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Biogeosciences Discussions

Interactive comment on "Carbon isotopic composition of branched tetraether membrane lipids in soils suggest a rapid turnover and a heterotrophic life style of their source organism(s)" *by* J. W. H. Weijers et al.

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We would like to thank both reviewers for their comments and suggestions on our manuscript. Both reviewers are generally supportive of our work which they regard as being of interest to the readers of Biogeosciences and of which they qualify the results on the turnover time of branched GDGTs in soils as a 'real novel aspect' and a 'substantial finding'. Below we discuss the comments of each reviewer in detail.

Referee #1 (Anonymous)

Heterotrophic lifestyle. This reviewer mentions that a heterotrophic lifestyle of the branched GDGT-synthesing organism has been suggested before and is therefore not the real novel aspect of this paper (in contrast to the turnover time). We would like to highlight that although this is correct, the heterotrophic lifestyle has been suggested only in two reports based on one peat sample (Pancost & Sinninghe Damsté 2003) and one soil sample (Oppermann et al., 2010), both clearly mentioned in the manuscript. Thus, although not providing the first ever reported value on the stable carbon isotopic composition of branched GDGTs, our study is the first to investigate this composition in more detail by means of a survey of multiple soils in different settings, which is necessary to reinforce these earlier suggestions.

n-Alkanes. The reviewer argues to leave out the isotopic data on plant leaf wax-derived n-alkanes as it would unnecessary lengthen the discussion, has partly already been published and distracts from the main point. Reply: An important aspect of the manuscript is to compare stable carbon isotopic compositions of branched GDGTs with those of vegetation. The reason that we included the n-alkane data is because they are indicative of the vegetation directly, whereas TOC represents the total pool of vegetation and microbes. In addition, the n-alkanes are one of the more recalcitrant types of lipids present in soils and as such an important comparison to branched GDGT-derived alkanes concerning the natural isotopic labelling experiments with two of the soils. It is correct that some of the n-alkane data is published before, this is also indicated in the text with the appropriate references. However, for a clear comparison these data are needed. We have decided to list the d13C values for all major n-alkanes individually in the table in order to enable future comparison by other researchers. Thus, we do not agree that the presence of the n-alkane data distract from the main point. We do agree, however, that the paragraph on the result of the n-alkanes could be slightly shortened, for example by leaving out the short discussion on the depth profile in the Rowden soil.

Fertilizer. The reviewer asks to include information on the amount of N-fertilizer applied to the soils as this might have implications for the discussion of the amounts of crenarchaeol observed in the soils. Reply: The reviewer is absolutely right here. These data are available from the literature and can be included, something we didn't think of earlier. As correctly observed by the reviewer, all occurrences of a relatively high crenarchaeol concentration are in the soils that received N-fertilizer. This information and observation will be added in the revised version of the manuscript.

Formulas for turnover time. The reviewer asks to include these in the manuscript, since the turnover time of the branched GDGTs is an important aspect of the study. Reply: Although we thought that a reference to the article describing these formulas would suffice, we see the point of the reviewer and will include these formulas in the Methods section of the revised manuscript.

Sample treatment. The reviewer wonders whether all samples have been pre-treated the same way and if not, if this would influence the results. Reply: The sample set has been compiled via collaboration with several research groups and are not all processed ourselves in terms of drying and grinding etc.. Some of the samples have been freeze dried while others might have been air or oven dried. Not all samples have been sieved, but if not sieved, roots, woody fragments and stones have been removed. It is expected that the way of drying the samples and removing larger fragments from them will not have an influence on the amount or isotopic composition of the branched GDGTs present.

Glycerol. The reviewer wonders if the exclusion of the glycerol moiety from the stable carbon isotopic analysis, as a result of the ether bond cleavage, could have affected the obtained results. Reply: We are not aware of any reports that have looked at the isotopic composition of glycerol moieties derived from GDGTs. Although for the biosynthesis of a glycerol unit different enzymes might be involved than for the biosynthesis of the alkyl chain, we do not expect large differences in the carbon isotopic composition of both GDGT components. Most importantly, however, is that if the glycerol moiety would possess a slightly different carbon isotopic composition, it would be negligible in relation to the whole tetraether lipid as the two glycerol moieties together comprise

only 6 carbon atoms as opposed to 60 carbon atoms for the two alkyl chains. This fact will be emphasized in the revised version of the manuscript at the appropriate place.

Length. The length of the manuscript is considered quite long by the reviewer who suggests to reduce this by writing in a more concise way. Reply: This is a similar comment as reviewer 2. For the revised version we will therefore carefully go through the manuscript again to make the text more concise were possible by omitting any redundancies and trying to reduce the amount of references.

All minor editorial comments of reviewer 1 will be accommodated according the reviewer's suggestions.

Referee #2 (R. Smittenberg)

Heterotrophic lifestyle. This reviewer mentioned that no conclusive insights are reached concerning the lifestyle of the branched GDGT-synthesizing bacteria (as opposed to the substantial finding of the decay rate of branched GDGTs). Reply: We do not fully agree with this as we do reach a conclusive insight. Although it is true that based on our data set alone it is difficult to distinguish between a pure heterotrophic or chemoautotrophic lifestyle, we do refer to the study by Oppermann et al. (2010) which provides additional evidence (via soils on and next to a naturally labeled CO2 vent) pointing to a heterotrophic lifestyle. This will be made more clear in the revised manuscript.

Length. Similar to the first reviewer, this reviewer suggests to reduce the amount of references. In addition reduction of the text dedicated to the history of the GDGT lipids is suggested as this is already covered in many other publications. Reply: Both the introduction at this place and the amount of references will be shortened in the revised manuscript.

Table 2. As also noted by reviewer 1, column d is missing and e to h should be moved a column to the right. Reply: This seems to have gone wrong during typesetting to the

BGD format and has escaped our attention during review of the type-set manuscript. In addition, the indent of 'C4 vegetated soil' is wrong in both tables 1 and 2 as well as the indent of 'Grassland soil' in table 1. This will be taken care of in the revised manuscript.

Response factor. The reviewer notes that we assume a 1:1 response factor ratio between crenarchaeol and branched GDGTs for the HPLC/MS quantification and suggests this to be acknowledged in the text. Reply: The reviewer is correct in this. The response factor for crenarchaeol and branched GDGTs in the MS might not be exactly the same. But as correctly mentioned by the reviewer, due to a lack of authentic standard this potential difference is not quantifiable. However, as both compounds are closed, macrocyclic, tetraether structures, this difference is not expected to be very large. Although this is usually assumed without explicit mentioning, we will add a sentence to the methods section dealing with this issue.

GDGT indices. The reviewer asks to include the GDGT based indices (BIT, MBT, CBT) as they are increasingly used as proxy in palaeoenvironmental reconstruction studies. Reply: Although we could add these data we have chosen not to do so as it will not add any information to our study. More importantly, if we would include them, this means that we also have to introduce them first, i.e. explain them in the text, which would further lengthen the manuscript. Although the reviewer mentioned that we do not need to discuss the numbers in great detail, in practice any differences in values amongst the soils with values expected based on the global soil calibration set, especially for the managed and fertilized soils, would urge us to include a small explanation. This would all distract the reader from the main point of this study and is outside the scope of our manuscript. In addition, such data for many soils have by now been published in studies specifically aimed at that, i.e. from Svalbard (Peterse et al., 2009) to soils around tropical lakes (Tierney and Russel, 2009; Sinninge Damsté et al., 2009). The benefit of adding these 10 soils is therefore very small compared to the text and explanation needed.

Figure 4. The reviewer points to a confusing use of terms and a mistake in the figure

concerning the fractionation during diffusion of soil CO2 to respired CO2. Reply: We are indebted to the reviewer for correctly noting the mistake in the figure and in the use of the term 'soil CO2'. Also the term 'respired CO2' has been confusing as we used it with respect to bacterial respiration whereas it is also used with respect to soil respiration. The mistake in the figure (a fractionation of -4.4 per mille instead of +4.4 for diffusing CO2) and the terminology, have already been corrected in both figure and text. The comment of the reviewer that soil-respired CO2 is not part of the carbon flow toward chemo-autotrophy is only correct if soil-respired CO2 is defined as solely the CO2 above the soil. There is a diffusive gradient, of course, from soil CO2 to soil-respired CO2, and a mixture of these two might still be used towards chemo-autotrophy.

Crenarchaeol. The reviewer suggests to remove the claim that 'all crenarchaeol biphytanes except one have lower d13C values than the branched GDGTs', because within error two of the five are equal. Reply: For this comparison we used the average numbers as given in Table 2. In the text we first state that 'crenarchaeol derived biphytanes are slightly depleted relative to branched GDGT-derived alkanes', followed by '…the differences, given the associated errors, are not large…'. We have the opinion that this phrasing already clearly shows that this claim is not as strong as suggested by the reviewer and that, as the reviewer noticed, within error they are not that much different.

Restructuring of discussion. The reviewer advises to consider restructuring the order of discussion at certain points in paragraphs 4.4 and 4.5. The main suggestions are to swap the discussion on chemoautotrophy and heterotrophy within paragraph 4.4 and then to swap paragraph 4.4 with 4.5 so that the carbon isotopic composition of the isoprenoid GDGTs will be discussed first, followed by the branched GDGTs. Reply: Both suggestions seem reasonable for the reasons given by the reviewer and will therefore be considered in the revised manuscript. The smaller suggestions regarding restructuring made by the reviewer will be considered as well, i.e. bringing the information from Opperman et al. (2010) more to the beginning. In some cases the suggested changes seem less practical, as information from the first discussion part is used in the second part of the discussion, e.g. swapping GDGT-0 with crenarchaeol in paragraph 4.5. While restructuring, attention will be paid as well to reducing the length of the discussion as mentioned by both reviewers.

Graph with GDGT d13C data. The reviewer suggests to add a graph that shows all GDGT d13C data as well as all TOC and n-alkane data, which might be integrated with figure 4. Reply: Assuming the reviewer suggest to include all measured data in Figure 4, we do not agree. Figure 4 is mentioned to be a very simplified (this will be emphasized in the revised version) schematic of the heterotrophic and chemoautotrophic pathways for carbon assimilation by the branched GDGT-synthesizing bacteria based on average numbers obtained from our study. Besides making it a very busy and more difficult to read figure, it also would suggest the picture being a precise and accurate representation of all the steps involved in the carbon assimilation pathway, opposite to the schematic display we envision.

Accumulating biomass. The reviewer does not agree with the conclusion that no biomass / GDGTs is accumulating. Reply: It has not been our intention to conclude that nothing will accumulate. Indeed, as correctly noted by the reviewer, we mention later on that (on longer timescales) other preservation factors play a more important role (indeed more important than first order decay as noted by the reviewer). We understand the potential confusion and will rephrase this conclusion in the revised version.

Discussion. According to the reviewer the sentence on the role of branched GDGT-synthesizing bacteria in organic matter degradation comes out of the blue in the discussion. Also the reviewer would refrain from extrapolating the turnover time in arable, managed soils to all soils. Reply: the potential role of branched GDGT-producing bacteria in the last steps of organic matter degradation has been suggested before (Pancost & Sinninghe Damsté 2003). We have, however, not mentioned this earlier in the discussion which should have been the case rather than only in the discussion. This will be changed in the revised version. The reviewer implies that we extrapolated our results from managed soils to all soils. This is, however, not the case. We state in the

text that '. . .the natural labeling experiments studied here suggest a turnover time of ca. 17 years for branched GDGTs in arable soils in temperate climates'. Then we only remark that this '. . .fits well with the emerging view that most microbial biomass in soils . . . is relatively short lived'. So it is an observation and not an extrapolation.

All smaller editorial suggestions made by this reviewer will be accommodated accordingly in the revised manuscript.

On behalf of all authors,

Johan W.H. Weijers

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