated we could not find a

correlation.

P 9055, lines 21âĂŘ23. I have read this sentence numerous times but I really don't understand the argument.

*We believe Dr. Sinninghe-Damste refers to pg 9056: This was modified according to reviewer suggestions: The correlation with MAPim is slightly higher for brGDGT abundances (R2=0.68) than for the MATest residuals (R2=0.59) (Fig. 4 a and b), thus suggesting that brGDGT abundance is not the key factor explaining the MBT'-MATest scatter in our dataset.

P 9057 Figure 4 really forms the message of this manuscript, i.e. the deviation of calculated MAT (using the global soil dataset) is negatively correlated with MAP and AI. This message would, however, be much stronger if the authors could demonstrate a direct relationship between brGDGT distribution and rainfall and aridity. In the way they present it now it heavily relies on the calculation of MAT using a correlation that they claim does not work (at least not for their region). If rainfall and aridity exert such a strong control on brGDGT distribution as claimed by the authors, it should be possible to reveal this in an independent way.

*We do not believe that T has no control on the MBT' index, so we have used the residuals of that relationship to identify the additional factors affecting MBT in our dataset. It just turns out that in arid regions hydrology can seemingly override the T signal on MBT', which we believe is an important finding. However, we did plot MBT' vs MAP (Fig 1f), where it is evident that rainfall amount explains more than half of the variability in the MBT (r2=0.55, as opposed to the r2 of 0.44 in the global dataset, where this observation had been explained by a co-variation of T and MAP, which is not present in our regional dataset – hence the explanation of this effect must be different – see our conclusions). We however thank the Reviewer for pointing this out and have included a plot of MBT' vs AI to make it clear that AI has a direct effect on the MBT' and not just the residuals, which shows that 53% of the variability in the MBT' in the Iberian soils

can be explained by the AI index.

It is not clear to us what an independent way this would be. If we aim to assess the effect of moisture conditions on the MBT'/CBT we do not see how we can exclude the MAT calculations. The correlation we use or MAT values we obtain are insubstantial as there is no correlation with temperature and we make this clear in the discussion and conclusions.

Interactive comment on Biogeosciences Discuss., 10, 9043, 2013.

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