“Distribution of tetraether lipids in agricultural soils – differentiation between paddy and upland management” by C. Mueller-Niggemann et al.

Response to the comments by anonymous reviewer #2

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.?

*Reply: Our study does not aim to characterize soils on a global scale as indicated by the reviewer (such studies are indeed available already) but focuses on paddy soils. These represent highly specific agro-ecosystems characterized by man-made episodic flooding and associated fluctuations in microbial community composition and activity as a response to shifts in Eh and pH and other environmental variables. Paddy agroecosystem are widespread and considered to contribute substantially to greenhouse gas emissions via microbial metabolism. Studies on the GDGT content of paddy soils are extremely few with only 2 publications known to us (Bannert et al., 2011; Ayari et al., 2013,) and our study is the first addressing this issue in sufficient depth. We were confident of having expressed the importance of paddy agro-ecosystems and of our motivation and approach to study these by molecular lipid biogeochemistry in the original submission but seem to have failed convincing at least one reviewer. The final version of the manuscript will point out in even more detail the need to study paddy agro-ecosystems and highlight the role of lipid geochemistry in such investigations.*

*We do not agree with the reviewer´s position that the paper does not list any conclusion, as we listed at least eight separate conclusion regarding occurrence of GDGTs in different agro-ecosystem environments as well factors affecting their abundance and distribution in space and time.*

*With the aim to emphasize the direction of and motivation for our research more convincingly we will add the following information to the manuscript:*

*Microbial presence and activity in soil ecosystems is dependent on natural factors, in particular climate and organic substrate and on anthropogenic influences in agro-ecosystems. In the latter human activities will control microbial to variable degrees depending on type and intensity of management practices, e.g. crop type, irrigation, fertilization, soil aeration by tilling, and various other effects. Rice paddies, represent an agro-ecosystem, where human influence is most pronounced due to episodic flooding. This leads to rapidly fluctuating redox and pH-regimes and favours microbial communities able to cope with such environmental stress. To cover a range of natural ecosystem properties we analysed a variety of paddy agro-ecosystems from tropical to subtropical climate settings and soil substrates. To identify anthropogenically induced ecosystem properties, reflected in the respective microbial community structures, we also studied adjacent upland fields, showing identical natural ecosystem properties but differing management practices. Management practices exert a major control on the duration and frequency of anoxic-oxic cycles, dependent one whether 1, 2, or 3 rice growth period per annum occurred. The question whether natural or human-induced variation in ecosystem properties dominate the microbial community association was addressed in this study, based on the distribution of GDGT*

*biomarkers derived from archaea vs. bacteria and relative distribution of archaeal GDGTs, which are interpreted towards a preferential methanogenic euryarchaeal or ammonium-oxidizing thaumarchaeal life style. From the literature a wide range of biomarker proxies based on GDGT distributions is available that are used to infer soil pH and air temperature in upland soils. In this study we determined these GDGT-palaeoproxies in upland soils, comparable to previously generate data sets and compared those to (episodically) subaquatic soils, which are yet largely unexplored with respect to their GDGT distributions.*

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?

*As specified above, we have been investigating an extremely important and yet lipid-geochemically uncharacterized agroecosystem, namely episodically flooded paddy soils. Such systems have not been analysed before using GDGT distributions in order to follow the evolution of paddy soil microbial communities over (cultivation) time and in response to management and climate change. We, therefore, addressed the question listed above in our response to the general comments.*

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.

*We will rephrase the sentence to make sure that no unintended potential misinterpretation by any reader may occur. The term "characteristic" was used with reference to the term "cell membrane" followed by the information, in which organisms these cell membrane lipids may occur. Postulated origins for "orphan" branched GDGTs are addressed on page 16712 line 17ff. and further information, in particular on sn-stereochemistry (see below) will be added here as well to explain why branched GDGTs can be attributed to unknown and uncultivated bacteria rather than archaea.*

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

*Reply: We will add here or in the text on page 16712 the opposite stereochemical configuration of glycerol backbones in archaea (2,3-di-O-alkyl-sn-glycerol) vs. bacteria (1,2-di-O-alkyl-sn-glycerol) as originally described in the paper by Weijers et al. (2006).*

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.

*Reply: We address a very broad readership in Biogeosciences and assume that not all readers are experts in GDGT occurrences in the bio- or geosphere. Hence, we intended to shortly list the current knowledge on GDGT distributions in the biogeosphere. None of the other two reviewers found this too detailed and hence we prefer to keep this information at least for the terrestrial realm and leave out the marine settings.*

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.

*Reply: We will integrate the proposed references such as Stahl and de la Torre (2012) and Offre et al. (2013).*

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.

*Reply: We agree with the reviewer. It is correct that no GDGT have yet been reported from MG-II Euryarchaeota in pure cultures but some authors related their detection to MG II Euryarchaeota (Lincoln et al., 2014), which has been challenged by Schouten et al. (2014). The designation of Groups I or Group II in brackets facilitated misunderstanding and will be avoided. We apologize for using the outdated term Marine Group I Crenarchaeota instead of 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.

*Reply: As stated before, the designation of Group I or Group II in brackets has caused misunderstanding. We had no intention to indicate that MG-I do not belong to the Thaumarchaeota and will rephrase to prevent misinterpretation.*

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.

*Reply: We are somewhat surprised by the reviewer's comment. Although there is indeed little information on the GDGT distribution in soil-living methanogens, there is ample evidence for the presence of GDGT-0 in representatives of the lineage Methanosarcinales (see Schouten et al., 2013; Bauersachs et al. (2015) and references therein). We will provide more detailed information on the distribution of GDGTs in methanogenic Archaea as requested by the reviewer and will rephrase this. It is correct that the presence of methanogens in soils has not yet been investigated using cultured Euryarchaeota to determine the GDGT-0 vs. crenarchaeol ratio. However, this ratio in conjunction with stable isotope analysis has been applied successfully in soils, sediments and water column of Lake Rotsee (Naeher et al., 2014) to identify methanogenic conditions. In a study even more applicable to our investigation Ayari et al. (2013) have shown that in a rice field where samples were collected before and after flooding, the ratio of GDGT-0 vs. crenarchaeol released after base hydrolysis was around 1 during the dry stage and increased to values of 2-7 upon flooding, when methanogenic conditions had been established. We take this as evidence that the GDGT-0 vs. crenarchaeol ratio in soils can be applied to identify higher contributions from*

*methanogenic Euryarchaeota, even if the methanogens in soils or cultures have not been identified. The presence of a wide group of methanogenic archaea (in particular Rice cluster I and II) in paddy soils has been documented and the methane emission in paddy soils are of global environmental concern. Hence, we find it justified to assess the degree of methanogenesis in paddy vs. upland soils studied here by using the GDGT-0 vs. crenarchaeol ratio.*

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).

*Reply: We will delete iterative information and clarify that all GDGT synthesizing archaea produce GDGT-0.The line of argumentation goes towards the GDGT-0 vs. crenarchaeol ratio that will commonly increase in methanotrophic settings.*

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?

*Reply: We will delete some of the not needed references.*

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

*Reply: It is correct that no culture experiments using brGDGT-synthesizing bacteria have been conducted yet but the empirical evidence for bacterial adaptation of cell membranes in response to habitat conditions has been stated already by Weijers et al. (2007): “Our results, however, strongly suggest that these soil bacteria adjust their cell membrane to changes in ambient pH by changing the amount of cyclopentyl moieties in their branched GDGT membrane lipids”. Despite of this, we will rephrase to tone down this statement.*

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.

*Reply: We will add some information about environmental influences on iGDGT distribution in archaea, such as growth temperature, pH, oxygen, salinity (Wuchter et al., 2004; Ayari et al., 2013; Elling et al., 2015; Qin et al., 2015).*

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

*Reply: We will add some references to microbial response on soil moisture, pH and temperature, such as: Frostegård et al. (1993) and Aanderud et al. (2015).*

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.

*Reply: As stated above in the reply to the general comments we consider it of great importance to study the presence of microbes and their adaption to ecosystem change in rice paddies for the following reason. First, there is hardly an information on the GDGT distribution in paddy soils and their variation with environmental parameters. Second, rice paddies constitute extremely dynamic ecosystems inhabited by a complex community of microbes, which can be and have been analysed by a variety of techniques. Lipid geochemistry is only one of these techniques and as yet has not been applied in detail to rice paddies and complementary dry cultivation soils. GDGTs can be applied to follow some trends in microbial community structure and adaption to ecosystem properties. Investigations based on other lipids (e.g. FAMEs) due to the complexity of paddy ecosystems do not provide a deeper insight or clearer results. The advantage of core GDGT analysis lies in the time-integrative approach, giving a higher representativeness compared to e.g. molecular genetic analysis that gives a snapshot of the microbial community structure. Based on the results shown here, we obtain information on whether episodically flooded soils behave more like lakes or wetland or more like dry upland soil. The study of agro-ecosystems is of particular interest as we can investigate man-made environmental constraints in addition to natural ones.*

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.

*Reply: We found no evidence for GDGT-4 (e.g. by checking m/z = 1294 vs. 1292 mass traces), which agrees well with the lack of GDGT-4 in Chinese soils reported by Yang et al. (2014). To the best of our knowledge, the current literature does not provide ample evidence for substantial abundance of GDGT-4 in soil.*

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.

*Reply: We consider the results section appropriate in length and detail and prefer to present the measured data separate from the interpretation.*

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.

*Reply: The statement we made is statistically significant ($p < 0.01$) and documents that the iGDGT content in tropical soils (including Philippines, Vietnam, Indonesia (Sumatra and Java), $n = 116$) was lower than in subtropical soils (including China and Italy, $n = 51$). Therefore, we consider our conclusion as justified. In addition, the number of locations covered in this study in most cases exceeds those presented in other studies of GDGTs in Asian soils (e.g. Ayari et al., 2013; Yang et al., 2014; Wang et al., 2014; Menges et al., 2014; Ding et al., 2015; Xiao et al., 2015) and thus certainly allows a comparison of GDGT abundances between different regional settings.*

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

*Reply: This statement is based on the different iGDGT compositions of upland vs. paddy soils. To discern influences of management we chose couplets of directly adjacent fields, differing in management practise only (identical soil substrate and climate). We consider this a valid strategic approach and interpretation. Results from non-parametric Mann-Whitney U-test indicate no significant difference in pH between paddy and upland usage on the same area, except for two locations in Indonesia (Ngawi and Sukabumbi ($p > 0.05$)). Significant (p*

*< 0.05) differences of relative iGDGT distributions between paddy versus upland suggest management (flooding, oxygen availability, manuring and cropping plants) as driving factor controlling the archaeal community and preservation of tetraether lipids.*

P16719 L22-24: Also input of fossil GDGTs?

*Reply: Yes. We will add information that the paddy characteristic redox regime may also favour an improved preservation of fossil isoprenoid and branched GDGTs compared to aerated upland soil.*

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

*Reply: We will add reference to soil studies, in particular those that became available only recently (Ayari et al., 2013; Yang et al., 2015) and slightly rephrase the paragraph to place the focus on soil archaea.*

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.

*Reply: Thaumarchaeota do indeed occur in soils including groups I.1.a,b,c and I.3. For groups I.1a and 1b GDGTs have been reported. We thus agree with the reviewer and will tone down to preferentially aquatic for group 1.1a Thaumarchaeota and an overall predominance of I.1b over I.1a in terrestrial 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).

*Reply: A quote from the publications says "This indicates that relatively high abundances of the crenarchaeol regioisomer (>10 to 20%) (Table 3) maybe indicative for group I.1b thaumarchaeota. This is consistent with environmental GDGT data (Table 3) since soils, which host in addition to group I.1a thaumarchaeota group I.1b thaumarchaeota, have in general higher abundances of the crenarchaeol regioisomer relative to crenarchaeol than marine and lacustrine samples, where group I.1b thaumarchaeota are far less common than group I.1a thaumarchaeota." The authors have analysed enrichment cultures but include sediments into their discussion. Nevertheless, to avoid a misleading statement we will rephrase this sentence.*

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).

*Reply: GDGT-0 occurs ubiquitously in archaea but high relative proportions of this component to the total GDGT pool seem to be indicative for methanogens. To be safe from oversimplification we will rephrase to:*
*"Despite GDGT-0 is a common component in many archaea, an elevated ratio of GDGT-0/crenarchaeol with a >2 threshold was used previously to indicate a potentially higher contribution of methanogenic archaea derived GDGT-0 than from additionally crenarchaeol synthesizing Thaumarchaeota. This suggestion was primary made for lake sediments, where the similar threshold (GDGT-0/crenarchaeol >2) have been implicated to methanogenesis that often occur under anoxic and organic matter rich conditions (Blaga et al., 2009; Naeher et al., 2014). Paddy soils are known to release high amounts of methane during flooding period. Therefore, Ayari et al. (2013) suggested that the 3 to 6 fold increase of the GDGT-*

*0/crenarchaeol ratio, using the intact polar lipid fraction, in paddy soils after flooding should be also associated with GDGT-0 synthesising archaea of methanogenic origin. We adopted this assumption and compared different kinds of soil management concerning their iGDGT composition."*

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.

*Reply: We have rephrased our GDGT-0/crenarchaeol ratio discussion and now state that the ratio should be applied with some caution in soils due to the lack of information on GDGT distributions in cultured soil archaea. Nonetheless, we consider it as very likely that this ratio can successfully be applied in flooded soils.*

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).

*Reply: We will add some more information on the relationship between $TEX_{86}$ and temperature in soils based on current literature (e.g. Liu et al., 2013; Dirghangi et al., 2013; Coffinet et al., 2014; Yang et al., 2015).*

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)

*Reply: We noted the most significant ($p < 0.05$) differences between paddy and upland soils using the $TEX_{86}$ values instead of the ring index and thus prefer to keep the $TEX_{86}$ in our discussion. This will also allow comparison with the above mentioned studies reporting on $TEX_{86}$ in soils. As we do report fractional abundances for the GDGTs, the reader is free to calculate other proposed environmental proxy ratios beside the RI, e.g. MI, AI or TI. We prefer to provide the raw data, allowing to perform individual calculations rather than calculating the many proposed ratios itself.*

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.

*Reply: When using the term "explained" it can be interpreted as "experimentally measured" but obviously, this was not intended in our reference. We will replace the term "explained by Weijers" with "proposed by Weijers" to avoid any overinterpretation by the reader.*

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

*Reply: Actual temperatures. Any textbook on soil science will serve as a suitable reference here, as soil moisture and soil temperature are important soil properties used in all modern soil classification systems. We will refer to a review paper by Seneviratne et al., (2010) that elaborates on relationships between soil temperature and moisture.*

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

*Reply: We could not measure soil temperature and its fluctuation over the year using e.g. temperature loggers as these would have suffered from field management (ploughing and puddling). We used here mean annual air temperatures from nearby meteorological stations. We will rephrase the sentence to point out that the temperatures inferred from brGDGT patterns , i.e. $T_{MC}$ values were generally lower in paddy soils compared to the adjacent upland soils (Table 1), suggesting that $T_{MC}$ reflects mean annual soil temperature rather than air temperature. Vegetation cover and soil moisture affect soil temperature, in particular in surface soils (Liu et al., 2014; Awe et al., 2015). This led us to hypothesize that soil moisture and/or soil temperature regulates composition of brGDGTs in adjacent subaquatic and upland soils of identical air temperature as recognized by their respective $T_{MC}$.*

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.

*Reply: This statement refers to the wide use of the BIT in nearshore marine environments to identify the influx of terrestrially derived versus marine produced GDGT. When trying to apply the BIT in soils, where all GDGTs are terrestrially derived, variations in BIT governed by microbial input will be less well constrained due to our incomplete knowledge of GDGT distributions in terrestrial microbes.*

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.

*Reply: These arguments are valid, as differential GDGT preservation or changes in community structure over time may exert a control on core GDGTs in soil. If this is considered a critical limitation, all soil derived core-GDGT interpretation is invalidated as well, due to suffering from the same time-integrative phenomenon. We assume that it is not intended to devalue all previous work on core GDGTs in soils following that rationale.*

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

*Reply: Correct, we will rephrase.*

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.

*Reply: We will rephrase to: "Peterse et al. (2015) performed a 152 day experimental study, where soils were incubated under water to simulate the development of an aquatic environment under aerobic conditions. Contrastingly to our observations, decreased BIT values were measured in flooded soils, potentially due to a higher production of crenarchaeol while brGDGTs remained unchanged until the end of the experiment."*

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

*Reply: The words "control on" were missing.*

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

*Reply: We now deleted "living".*

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

*Reply: We elaborate on our hypothesis in the following paragraph. Environmental variables that are known to affect GDGT distributions can be ruled out due to lack of correlation with measured soil properties (e.g. pH) and climate factor (e.g. precipitation, air temperature) or are invariant at a given location for paddy/upland soil couplets. Consequently other environmental properties must cause the differences in paddy/upland couplets at a given location. The main ecological difference between paddy and upland soil is the water budget and thus we interpret this environmental variable to cause the offset in GDGTs. Other environmental variables could be inferred as well, e.g. nutrient supply but water flooding is the common denominator affecting all paddy soils in the same manner.*

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?

*Reply: This is an unsolvable problem when taken to the point that management type controls soil temperature/moisture and this then will cause a temperature response in microbial cell membranes, recognized in GDGT.*

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).

*Reply: We will extend our discussion on the abundances/activity of Thaumarchaeota in subtropical and tropical soils for comparison, although in our study we can only refer to relative proportions of tetraether lipids.*

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.

*Reply: We referred to the "relative proportions" that were mentioned in preceding sentence. However, we will rephrase the sentence to avoid confusion.*

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.

*Reply: We did not monitor for (hydroxyl)archaeol in our SIM-HPLC-MS protocol. We therefore rephrase to: "In subaquatic paddy soils, the lower proportion of crenarchaeol compared to other iGDGTs indicates an enhanced presence of methanogenic archaea compared to ammonia oxidizing Thaumarchaeota, which were apparently more abundant in dry upland soils."*

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

*Reply: We did not detect any relationship ($r^2$ < 0.160) of pH values to e.g. relative abundances of crenarchaeol, crenarchaeol regioisomer and the thaumarchaeota index (TI) as proposed by Xie et al. (2015). This is why we did not discuss this relation in the manuscript.*

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.

*Reply: Full explanation of abbreviations in Table 1 as well as in Table S1 will be given. As we provide the data in an open access journal, the data will be available to the public but we will consider depositing the data in a repository as well.*

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

*Reply: We will add an appropriate description of the symbols' meanings in the caption. The symbols (circle or asterisk) denote outliers that are more than 1.5 (or 3) box lengths from one hinge of the box. Yes, the numbers explain the outliers. We left the numbers in the figures, so that the reader would have the opportunity to see which samples diverge from the general trends.*

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"?

*Reply: We will add more tick marks on the logarithmic y-axis. There is no reference for the statement that $TEX_{86}$ <0.6 may be diagnostic for methanogens. Based on our data set, we propose that below this threshold methanogens dominate over Thaumarchaeota. For a more detailed explanation, which will be further extended in the final manuscript, see* P16722 L20-23*. We will delete the doubling in the caption for panel b.*

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

*Reply: We will add more tick marks on logarithmic y-axis. The location of dotted line is rather 6.2 that was based on observation in Fig. 6a, where CBT of paddy and upland soils with pH values > 6.2 showed an offset. We adopted this position but will insert a shaded zone indicating neutral soil conditions, which will cover the interval between 6.6 to 7.3 pH units.*

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

*Reply: We will add more tick marks on logarithmic 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

*Reply: We will correct these technical mistakes.*

**References**


Aanderud, Z. T., Jones, S. E., Fierer, N. and Lennon, J. T.: Resuscitation of the rare biosphere contributes to pulses of ecosystem activity, Front. Microbiol., 6, 1–11, 2015.

Awe, G. O., Reichert, J. M. and Wendroth, O. O.: Temporal variability and covariance structures of soil temperature in a sugarcane field under different management practices in southern Brazil, Soil and Tillage Research, 150, 93–106, 2015.

Ayari, A., Yang, H. and Xie, S.: Flooding impact on the distribution of microbial tetraether lipids in paddy rice soil in China, Front. Earth Sci., 7, 384–394, 2013.

Bauersachs, T., Weidenbach, K., Schmitz, R. A. and Schwark, L.: Distribution of glycerol ether lipids in halophilic, methanogenic and hyperthermophilic archaea, Org. Geochem., 83-84, 101–108, 2015.

Blaga, C. I., Reichart, G. J., Heiri, O. and Sinninghe Damsté, J. S.: Tetraether membrane lipid distributions in water-column particulate matter and sediments: A study of 47 European lakes along a north-south transect, J. Paleolimnol., 41, 523–540, 2009.

Coffinet, S., Huguet, A., Williamson, D., Fosse, C. and Derenne, S.: Potential of GDGTs as a temperature proxy along an altitudinal transect at Mount Rungwe (Tanzania), Org. Geochem., 68, 82–89, 2014.

Dirghangi, S. S., Pagani, M., Hren, M. T. and Tipple, B. J.: Distribution of glycerol dialkyl glycerol tetraethers in soils from two environmental transects in the USA, Org. Geochem., 59, 49–60, 2013.

Elling, F. J., Könneke, M., Mußmann, M., Greve, A. and Hinrichs, K.-U.: Influence of temperature, pH, and salinity on membrane lipid composition and TEX86 of marine planktonic thaumarchaeal isolates, Geochim. Cosmochim. Acta, 171, 238–255, 2015.

Frostegård, Å., Bååth, E. and Tunlio, A.: Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis, Soil Biol. Biochem., 25, 723–730, 1993.

Lincoln, S.A., Wai, B., Matthew, J.M., Church, J., Summons, R.E. and DeLong, E.F.: Planktonic Euryarchaeota are a significant source of archaeal tetraether lipids in the ocean, Proceedings of the National Academy of Sciences USA, 111, 9858–9863, 2014.

Liu, W., Wang, H., Zhang, C. L., Liu, Z. and He, Y.: Distribution of glycerol dialkyl glycerol tetraether lipids along an altitudinal transect on Mt. Xiangpi, NE Qinghai-Tibetan Plateau, China, Org. Geochem., 57, 76–83, 2013.

Liu, Y., Wang, J., Liu, D., Li, Z., Zhang, G., Tao, Y., Xie, J., Pan, J. and Chen, F.: Straw mulching reduces the harmful effects of extreme hydrological and temperature conditions in citrus orchards, PLoS ONE, 9, e87094, 2014.

Menges, J., Huguet, C., Alcañiz, J. M., Fietz, S., Sachse, D. and Rosell-Melé, A.: Influence of water availability in the distributions of branched glycerol dialkyl glycerol tetraether in soils of the Iberian Peninsula, Biogeosciences, 11, 2571–2581, 2014.

Naeher, S., Peterse, F., Smittenberg, R. H., Niemann, H., Zigah, P. K. and Schubert, C. J.: Sources of glycerol dialkyl glycerol tetraethers (GDGTs) in catchment soils, water column and sediments of Lake Rotsee (Switzerland) - Implications for the application of GDGT-based proxies for lakes, Org. Geochem., 66, 164–173, 2014.

Offre, P., Spang, A. and Schleper, C.: Archaea in Biogeochemical Cycles, Annu. Rev. Microbiol. 67, 437–457, 2013.

Peterse, F., Moy, C. M. and Eglinton, T. I.: A laboratory experiment on the behaviour of soil-derived core and intact polar GDGTs in aquatic environments, Biogeosciences, 12, 933–943, 2015.

Qin, W., Carlson, L. T., Armbrust, E. V., Devol, A. H., Moffett, J. W., Stahl, D. A. and Ingalls, A. E.: Confounding effects of oxygen and temperature on the TEX 86 signature of marine Thaumarchaeota, Proc. Natl. Acad. Sci., 112, 2015.

Schouten, S., Hopmans, E. C. and Sinninghe Damsté, J. S.: The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: A review, Org. Geochem., 54, 19–61, 2013.

Schouten, S., Villanueva, L., Hopmans, E. C., van der Meer, M. T. J. and Sinninghe Damsté, J. S.: Are Marine Group II Euryarchaeota significant contributors to tetraether lipids in the ocean?, Proc. Natl. Acad. Sci. U. S. A., 111, E4285, 2014.

Seneviratne, S. I., Corti, T., Davin, E. L., Hirschi, M., Jaeger, E. B., Lehner, I., Orlowsky, B. and Teuling, A. J.: Investigating soil moisture-climate interactions in a changing climate: A review, Earth-Science Rev., 99, 125–161, 2010.

Stahl, D. A. and de la Torre, J. R.: Physiology and diversity of ammonia-oxidizing archaea. Annu. Rev. Microbiol., 66, 83–101, 2012.

Wang, H., Liu, W. and Zhang, C. L.: Dependence of the cyclization of branched tetraethers (CBT) on soil moisture in the Chinese Loess Plateau and the adjacent areas: implications for palaeorainfall, Biogeosciences, 11, 6755–6768, 2014.

Weijers, J. W. H., Schouten, S., Spaargaren, O. C. and Sinninghe Damsté, J. S.: Occurrence and distribution of tetraether membrane lipids in soils: Implications for the use of the TEX86 proxy and the BIT index, Org. Geochem., 37, 1680–1693, 2006.

Weijers, J. W. H., Schouten, S., van den Donker, J. C., Hopmans, E. C. and Sinninghe Damsté, J. S.: Environmental controls on bacterial tetraether membrane lipid distribution in soils, Geochim. Cosmochim. Acta, 71, 703–713, 2007.

Wuchter, C., Schouten, S., Coolen, M. J. L. and Sinninghe Damsté, J. S.: Temperature-dependent variation in the distribution of tetraether membrane lipids of marine Crenarchaeota: Implications for TEX 86 paleothermometry, Paleoceanography, 19, PA4028, 2004.

Xiao, W., Xu, Y., Ding, S., Wang, Y., Zhang, X., Yang, H., Wang, G. and Hou, J.: Global calibration of a novel, branched GDGT-based soil pH proxy, Org. Geochem., 89-90, 56–60, 2015.

Xie, W., Zhang, C. and Ma, C.: Temporal variation in community structure and lipid composition of Thaumarchaeota from subtropical soil: Insight into proposing a new soil pH proxy, Org. Geochem., 83-84, 54–64, 2015.

Yang, H., Pancost, R. D., Dang, X., Zhou, X., Evershed, R. P., Xiao, G., Tang, C., Gao, L., Guo, Z. and Xie, S.: Correlations between microbial tetraether lipids and environmental variables in Chinese soils: Optimizing the paleo-reconstructions in semi-arid and arid regions, Geochim. Cosmochim. Acta, 126, 49–69, 2014.

Yang, H., Pancost, R. D., Jia, C. and Xie, S.: The Response of Archaeal Tetraether Membrane Lipids in Surface Soils to Temperature: A Potential Paleothermometer in Paleosols, Geomicrobiol. J., 0451, 1–12, 2015.