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Interactive comment on "Distribution of tetraether lipids in agricultural soils – differentiation between paddy and upland management" *by* C. Mueller-Niggemann et al.

Anonymous Referee #2

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General comments

In this manuscript, C. Mueller-Niggemann and colleagues present results on the distributions of branched and isoprenoid GDGTs in a global soil sample set and the inferred influence of agricultural practices on these distributions. While the manuscript is well written and technically sound, the authors fail to convey the motivation for their research and significance of their findings. For instance, their initial statement in the abstract that "Insufficient knowledge of the composition and variation of isoprenoid and branched GDGTs in soil exists" is not in itself a compelling justification for their study. Similarly, the manuscript lacks real conclusions and impact: What is the actual significance of this work for ongoing and future research in the area of GDGT biomarkers,

GDGT-based proxy applications, and soil microbiology etc.?

Although I do think that the presented manuscript addresses a topic within the scope of Biogeosciences, the work still needs major improvement (with respect to the authors' explanations, data synthesis and conclusions, which I outline in detail below), until a final decision on the manuscript can be made.

Specific comments

P16710 L1-5: Insufficient knowledge of the GDGT composition in agricultural soils is not a compelling motivation in itself. Please demonstrate in the abstract the significance of this work and its relation to prior research. Consider restructuring the abstract according to the following points: What is the general theme of this study and what prior works have motivated you to perform this research. Why is it relevant to study the GDGT distribution in agricultural soils and what could be gained from this knowledge?

P16711 L4-6: Rephrase. GDGTs are not characteristic for bacteria. They are extremely rare in cultivated bacteria. Only one GDGT (GDGT-Ia) has been found in Acidobacteria and two (GDGT-Ia, GDGT-IIIa) have been found in Thermotogales.

P16711 L6-8: Consider mentioning the fundamental differences in glycerol stereochemistry of bacterial and archaeal GDGTs.

P16711 L9-20: This sentence is littered with citations and hard to read. Consider reducing these to a few key references and a more general statement, such as "e.g. in the water column and sediments of oceans and lakes, peat bogs, and soils", to enhance readability.

P16711 L21-24: These citations, with the exception of Leininger et al., are not appropriate for the referenced statement. Please choose more appropriate references for archaeal metabolisms such as Stahl and de la Torre 2012 (Ann. Rev. Microbiol.), Offre et al., 2013 (Ann. Rev. Microbiol.) etc. or refer to the first report of archaeal involvement in each mentioned process.

P16711 L24-27: This is misleading, MG-II Euryarchaeota were not in the focus of GDGT research until very recently and these groups were never distinguished according to their lipids but based on (meta-)genomic evidence. Furthermore, the Marine Group I Crenarchaeota moniker is obsolete. Please rephrase to Marine Group I Thaumarchaeota.

P16711 L24-P16712 L2: Rephrase. MG-I archaea form a part of the phylum Thaumarchaeota and are not separate from them. If you want to introduce different archaeal phyla, do this in a concise way. Currently, this section is very confusing.

P16712 L5-L7: This sentence is important for understanding the authors' narrative of methanogens versus Thaumarchaeota in the discussion but is misleadingly written. The authors are correct that GDGT-0 is the most common GDGT in methanogens. However, GDGTs only occur in some methanogens, most of which are thermophilic. In (agricultural) soils, there are, among others, two important methanogenic lineages, Methanosarcinales and Methanocellales. There is no conclusive evidence for the occurrence of GDGTs in Methanosarcinales and the lipids of the Methanocellales have not been studied yet. In this way, this statement is very misleading in implying that GDGT-0 is a common membrane lipid in (environmentally relevant) methanogens. Please revise this section to acknowledge the current knowledge of lipid distribution among methanogens.

P16712 L12-16: This section is repetitive and misleading. It has already been stated a few lines above that Thaumarchaeota produce GDGTs 0-4 and crenarchaeol, please omit this repetition. Why is the special structure of crenarchaeol explained here and not when it is first mentioned above? This sentence is misleading in that the authors imply that all mesophilic archaea produce GDGTs when in fact only one non-methanogenic archaeal lineage has been cultured, the Thaumarchaeota. This sentence further implies that these mesophilic archaea produce no or only low amounts of GDGT-0. In fact, all archaea that synthesize GDGTs also produce GDGT-0 as a major membrane lipid (including Thaumarchaeota).

P16712 L19-21: Reduce references to a couple key references. Did all of these referenced works contribute new information on the biological origin of brGDGTs?

P16713 L27-29: Replace "the bacterial cell membrane" with "soils". There is no direct evidence on adaptation mechanisms in the brGDGT-producing organisms.

P16713 L15-16: This and the last paragraph are dealing exclusively with environmental influences on brGDGT composition in soils. What is known about archaeal abundance/community composition/lipid patterns in soil and the influencing parameters? Furthermore, as these organisms have been in culture for 10 years: What is known about lipid adaptation in cultivated Archaea/Thaumarchaeota. This section is a good opportunity to reflect on the state of the art.

P16713 L25-27: Please provide references for these statements.

P16714 L26-P16715 L4: Why is it important to study tetraether lipids in soils? Even though the introduction is quite extensive, the authors have not made a case for the necessity of their study. The current problems and research question in this field of study have not been formulated at all.

P16716 L20-24: Did you detect GDGT-4? As far as I know, GDGT-4 and crenarchaeol co-elute using this HPLC method. This is not much of an issue for many marine samples but GDGT-4 is abundant in soil Thaumarchaeota and therefore might lead to an overestimation of crenarchaeol abundances. Did you employ any correction for this effect (e.g. for isotope peaks)? If so, this should be stated here.

P16718 L12-P16719 L4: The Results section is too short and the Discussion section is too descriptive. Please consider combining the results and discussion sections or extend results section and minimize redundancy between results and discussion sections.

P16719 L12-14: I am not convinced that this conclusion is supported by the few locations presented in this study. Change phrasing or tone down.

P16719 L18-19: How did you derive at this conclusion? Can this be statistically proven?

P16719 L22-24: Also input of fossil GDGTs?

P16719 L25-P16720 L1: None of these references deal with soil archaea or iGDGTs in soil, please rephrase or replace with appropriate references.

P16720 L5-7: This statement is oversimplified. 1.1a Thaumarchaeota as well as the closely related SAGMGC-1 lineage (Nitrosotalea devanaterra) also occur in soils.

P16720 L9-12: This sentence is phrased misleadingly. Sinninghe Damste et al. observed higher crenarchaeol regioisomer abundances in soils than in marine and lake sediments, but they did not investigate the production of this compound in soil and the composition of the microbial community in these sediments (i.e., I.1a vs. I.1b Thaumarchaeota).

P16721 L1-3: GDGT-0 is also a major component in Thaumarchaeota and many other archaea. Given the prevalence of GDGT biosynthesis in archaea, many of the uncultured archaeal clades in soils and sediments may contribute GDGT-0. I would urge the authors to oversimplify the complexity of archaeal assemblages (Thaumarchaeota vs. methanogens).

P16721 L5-7: While it might be true that methanogenic environments have high GDGT-0 to crenarchaeol ratios, this is not an established fact. The ratio was conceptualized by Blaga et al. for lakes and the >2 threshold is actually based on the ratio of GDGT-0/crenarchaeol in marine surface sediments. This ratio has been used by Blaga et al. and Naeher et al. for lake sediments but has not been established for soils. There is to my knowledge no published additional (e.g. metagenomic) evidence supporting the claimed threshold between methanogenic and thaumarchaeal dominance. This is even more worrying as insufficient knowledge on the occurrence of GDGT-0 in major methanogenic lineages in soil exists and additional archaeal lineages might produce

these lipids (see comments to P16712 L5-L7 and P16721 L1-3). I would urge the authors to be careful when discussing this ratio in the manuscript.

P16721-L24 P16721 L27: The connection between TEX86 and temperature was not made previously. Please describe the state of the art on this topic here or in the introduction (focus on soils and cultures).

P16722 L4-6: Is TEX86 really an appropriate/the best metric to use here? TEX86 is an arbitrary ratio that was established for marine environments and is a metric of GDGT-1 versus the other low-abundance GDGTs. Given that soil thaumarchaeota seem to have lipid compositions different from their marine relatives (as the authors also state themselves in the manuscript), it would be more appropriate to use a more generalized metric, such as a ring index of all GDGTs or of the low abundance compounds, e.g.: (GDGT-1+2*GDGT-2+3*GDGT-3+5*Cren regioisomer)/(GDGT-1+GDGT-2+GDGT-3+Cren regioisomer)

P16723 L19-22: This is stated as a fact here but is far from proven. There are no published experiments on the function of cyclopentyl rings in branched GDGTs or their potential biophysical properties. The hypothesis of Weijers et al. might prove correct, but lacks experimental evidence apart from the analogy to the function of cycloalkyl rings in archaea.

P16724 L16-17: Influencing GDGT-reconstructed temperatures or actual temperatures? References?

P16724 L17-19: This sentence is unclear, especially the relation to lines 16-17. Have you actually measured soil temperatures or only air temperatures?

P16725 L8-10: How are the crenarchaeol abundances of terrestrial Thaumarchaeota less constrained than in aquatic environments? Lakes and the ocean harbor a huge, mostly uncultured thaumarchaeal diversity with unknown crenarchaeol abundances.

P16725 L21-23: This conclusion is not supported by the presented data. The only

observation that you made is, that brGDGTs are more abundant than iGDGTs. Remember that these are fossil lipids and not intact polar lipids associated with living organisms. Therefore, multiple explanations exist. E.g. brGDGTs could accumulate over long time spans (or faster due to higher activity), while iGDGT producers might be less active or slower growing (or their lipids are degraded faster or recycled). As long as there is nearly nothing known about the producing organisms of brGDGTs, I would be very careful with equating the actual living organisms with their fossil biomarkers.

P16725 L24-25: Relative to branched GDGTs. This is important to state as you are reporting only relative but not absolute abundances.

P16725 L25-P16726 L1: It is important to note that no production of branched GDGTs was observed by Peterse et al. This is different from simply stating that the iGDGT production rate was higher than that of branched GDGTs.

P16726 L5-6: Something seems to be missing here. Rephrase?

P16726 L20-21: What does “ANME living archaea” mean?

P16727 L10-11: What is your basis for this assumption? References?

P16731 L10-11: This seems to be circular reasoning. Wouldn’t it be much more reasonable to assume that management type affects the composition and/or physiological response of soil bacteria and therefore lead to an altered MBT’-reconstructed temperature (i.e., a bias versus actual temperature) but not to an actual change in soil temperature?

P16731 L15-17: There is a large number of (mostly metagenomic) studies on the abundance of Thaumarchaeota in soils. Please reflect here or in the discussion if there is any evidence in the literature supporting this specific conclusion (abundances/activity in subtropics versus tropics).

P16731 L17-18: Only relative to archaea. You can’t conclude if brGDGT producers are more or less abundant between different sampling areas except if you use another

metric, e.g. relative to the total microbial/bacterial community or as lipids per gram soil etc. Rephrase.

P16731 L20: This is a bit of an overstatement. You only have one biomarker for methanogens that is in addition not very specific. Rephrase. Did you look for other more specific biomarkers such as hydroxyarchaeol or archaeols in general? These should be detectable by the employed HPLC-MS method. If these data are not available or not obtainable, I would like to urge the authors to consider archaeal and bacterial biomarkers other than the “standard” iGDGTs and brGDGTs in future studies as much can be gained from investigating these lipids.

P16731 L25: pH is also an important factor shaping thaumarchaeal communities (e.g., relative importance of group I.1b and SAGMGC-1 Thaumarchaeota). It would be worthwhile to investigate if there are any patterns in iGDGT abundances or iGDGT metrices that are correlated or dependent on soil pH that could possibly be explained by shifts in thaumarchaeal community composition. Tables and Figures

Table 1: Please explain abbreviations in caption (e.g., MAT, MAP, SOC). Please consider depositing these data as well as those in Table S1 in a repository such as Pangaea to make them easily accessible to other researchers.

Figure 2: What do the different symbols represent? Why are the numbered samples important? Are these outliers as in Fig. 4?

Figure 3: Please add more tick marks on the y-axis of panel b). Please add a reference to the statement that TEX86 <0.6 is diagnostic for methanogens. There seems to be a mistake in the caption for panel b: “lower concentrated iGDGTs as TEX86 and lower concentrated iGDGTs. . .as TEX86”?

Figure 5: Please add more tick marks to the y-axis. Why is the separation of neutral and alkaline soils not at pH 7?

Figures 10 and 11: Please add more tick marks on x-axes.

Technical corrections:

P16711 L9: Check grammar: “to be preserved”

P16714 L2: microorganisms

P16714 L3: metabolic reactions

P16716 L21: Insert “HPLC” after “Alliance 2690”

P16717 L5: “selected ion recording”, not “selective ion recording”

P16717 L8-9: Please refer to specific appendix figure instead of just pointing to the appendix here and elsewhere.

P16720 L3: biological marker

P16721 L4: aerobic oxidation

P16722 L4: Replace “tetraether index” with “TEX86”

P16722 L22: Usage of “loading” unclear. Replace with “abundance”?

P16730 L22: microorganisms

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