Dear Prof. Kuzyakov,

First of all, we would like to thank the reviewers for their constructive comments and helpful suggestions on our manuscript “Distribution of tetraether lipids in agricultural soils – differentiation between paddy and upland management”.

Following the reviewers request, we rephrased especially the abstract and introduction part to improve the readability and emphasize the direction of motivation for our research.

Below we respond to the comments (*blue coloured in italic*) and indicate how we have modified the manuscript (page and line numbers of the corresponding adjustments in the manuscript). All changes are highlighted in the revised manuscript.

We hope that you find the revised manuscript suitable for publication in *Biogeosciences*.

Yours sincerely,

Cornelia Müller-Niggemann

Reviewer #1: J. Tuo

This manuscript fall into the scope of BG and it contains sufficient scientifically merits and can be published in BG. The Figs and Tables were well organized and the results discussed in an appropriate and balanced way.
In this manuscript, Dr Mueller-Niggemann et al collected comparable soil samples in various locations from tropical (Indonesia, Vietnam and Philippines) and subtropical (China and Italy) sites to compare the local effects on GDGT distribution patterns and determined the influence of different soil management types on the GDGT composition in paddy (flooded) and adjacent upland (non-flooded) soils, bushland and marsh soils. The results indicated that Agricultural management was a major factor that controlled the distribution of the archaeal community in soils. Management induced variations of GDGT containing microorganism and also induced differences in the archaeal community structure. Monocyclization of brGDGT is strongly controlled by pH. Moisture is an important environmental variable affecting the distribution of brGDGT in soil. Moisture is also known to affect soil temperature, in particular in surface soils. Management type affects the MST, which in turn controls the membrane lipid composition of brGDGT producing bacteria. Warm and humid environments favour the growth of bacteria that produce brGDGT. The pH value and the soil moisture controlled the distribution of brGDGT during long-term paddy soils usage.

We Thank you again very much for your pleasant words to our manuscript and hope that the revised manuscript is also suitable for publication.

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.?

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.
We have improved the abstract and introduction section.

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.

We have rephrased the abstract: P1, line 1 - P2, line 25.

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.
P2, line 31 – P3, line 5.

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

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).
P2, line 31 – P3, line 5.

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.

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.

We will integrate the proposed references such as Stahl and de la Torre (2012) and Offre et al. (2013).
P3, lines 20-21.

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.

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.
P3, lines 23-30.

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.

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.
P3, lines 23-30.

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.

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.
P3, line 32 – P4, line 22.

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).

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.
P4, lines 26-30.

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?

We will delete some of the not needed references.
P5, lines 18-19 is now deleted.

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

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.
P6, line 22.

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.

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).
P4, line 31 – P5, line 14.

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

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).
P6, line 21.

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.

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.
P7, line 12 – P8, line 6.

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.

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.

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.

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.
P12, lines 4-7.

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

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.
P12, lines 11-13.

P16719 L22-24: Also input of fossil GDGTs?

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.
P12, lines 16-18.

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

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.
P12, lines 19-23.

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.

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.
P12, line 26 – P13, line 7.

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).

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.
P13, lines 8-10.

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).

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."
P13, line 28 – P14, line 10.

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.

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.
P13, line 28 – P14, line 10.

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).

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).
P5, lines 6-14.

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)

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.

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.
P16, lines 12-16.

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

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.
P17, lines 5-14.

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

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}.
P17, lines 5-14.

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.

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.
P18, lines 4-8.

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.

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.
P18, lines 20-22.

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

Correct, we will rephrase.
P18, lines 22-24.

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.

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."
P18, line 24 – P19, line 3.

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

The words "control on" were missing.
P19, line 6.

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

We now deleted "living".
P19, lines 19-20 is now deleted.

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

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.
P20, lines 5-10.

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?

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).

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.
P24, lines 1-3 and P12, lines 28-32.

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.

We referred to the "relative proportions" that were mentioned in preceding sentence. However, we will rephrase the sentence to avoid confusion.
P23, line 31 – P24, line 1.

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.

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."
P24, lines 5-8.

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

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.

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.
P34, line 1.

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

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.
P35, lines 12-14.

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"?

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.*
P35, lines 18-19.

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

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.
P36, lines 3-4.

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

We will add more tick marks on logarithmic x-axes.
See figures 10 and 11.

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

We will correct these technical mistakes.
Done.

Anonymous reviewer #3

The presented paper describe the occurrence of tetraether lipids in agricultural soils from various locations in the tropical and subtropical area. Despite the article is well presented, it is difficult to understand which is the motivation behind this research. The argument is novel, but it seems only to report the data about tetraether lipids without giving any exhaustive explanation about which are the implications and the importance of their occurrence in soil. Which is the relevance of the study? I think it can be relevant, but it should be made clear. The research questions as well as the findings of the study should be improved.I think the article needs major revisions so to better explain the importance of this study. The lack of data is not a research question. The discussion should indicate clearly the relevance of the findings from this study, not only indicate that now there are more data concerning this topic. Which are the implications related to a different content of GDGT in soils? Which are the implication also for other types of environments? and of managements?

With the aim to emphasize the direction of and motivation for our research more convincingly, a point raised by the 3rd reviewer in a manner comparable to the 2nd reviewer, we will add the following information:

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 favors microbial communities able to cope with such environmental stress. To cover a range of natural ecosystem properties we analyzed 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 in parallel 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 soil, comparable to previously generate data sets and compared those to (episodically) subaquatic soils, which are yet unexplored with respect to their GDGT distributions.
We have improved the abstract and introduction section.

Below are reported some specific comments:

Page 16712 line 10: What does ANME-1 means? Please give the full name and then the acronim.

ANME refers to anaerobic methanotrophic archaea. This was already indicated in the submitted manuscript. ANME-1 is one of the three clusters that have been defined based on 16S rRNA gene sequences.
P4, lines 23-24.

Page 16714 line 4: remove e.g. Alternatively rephrase the sentence to indicate some parameters, other than to soil moisture, affecting Eh.

We will add a further sentence to make clear that beside by soil moisture Eh is also affected by other parameters. As individual microorganisms are adapted to specific Eh conditions, an increase in e.g. soil moisture is accompanied by a decrease in Eh because of the consumption of oxygen by microbes (Husson, 2013).
P6, lines 29-31.

Page 16714 line 20-29: here you should state clearly which are the motivation of your research and the scientific questions you want to address. Also the global implication should be made clear. The lack of data concerning the topic is not enough for conducting a reaserch.

Although we do not agree with the statement that a lack of data (and thus knowledge) does not warrant research, but rather assume the opposite, we will add more research questions as pointed out in the comment to the general statement, presented above.
P7, line 12 – P8, line 6.

Page 16715 line 8. I am not sure if the Italian site can be considered in this study as representative of a subtropical location. Which kind of climtic subdivision it was used to identify the sites?

We disagree, because after Köppen climate classification both Italian sites are located in a humid subtropical climate zone.

Page 616715 line 24: Please be clearer. Are you referring to the dike chronosequence in CIXI for this composite sample? Or in general to all the sites? Which was on average the area covered by each field? You state that seven samples were representative for the complete field but you should specify the area covered by the fields.

This passage deals with the description of soil samples from the Cixi chronosequence that has been previously studied in detail, in particular addressing the question of sample representativeness by Mueller-Niggemann et al. (2012). We will add information on the area covered by each field to the final manuscript.
P8, lines 26-28.

Page 16716 line 13: have you analysed the three composte samples for each site? Please specify the number of samples you analysed in each area.

Only for the Cixi chronosequence, we had composite samples available. Those were measured individually. For all other sites we analysed between 1-10 samples as listed in Table 1.

Page 16718 line 18: Why percentage symbol in reporting pH values? It is not the correct way to report pH values. Remove the % symbol.

We accidentally added the % symbol to the pH values. This will be removed in the final manuscript.
P11, lines 13-15.

Page 16718 line 22: you mean comprised between 20 and 80? it is not clear

We will provide a more specific description: "The brGDGT/iGDGT ratio was >80 in Indonesian paddy soils (Jasinga), varied between 20–80 in forest and bushland soils, and was as low as 1.9 in the remaining soils (Supplement, Fig. S1)."
P11, lines 17-18. We have corrected the range of brGDGT/iGDGT ratio for forest and bushland soils.

Page 16719 line 3: report first the full name than the acronim in brackets

We will now first report the full name and then the acronym.
P11, line 24.

Figure 1: Pay attention that in Italy the blue zone is exactly covering all the Alpine area, where climate is not "subtropical".

We are thankful for the reviewer's comment and will redraw the area affected by subtropical conditions in Italy (Fig. 1).
See figure 1.

Figure 2: what does numbers in the gap means?

The meaning of numbers was designated in the figure caption: "Numbers in all plots indicate samples listed in Table S1." We left the numbers in the figures, so that the reader can easily figure out which samples do not follow the trend. In addition we will add information to the

different symbols representing outliers: “The symbols (circle or asterisk) denote outliers that are more than 1.5 (or 3) box lengths from one hinge of the box.”
P35, lines 12-14.

Distribution of tetraether lipids in agricultural soils – differentiation between paddy and upland management

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Abstract

~~Insufficient knowledge~~Rice paddies constitute almost a fifth of global cropland and provide more than half of the ~~composition and variation of isoprenoid~~world´s population with staple food. At the same time, they are a major source of methane and ~~branched glycerol dialkyl glycerol tetraethers (GDGTs)~~therewith significantly contribute to the current warming of Earth's atmosphere. Despite their apparent importance in ~~agricultural soils~~ the cycling of carbon and other elements, however, the microorganisms thriving in rice paddies are insufficiently characterized with respect to their biomolecules. Hardly any information exists~~,~~ ~~despite of the potential effect of different~~ on human-induced alteration of biomolecules from natural microbial communities in paddy soils through varying management types (affecting e.g. soil/water and redox conditions, cultivated plants~~) on GDGT distribution.~~). Here, we determined the influence of different ~~soil management~~land use types on the ~~GDGT composition in paddy (~~distribution of glycerol dialkyl glycerol tetraethers (GDGTs), which serve as molecular indicators for microbial community structures, in rice paddy (periodically

flooded) and adjacent upland (non-flooded) soils, and ~~if available also~~for further comparison forest, bushland and marsh soils. To ~~compare the~~differentiate local effects on GDGT distribution patterns, we collected ~~comparable~~ soil samples in ~~various~~ locations from tropical (Indonesia, Vietnam and Philippines) and subtropical (China and Italy) sites. We found that differences in the distribution of isoprenoid GDGTs (iGDGTs) as well as of branched GDGTs (brGDGTs) are predominantly controlled by management type and only secondarily by climatic exposition. In general, upland soil had higher crenarchaeol contents than paddy soil, which on the contrary was more enriched in GDGT-0. The GDGT-0/crenarchaeol ratio ~~was 3-27 times higher in paddy soil and indicates~~, indicating the enhanced presence of methanogenic archaea, ~~which were additionally linked~~was 3-27 times higher in paddy soils compared to other soils and increased with the number of rice cultivation cycles per year ~~(higher number of cycles was coupled with an increase in the ratio). The~~. TEX_{86} values were 1.3 times higher in upland, bushland and forest soils than in paddy soils~~.~~, potentially due to differences in soil temperature. In all soils brGDGT predominated over iGDGTs~~,~~ with the relative abundance of brGDGTs increasing from subtropical to tropical soils. Higher BIT values in paddy soils compared to upland soils together with higher BIT values in ~~soil~~soils from subtropical climates ~~indicate~~indicated effects on the amounts of brGDGT ~~through~~induced by differences in management as well as ~~climatic zones.~~climate. In acidic ~~soil~~soils CBT values correlated well with soil pH. In neutral to alkaline soils, however, no ~~apparent~~ correlation but an offset in CBT between paddy and upland managed soils was detected~~, which may suggest that~~. This is interpreted to indicate soil moisture ~~may exert~~exerting an additional control on the CBT in these soils. Lower MBT' values and ~~calculated~~ temperatures calculated therefrom (T_{MC}) in paddy soils compared to upland soils ~~may indicate~~are attributed to a management (e.g. ~~-~~enhanced soil moisture ~~through~~via flooding practises) induced effect on mean annual soil temperature (MST).

1 Introduction

~~Glycerol~~ Isoprenoid and branched glycerol dialkyl glycerol tetraethers (GDGTs) are ~~characteristic~~principal constituents of the prokaryotic cell membrane ~~lipids of archaea~~ (Pearson and Ingalls, 2013; Schouten et al., 2013 and references therein~~) and bacteria (Weijers et al., 2006a; Sinninghe Damsté et al., 2011). The~~). Differences in the GDGT core structures ~~differ in both domains,~~are crucial for distinguishing archaeal and bacterial origins

of these components with isoprenoid alkyl chains and a 2,3-di-*O*-alkyl-*sn*-glycerol stereoconfiguration being specific for archaea and branched alkyl chains ~~for bacteria (~~and a 1,2-di-*O*-alkyl-*sn*-glycerol stereoconfiguration for ~~structures see Appendix).~~bacteria (Weijers et al., 2006a). Both types of tetraether lipids have a high potential to ~~preserve~~be preserved in the sediment record (Schouten et al., 2013) and have been reported in abundance from terrestrial and marine environments, e.g. in the water column and sediments of oceans and lakes (Hopmans et al., 2000, 2004; Schouten et al., 2012; Tierney and Russel, 2009; Zink et al., 2010; Naeher et al., 2014), in ponds (Tierney et al., 2012; Loomis et al., 2014; Huguet et al., 2015), in hot springs (Pearson et al., 2004; ~~Peterse~~Reigstad et al., ~~2009a~~2008; Pitcher et al., 2009), in geothermally heated soils (Peterse et al., 2009a), in peat bogs (Sinninghe Damsté et al., 2000; Weijers et al., 2006a, 2010), in grassland soils (Weijers et al., 2007, 2010; Naeher et al., 2014), in forest soils (Hopmans et al., 2004; Weijers et al., 2007, 2010), in permafrost soils (Peterse et al., 2009b; Bischoff et al., 2014), in loess soils (Huguet et al., 2012), in Podzols (Huguet et al., 2010), in garden and agricultural soils (Leininger et al., 2006; Weijers et al., 2010; Sinninghe Damsté et al., 2012) as well as in paddy soils (Bannert et al., 2011; Ayari et al., 2013).

It is well known that archaea are involved in biogeochemically important processes, including methanogenesis, anaerobic methane oxidation (AMO) and aerobic ammonia oxidation (~~Kuypers~~Boetius et al., ~~2001; Pancost et al., 2001~~2000; Leininger et al., 2006; ~~Pearson~~Thauer et al., 2008; Stahl and ~~Ingalls,~~de la Torre, 2012; Offre et al., 2013). Distributions of isoprenoid GDGTs (iGDGTs) were initially used to characterize archaeal communities in marine environments with two major groups of archaea being distinguished: *Thaumarchaeota* (formerly recognized as mesophilic *Crenarchaeota* ~~(Group I~~) and *Euryarchaeota* (~~Group II)~~ ~~(~~see Pearson and Ingalls, 2013 and reference therein). ~~An additional archaeal phylum comprising the ammonia~~Ammonia-oxidizing ~~*Thaumarchaeota* has been identified more recently (Brochier-Armanet et al., 2008; Spang et al., 2010). Members~~members of ~~this phylum~~the *Thaumarchaeota* are currently the only known biological sources of crenarchaeol ~~and in~~, a GDGT structure that contains four cyclopentane ring systems and an additional cyclohexane ring moiety (Sinninghe Damsté et al., 2002). In addition ~~they~~, *Thaumarchaeota* contain varying amounts of ~~tetraether lipids~~GDGTs with 0 to 4 cyclopentane rings (Sinninghe Damsté et al., 2012; Schouten et al. 2013; Pearson and Ingalls, 2013). ~~Tetraether lipids of methanogenic archaea generally contain GDGT-0 (Koga et al., 1998; Koga and Morii, 2005;~~

~~Pearson and Ingalls, 2013, Schouten et al., 2013), although in some instances iGDGTs with cyclopentyl moieties have been reported (De Rosa 1986; Bauersachs et al., 2015). iGDGTs with cyclopentane rings were also reported from methanotrophic archaea of the ANME-1 cluster, *Thaumarchaeota* as well as~~

GDGT-0 is another common tetraether lipid that is present in a majority of archaea (Pearson and Ingalls, 2013; Schouten et al., 2013 and references therein, Villanueva et al., 2014), including for example mesophilic methanogens (Koga et al., 1998; Koga and Morii, 2005; Villanueva et al., 2014; Bauersachs et al., 2015). In addition, the presence of high abundances of GDGT-0 at sites with active AMO suggest a close relationship between microbial consortia involved in the production and consumption of methane (Pancost et al., 2001; Blumenberg et al., 2004; Schouten et al., 2013). In periodically flooded soils (paddy soils) methanogenic lineages, such as *Methanosarcinales*, *Methanocellales*, *Methanobacteriales* and *Methanomicrobiales* were found (Liesack et al., 2000; Watanabe et al., 2006, 2013) with varying abundances in continuously flooded as well as in alternating flooded and dried paddy fields (Watanabe et al., 2013). The distribution of methanogens in soils has not yet been extensively studied by using 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. Likewise, Ayari et al. (2013) have shown that in a rice field, where samples were collected before and after flooding, the ratio of GDGT-0/crenarchaeol increased upon flooding, when methanogenic conditions had been established.

iGDGTs with multiple cyclopentane rings have been reported from anaerobic methanotrophic archaea (ANME) of the ANME-1 cluster as well as *Thaumarchaeota* and extremophilic *Euryarchaeota* and *Crenarchaeota* (Blumenberg et al., ~~2004; Pearson and Ingalls, 2013, Schouten et al., 2013 and references therein). The cell membrane of mesophilic archaea consists, among others, of iGDGT structures usually containing 1 to 4 cyclopentyl moieties (GDGT-1 to GDGT-4) with members of the *Thaumarchaeota* also possessing crenarchaeol, a GDGT structure that contains four cyclopentane ring systems and an additional cyclohexane ring moiety (Sinninghe Damsté et al., 2002~~2004; Pearson and Ingalls, 2013, Schouten et al., 2013 and references therein). The presence of iGDGTs has been predominantly investigated in marine, limnic or other aquatic habitats, but they have also been reported from soils. Here, the specific environmental conditions controlling their distribution are less well studied

(Weijers et al., 2006b; Leininger et al., 2006; Sinninghe Damsté et al., 2012; Ayari et al., 2013). An improved knowledge of environmental factors influencing iGDGT compositions has been gained from cultivation experiments, which demonstrated that growth temperature, pH and oxygen content affect GDGT synthesis (Wuchter et al., 2004; Elling et al., 2015; Qin et al., 2015). Probably the most commonly used archaeal-based proxy in marine systems is the TEX_{86} (tetraether index of *Thaumarchaeota* derived tetraethers consisting of 86 carbons), which correlates well with surface water temperatures (Schouten et al., 2002). Culture experiments revealed the effect of increasing temperature to raise the number of cyclopentane rings (Schouten et al. 2013 and references therein). Regional studies on altitudinal mountain transects confirmed a dependency of the iGDGT cyclization on temperatures in soil systems (Liu et al., 2013; Coffinet et al., 2014; Yang et al., 2016), but additional factors as e.g. pH or soil moisture may influence the archaeal community and therefore the lipid composition found in soils as well (Wang et al., 2013; Xie et al., 2015).

High abundances of branched GDGT (brGDGTs) have previously been reported from soils worldwide (Weijers et al., 2007, 2010; Peterse et al., 2009a; Huguet et al., 2010, 2012). Information on the biological sources of these components, however, is still very limited (Hopmans et al., 2004; Weijers et al., 2007, 2010~~; Peterse et al., 2009b, 2009c; Tierney and Russell, 2009; Huguet et al., 2010, 2012; Tierney et al., 2012).~~). Molecular investigations in peat bogs demonstrated that brGDGTs occurred in highest concentrations in the catotelm, the bottom layer of peats (Weijers et al., 2006a, 2010~~), which suppose an~~). This was used to infer anaerobic and acid tolerant bacterial species as ~~origin~~brGDGT sources, e.g. microbes belonging to *Acidobacteria* the most abundant bacteria in this environment (Weijers et al., 2006a, 2009, 2010). This is supported by the presence of ~~the~~a tetra-methylated brGDGT that was recently identified in two cultured acidobacterial strains (Sinninghe Damsté et al., 2011). In addition, ~~the~~ ether-bound 5-methyl *iso*-diabolic acid was detected in four mesophilic species of the subdivision 4 of the *Acidobacteria* as a potential breakdown product of penta-methylated brGDGT (Sinninghe Damsté et al., 2014). ~~It has consequently been suggested that~~Soil bacteria producing ~~these lipids are~~brGDGTs have been proposed to be obligate anaerobes ~~and follow~~following a heterotrophic mode of life (Oppermann et al., 2010; Weijers et al., 2006a, 2010). The presence of brGDGTs in oxic soils ~~does not exclude that~~infers aerobically living bacteria to produce these lipids, but anaerobic bacteria residing in anoxic microhabitats ~~are also~~may be possible sources as well (Schouten et al., 2013). The distribution

of brGDGTs in soils is related to growth temperature (mean annual air and soil temperature) and soil pH (Schouten et al., 2002; Weijers et al., 2007, 2009; Peterse et al., 2009a, 2012). Indices which denote the degree of methylation and cyclization of brGDGTs, the MBT and the CBT indices, have previously been employed to reconstruct mean annual air temperatures (MAT) using a global soil calibration (Weijers et al., 2009). More recently, Peterse et al., (2012) defined the MBT', which represents the ratio of tetra-methylated brGDGT (GDGT-Ia, Ib and Ic) vs. the seven most abundant brGDGTs (GDGT-Ia, Ib, Ic, IIa, IIb, IIc and IIIa).

However, factors other than temperature and pH also seem to affect the distribution of brGDGTs in natural ecosystems. For example, the relative broad scatter of calculated MAT in arid soils (Peterse et al., 2012) as well as values deviating from the trend in the highest elevations of a transect sampled on Mt. Kilimanjaro (Sinninghe Damsté et al., 2008) ~~are~~have been interpreted to indicate an influence of water content and vegetation type on the brGDGT pool. In addition, several authors noted that changes in the distribution of brGDGT are strongly correlated with MAT on local scales as, for example, in altitudinal transects of Mt. Rungwe and Mt. Gongga (Peterse et al., 2009c; Coffinet et al., 2014). In agricultural soils from the same area, the type of soil management and the vegetation cover can differ, leading to variable soil water contents and soil temperatures (Liu et al., 2014; Awe et al., 2015), which affect the local microbial community. ~~In addition, soil~~Soil microbes respond to environmental ~~stresses~~stress induced by e.g. starvation, oxygen limitation or acidification~~.~~ (Frostegård et al., 1993; Aanderud et al., 2015). The latter results in the predominance of brGDGTs without cyclopentyl moieties in ~~the bacterial cell membrane~~soils and ~~explain~~explains the dependency of soil pH and CBT (Weijers et al., 2007).

~~In addition to the~~Besides pH, the redox potential (Eh) is an important factor that affects the diversity and abundance of soil ~~microorganism.~~microorganisms. The Eh expresses the activity of electrons ~~(measured in volts),~~ which influence microbial ~~metabolism~~metabolic reactions in soils. As individual microorganisms are adapted to specific Eh conditions, an increase in e.g. soil moisture is accompanied by a decrease in Eh because of the consumption of oxygen by microbes (Husson, 2013). Further parameters, which regulate the Eh are temperature, organic matter content, or soil tillage, the latter modifying the soil structure and soil aeration (Husson, 2013 and references therein). Agricultural management therefore may contribute to control redoximorphic conditions. In contrast to upland soil, i.e. without water flooding and associated crop plants, including corn/maize, wheat, barley, rape, cassava, sugar cane, cotton,

banana and other vegetables, rice paddy soil management with repetitive puddling of the surface soil as well as frequent flooding and alternating draining practices leads to a reduced Eh in the surface layer (Kögel-Knabner et al., 2010; Kölbl et al., 2014). Prevailing anoxic conditions are assumed to restrict the decomposition rate of organic matter (Lal, 2002; Sahrawat, 2005), leading to high activities of methanogenic archaea (Liesack et al., 2000) and in combination with the application of mineral fertilizer to high denitrification rates producing nitrous oxide (Xiong et al., 2007). In contrast, oxic conditions are associated with high Eh, as in upland soil and in paddy soil after draining where ammonia oxidation ~~occurs~~can occur. The latter is either performed by ammonia-oxidizing archaea (AOA) or bacteria (AOB) (Leininger et al., 2006) depending on the soil pH, with AOA being more active in acidic soils and AOB in alkaline soils (Jiang et al., 2015).

~~Here, we investigated the environmental controls that affected the tetraether lipid composition in soils of different~~Rice serves as major staple food for 50% of the world´s population and paddy rice cropland occupies an area of 157 million ha. This is equivalent to 18% of the agricultural land use area of the ten major crops worldwide and illustrates the importance of paddy agroecosystem utilization (FAO, 2003). This profound anthropogenic influence on aquatic agroecosystems will dictate their biogeochemical and geomicrobiological properties and processes, which determined from GDGT distribution warrants further investigation. Only limited information on microbial assemblages and their activity in paddy soils is currently available (Bannert et al., 2011; Ayari et al., 2013). The study of such agroecosystems is of particular interest for both, soil scientists and geochemists in similar way, as man-made environmental constraints can be compared to natural ones. To identify the anthropogenically induced ecosystem properties, reflected in microbial community structures, we studied the tetraether lipid composition in soils of different agricultural management systems, which developed in subtropical (Italy, SW-China) as well as in tropical (Indonesia, Philippines, Vietnam) climates. ~~Additionally~~Next to the management type, including differences in cropping style (upland crop plants vs. wetland rice), the intensity of the management and the duration of utilization were distinctive criteria in the investigation of effects on the microbial lipids in rice paddy soil (periodically flooded), upland~~, paddy~~ (non-flooded) and forest soils. ~~Only limited information on the distribution of tetraether lipids in paddy soils is currently available (Bannert et al., 2011; Ayari et al., 2013), although an area of 157 million ha, contributing 18% area to the ten major crops worldwide, is covered by rice~~

~~paddy management (FAO, 2003). To the best of our knowledge, this is the first~~This study~~, which~~ compares non-flooded and flooded agroecosystems of different agricultural use with respect to their GDGT composition~~. The variation in GDGT distribution patterns between soils with different agricultural usage will provide additional information~~ (including GDGT-palaeoproxies) to widen our knowledge on the sources and properties of GDGTs in terrestrial ~~ecosystems~~agroecosystems on local, regional and global scale.

2 Material and methods

2.1 Sampling

From 2008 to 2014, a total of 170 Indonesian, Vietnamese, Philippine, Chinese and Italian soils with different land-use systems were collected, including 119 paddy, 37 upland, 9 forest, 2 bushland and 3 marsh samples from the topsoil horizon (0-30 cm depth). The study sites are located in tropical as well as in subtropical climate zones (Fig. 1, Table 1) and agricultural soils were subject to different management techniques. Detailed soil characteristics and geographical positions of the sampling sites are given in Table S1 (Supplementary material). Topsoils were sampled with a soil auger as described by Klotzbücher et al. (2014).

In addition, successive land reclamation in the Chinese location Cixi via dyke construction on marine tidal flats over the last > 1000 yr (Feng and Bao, 2005) led to differently aged soils, which allow studying a 2000 yr chronosequence. Based on the time of dyke construction and information from the Edit Committee of Chorography of Cixi County (1992), differently aged marsh soils (10-35 yr) and agricultural soils under continuous non-irrigated upland use (50-700 yr) as well as wetland rice cultivation (50-2000 yr) were selected and sampled. The local cropping system on paddy fields is paddy-upland rotation, with one wetland rice season and one inter-crop (vegetables, wheat or cereals) season per year (Cheng et al., 2009). Paddy and upland topsoils were sampled with a soil auger. Three composite samples, composed of 7 sub-samples~~,~~ each (taken in an area of 1 m^2) and being representative for the ~~complete~~entire field (area of 120 m^2) were investigated at each location~~.~~ (for more details see Mueller-Niggemann et al., 2012).

2.2 Bulk geochemistry

All soils were lyophilized, sieved to a size < 2 mm and ground to a fine powder using agate pestle and mortar prior to analyses. Soil pH was measured in a suspension of the soil in 0.01 M $CaCl_2$, using a 1:2.5 (w/v) soil/liquid ratio. The pH was determined with a pH meter Model FG2-438 (Mettler-Toledo AG, Switzerland) at ambient temperature and atmospheric pressure. The total carbon (TC) and total nitrogen (TN) contents were measured on a CNS elemental analyser Vario EL III (Elementar Analysensysteme GmbH, Germany). The total inorganic carbon (TIC) content was determined using the Vario EL III elemental analyser coupled to SoliTIC module. The soil organic carbon (SOC) was calculated as the difference between TC and TIC.

2.3 GDGT preparation and HPLC-MS analysis

Core lipids of iGDGTs and brGDGTs were obtained by automated solvent extraction using an ASE 200 (Dionex, USA) at a temperature of 75°C and a pressure 5.0 x 10^6 Pa. Each sample was extracted for 20 min using a solvent mixture of dichloromethane (DCM)/MeOH (93:7, v/v). The total lipid extracts were separated over an aluminium oxide column into apolar and polar fractions using *n*-hexane/DCM (9:1, v/v) and DCM/MeOH (1:1, v/v) as respective eluents. The polar fractions were dried under a gentle stream of N_2, re-dissolved in *n*-hexane/2-propanol (99:1, v/v) and filtered through a 0.45 µm polytetrafluoroethylene (PTFE) filter prior to analysis.

All samples were analysed by high performance liquid chromatography coupled to atmospheric pressure positive ionisation mass spectrometry (HPLC/APCI-MS) using an Alliance 2690 HPLC (Waters, UK) and a Quattro LC triple quadrupole mass spectrometer (Micromass, UK) and following the analytical protocol described by Hopmans et al. (2000) and Schouten et al. (2007). Briefly, 10 µl of the filtered polar fractions were injected on an analytical Prevail Cyano column (2.1 x 150 mm, 3 µm particle size, Grace, USA), maintained at a temperature of 30 °C with a constant flow rate of 0.2 ml/min. Tetraether lipids were eluted isocratically with 99% *n*-hexane and 1% 2-propanol for 5 min, followed by a linear gradient to 1.8% 2-propanol in 36 min and subsequently to 10% 2-propanol in 5 min, after which the system was held isocratic for 5 min. The column was re-equilibrated with 99% *n*-hexane and 1% 2-propanol for 12 min before the next injection. The MS was operated as

outlined in Heyng et al. (2015) with isoprenoid and branched GDGTs being detected in ~~selective~~selected ion recording (SIR) mode of their protonated molecules $[M+H]^+$.

2.4 Calculation of GDGT indices

Acronyms in the below equations refer to ~~the relative abundance of~~ GDGTs displayed in ~~the~~ Appendix Fig. A1. The relationship between the ~~commonly less occurring~~ cyclopentane ring containing iGDGTs (GDGT-1 to GDGT-3 vs. the crenarchaeol regioisomer) was ~~considered with using~~used to calculate the TEX_{86} (tetraether index of tetraethers consisting of 86 carbons~~). The TEX_{86} was calculated according to~~) as described by Schouten et al. (2002):

$$TEX_{86} = (GDGT\text{-}2 + GDGT\text{-}3 + Cren\ regioisomer)/ (GDGT\text{-}1 + GDGT\text{-}2 + GDGT\text{-}3 + Cren\ regioisomer) \quad (1)$$

The Cyclization ratio of Branched Tetraethers (CBT) was calculated using the relative abundance of tetra- and penta-methylated brGDGT according to Weijers et al. (2007):

$$CBT = -\log ((Ib + IIb)/ (Ia + IIa)) \quad (2)$$

The Methylation index of Branched Tetraethers (MBT') index was calculated as ~~described by~~given in Peterse et al. (2012):

$$MBT' = (Ia + Ib + Ic)/ (Ia + Ib + Ic + IIa + IIb + IIc + IIIa) \quad (3)$$

The MBT' and CBT derived MAT (T_{MC}) was calculated after Peterse et al. (2012):

$$T_{MC} = 0.81 - 5.67 \times CBT + 31.0 \times MBT' \quad (4)$$

The Branched and Isoprenoid Tetraether (BIT) index was determined as given in Hopmans et al. (2004):

$$BIT = (Ia + IIa + IIIa)/ (Ia + IIa + IIIa + Cren) \quad (5)$$

2.5 Statistical analysis

Statistical analysis was conducted using the PASW Statistics 18 software. Principal component analysis (PCA) was performed on relative abundances of iGDGTs, brGDGTs and the different GDGT-based indices, to explore and characterize the variability within the GDGT distribution in differently managed soils. To identify relationships between variables, a correlation analysis was performed. Results were given as r for Pearson's correlation

regression coefficient together with the *p*-value (two-tailed test), which ~~denotes a significance if *p* is < 0.001~~is considered to be significant if *p* is < 0.001. The non-parametric Mann-Whitney *U*-test was used to investigate the significance of differences in soil properties depending on management or geographical locations. Differences are significant if *p* is < 0.05.

3 Results

SOC (Table 1) varied from 0.4 to 5.0% with highest contents present in paddy soils from the Philippine Ifugao (5.0%) and Laguna (4.0%), the Indonesian Sukabumi (4.4%) and the Vietnamese Tien Giang (4.4%) sites. The forest and bushland soils had a mean SOC of 2.7±0.9% (n = 11), which was higher than in most upland soils (1.6±0.9%, n = 37). The pH ranged from 3.7 in Tien Giang (Vietnam) to 8.2 in Cixi (China; Table 1). In general, no statistically significant differences in pH values were noticed for soils with paddy (5.3±1.0~~%~~, n = 119) or upland (5.3±1.1~~%~~, n = 37) management. Forest and bushland soils had the lowest mean pH of 4.5±0.5~~%~~ (n = 11).

Both iGDGT and brGDGT were detected in variable abundances in all soils. The brGDGT/iGDGT ratio was > 80 in Indonesian paddy soils (Jasinga), ~~>~~varied between 20-~~80~~55 in forest and bushland soils, and ~~as low as~~ between 20-1.9 in the remaining soils (Supplementary material, Fig. S1). The lowest proportion of brGDGT was noted in Italian upland soils, in very young Chinese marsh soils (< 30 yr) and upland soils. A specific feature of ~~soil~~soils from the Chinese Cixi area is ~~its~~their development ~~from~~on tidal wetland sediment. The GDGT signature of these soils was distinct from the one ~~in~~of other soils investigated in this study and represents a mixed signature of the parent substrate (tidal wetland sediments) and the recent ~~SOM (~~soil organic matter (SOM).

4 Discussion

4.1 Distribution of isoprenoid GDGTs in soils

iGDGTs constitute between 0.9 and 25.7% (and in soils of Cixi 35%) of all GDGTs (Table 1), indicating substantial contributions of archaeal lipids to most of the investigated soils. Forest

and bushland soils had lowest relative mean abundances of iGDGTs (5.8±2.6%), followed by tropical paddy (9.3±4.0%) and upland soils (9.8±6.0%). The proportion of iGDGTs was highest in Chinese and Italian upland soils (21.1±8.0%) compared to their adjacent paddy soils and all other remaining soils (13.3±5.0%). The fact that the iGDGT content was significantly ($p < 0.01$; Mann-Whitney *U*-test) lower in tropical soils (including Philippines, Vietnam, Indonesia, n = 116) compared to subtropical soils (including China and Italy, n = 51) suggests that the composition of the microbial consortia varies on regional to global scales. In addition, the differentiation between upland and paddy soils with higher amounts of iGDGTs in the former may indicate management (regulating the water regime, nutrient availability, oxygen availability and/or redox conditions) induced variations of GDGT containing microorganism. In general, at locations with the same climate and substrate, different management types best explain significantly different GDGT ~~distribution.~~distributions ($p < 0.05$; Mann-Whitney *U*-test). Regardless of whether paddy, upland or forest management, all soil types differ in their microbial lipid pattern that may be influenced by differing inputs of plant organic matter, differing fertilization practises and redox conditions. The latter is controlled by flooding and draining practises on paddy soils, which seem to favour growth and input of brGDGT containing bacteria and/or the improved preservation of fossil brGDGTs compared to the adjacently located aerated upland soils.

~~The~~iGDGT distribution ~~of iGDGTs in~~patterns described from cultured archaea (Koga et al., 1998; Pancost et al., 2001; Blumenberg et al., 2004; Koga and Morii, 2005) and their comparison with soils may provide ~~detailed~~ insights into the archaeal community structure and the biological processes that they mediate (~~Koga~~Ayari et al., ~~1998; Pancost~~2013; Yang et al., ~~2001; Blumenberg et al., 2004; Koga and Morii, 2005~~2016). The most abundant iGDGTs in our sample set are GDGT-0 and crenarchaeol. The latter is considered a highly specific biological ~~maker~~marker for ammonia-oxidizing *Thaumarchaeota* (Leininger et al., 2006; Pitcher et al., 2010; Sinninghe Damsté et al., 2012; Pearson and Ingalls, 2013~~). Molecular~~), which, in form of groups 1.1a,b,c and 1.3, have been reported to be present in soils worldwide (Pester et al., 2011; Oton et al., 2016). Differences in ammonia oxidizing archaea community composition of group 1.1b Thaumarchaeota in soils may be influenced by climatic conditions, as demonstrated in soils of various geographical origins (Pester et al., 2011). This dependency was not made for the relative abundance of crenarchaeol in soils investigated here using the Mann-Whitney *U*-test. To date, molecular investigations on cultivated *Thaumarchaeota*

revealed separation between group I.1a *Thaumarchaeota* (aquatic) and report GDGTs only for groups 1.1a and 1.1b (Pitcher et al., 2010; 2011; Sinninghe Damsté et al., 2012). Sinninghe Damsté et al., (2012) showed that group I.1b1.1a *Thaumarchaeota* (terrestrial/soil)marine and other environments) and group 1.1b *Thaumarchaeota* (soils and other environments) can be separated from each other based on the relative abundance of the crenarchaeol regioisomer. Abundances with the proportion of the crenarchaeol regioisomer < 5% arebeing indicative for group I1.1a and >10-20% for group I1.1b *Thaumarchaeota* (Sinninghe Damsté et al., 2012). The same authors demonstrated that in soils group I.1a *Thaumarchaeota* and group I.1b *Thaumarchaeota* produceobserved higher abundances of the crenarchaeol regioisomer in soils rather than in marine or lacustrine environments (Sinninghe Damsté et al., 2012). Crenarchaeol and its regioisomer are present in all analysed soil samples, which is in agreement with a previous study (Weijers et al., 2006b). The amount of crenarchaeol is generally higher in upland soils (46.4±12.9%, n = 37) compared to adjacent paddy soils (22.5±14.5%, n = 119; Fig. 2a), possibly suggesting management induced differences in the archaeal community structure. The abundance of the crenarchaeol regioisomer varies from 3 to 21% to that of crenarchaeol (mean value of 9±4%, n = 170), and shows no differences between soils and/or management types (Fig. S2).

Angel et al. (2012) observed that methanogenic archaea are ubiquitous in soils and being active only in anoxic, highly reducing environments, e.g. under flooded conditions. One distinct feature of paddy soil management vs. management of all other soils is the periodic flooding and draining of soils, which leads to highly variable redox conditions throughout the time course of a year (Kögel-Knabner et al., 2010; Kölbl et al., 2014). Paddy soils are known for high methanogenic activity and as significant sources of atmospheric CH_4 (Conrad, 2007; Thauer et al., 2008; Serano-Silva et al., 2014) without anywith little changes in the methanogenic community structure between floodingsflooding events (Krüger et al., 2005; Watanabe et al., 2006, 2009). In turn, this suggests that the overall lipid pool in paddies does not change significantly after draining the fields for harvesting.

TheDespite GDGT-0 being a common component in many archaea, an elevated ratio of GDGT-0/crenarchaeol, initially proposed for lake environments, may be with a threshold >2 has been used previously to indicate thea dominance of methanogenic archaea (Blaga et al., 2009) or of *Thaumarchaeota* in in a given sedimentary environment. The latter are members performing the first and rate-limiting step in nitrification: the aerobically oxidation of

~~ammonia (Stahl and de la Torre, 2012; Stieglmeier et al., 2014). In various studies, it~~This notion was ~~shown that a~~primarily made for lake sediments, where the threshold in GDGT-0/crenarchaeol ~~ratio > ~~>2 ~~is diagnostic for methanogens~~has been attributed to methanogenesis occurring under anoxic and organic matter rich conditions (Blaga et al., 2009; Naeher et al., 2014). ~~In~~Paddy soils are known to release high amounts of methane during flooding period (Thauer et al., 2008). Therefore, Ayari et al. (2013) suggested that the 3 to 6 fold increase in the GDGT-0/crenarchaeol ratio, determined on the ~~analysed~~intact polar lipid fraction, in paddy soils after flooding is associated with GDGT-0 synthesis by methanogenic Euryarchaeota. We adopted this presumption and compared different kinds of soil management with respect to their iGDGT composition. In the investigated soils, the GDGT-0/crenarchaeol ratio ranged from 0.1 to 121.6~~,~~ with highest ratios observed in Philippine and Vietnamese paddy soils (Fig. 2c, Table 1). In oxic upland and forest soils the mean GDGT-0/crenarchaeol ratio was ≤ 1, which indicates that methanogenic archaea are only a minor component of the microbial community at these sites. In addition, a few paddy soils (e.g. sites in Chinese Cixi and in Italy) had GDGT-0/crenarchaeol ratios comparable to those observed in upland soils, which can be explained by the management form including higher intensities of crop-rotation with upland crops under non-flooded conditions on these fields. However, if soils from the same region are compared, the ratio was generally 3-27 times higher in soils which are under paddy management compared to adjacent upland soils, indicating increased abundances and activity of methanogens in flooded soils.

~~The~~ TEX_{86} values ~~determined~~from all sites ranged from 0.3 to 0.9 (Fig. 2d, Table 1) without an apparent geographical trend. However, ~~within a region~~ TEX_{86} values were on average 1.3 times higher in upland, bushland and forest soils compared to the adjacent paddy soils~~.~~ ~~Highest values (~~ within the same region. For example, the ratios of upland~~/~~ and paddy~~-~~ soil TEX_{86} ~~= 1.5)~~values were ~~observed~~highest in the subtropical locations of Cixi and Italy ~~(~~(~1.5; Table 1). None or only minor differences in TEX_{86} values were noted in the Jasinga and Ngawi upland and paddy soils of Indonesia. Because of the relation between the TEX_{86} and temperature, one explanation for ~~the~~this difference could be that the periodic water layer on paddy soils may protect the soil surface from excessive heating and therefore results in lower mean annual soil temperatures (MST) in both soil types. Previous studies of altitudinal mountain transects support this suggestion, as the soil TEX_{86} was negatively correlated with elevation and therefore with decreasing temperatures e.g. in the Qinghai-Tibetan Plateau (r =

-0.81, r^2 = 0.65, p < 0.01; Liu et al. 2013) and Tanzania (r = -0.71, r^2 = 0.50, p < 0.0001; Coffinet et al., 2014).

In the soils investigated here, the relative proportion of GDGT-3 and the crenarchaeol regioisomer together with GDGT-1 mainly affected the ~~tetraether index.~~TEX_{86}. Low TEX_{86} values, as observed in paddy soils, are the result of high relative abundances of GDGT-1 and low proportions of GDGT-3. This suggests that paddy soil characteristics such as alternating redox conditions and higher water content control the presence of GDGT-1. High contents of cyclopentyl moieties in archaeal membrane lipids ~~were~~are known to be associated with anaerobic methanotrophic (ANME) archaea, which synthesize significant quantities of GDGT-1, GDGT-2 and GDGT-3 (Pancost et al., 2001; Blumenberg et al. 2004). Interestingly, two divergent trends in direction of increased TEX_{86} values were observed for GDGT-2 (Fig. 3a), with an increase of the GDGT-2 content to a TEX_{86} value of 0.70 and a subsequent decrease if values exceed this threshold (Fig. 3a). This change may again indicate that the archaeal community differs in dry upland/forest soils and flooded soils.

Fig. 3b shows that there is only a weak relationship between the relative abundance of GDGT-0 and TEX_{86} (logarithmic r = -0.67, r^2 = 0.45, p < 0.0001). However, both the TEX_{86} and the GDGT-0/crenarchaeol ratio show clear differences in soils under paddy (grey background in Fig. 3b) and upland management for adjacent sites suggesting that ~~they~~a comparison of both parameters may ~~be used to determine~~allow distinguishing anoxic or oxic conditions in soils. In general, paddy soils plotted within a field characterized by GDGT-0/crenarchaeol ratios > 2 and TEX_{86} values < 0.6 (Fig. 3b), possibly denoting a diagnostic area for the ~~loading~~abundance of methanogenic archaea. The GDGT-0/crenarchaeol ratio also differs between the various paddy soils, with exceptional high ratios in the Philippine Ifugao and Vietnamese Lào Cai soil (Table S1). At these sites, longer flooding periods (> 5 month per year) compared to Chinese and Indonesian soils are the likely explanation for the high ratios.

4.2 Distribution of branched GDGTs in soils

In the soils investigated here, the relative proportion of brGDGTs ~~within~~to the total GDGT pool was high and varied from 65.0 to 99.1% (Table 1). Forest soils generally contained the highest abundances of brGDGTs (> 92%), while they were significantly lower in upland and

paddy soils (Fig. 4a). Pearson's correlation analysis indicated that the SOC content was not related to the relative abundance of brGDGT ($r = 0.22$, $r^2 = 0.05$, $p < 0.01$).

In general, the tetra-methylated GDGT-Ia was the most abundant brGDGT in acidic soil and was the only brGDGT to increase in relative abundance with decreasing pH ($r = -0.75$, $r^2 = 0.56$, $p < 0.001$; Fig. 5). All other brGDGTs increased in relative abundance with pH ($p < 0.001$; Table S2), with the highest correlations observed for GDGT Ib ($r = 0.83$, $r^2 = 0.69$), GDGT IIb ($r = 0.79$, $r^2 = 0.62$) and GDGT IIIb ($r = 0.71$, $r^2 = 0.50$). Our results thus suggest that especially the monocyclization of brGDGT is strongly controlled by pH ($r = 0.86$, $r^2 = 0.74$, $p < 0.001$) with alkaline conditions favouring the synthesis of brGDGT with one cyclopentane moiety (Fig. 5). Similar observations have previously been made in a set of globally distributed upland soils (Weijers et al., 2007; Peterse et al., 2012).

Weijers et al. (2007) ~~explained~~proposed the lower number of cyclopentyl moieties in brGDGT as a protection mechanism of bacterial cell membranes within acidic soils. The decrease in the amount of cyclopentyl moieties in brGDGT is thought to be associated with a decrease in membrane permeability~~, which~~ that regulates the internal pH of bacteria under acidic conditions (Weijers et al., 2007). In soils investigated here, the CBT ratio varied between -0.04 to 2.13 (Table 1) and showed a negative correlation with increasing soil pH ($r = -0.81$, $r^2 = 0.65$, $p < 0.001$; Fig. 6a). In neutral to alkaline soils (with pH values > 6.5) CBT values stayed rather constant with an offset observed between paddy soils (mean 0.34) and upland soils (mean -0.01; Fig. 6a). Wang et al. (2014) also found no apparent correlation between pH and CBT in alkaline soils in a study of arid and subhumid Chinese soils. However, a predominant dependency of CBT with soil water content and the mean annual precipitation (MAP) was observed (Wang et al., 2014). In our study, varying degrees of soil moisture ~~could~~may be one ~~potential factor~~possible explanation for the varying CBT values in paddy and upland soil, especially under alkaline conditions (Fig. 6a).

The degree of methylation of brGDGTs (MBT') has previously been shown to correlate with MAT and pH (Weijers et al., 2007; Peterse et al., 2012). Our results demonstrate that the MBT' generally shows low values in paddy soils compared to the adjacently located upland soils, except for the Chinese soils of Cixi (Table 1). The difference in MBT' between soils from the same sampling area denotes a lower influence of MAT on the MBT' than on the pH, which was weakly related to the MBT' ($r = -0.55$, $r^2 = 0.31$, $p < 0.001$; Fig. 6b). The MBT'

was mainly controlled by the relative abundance of GDGT-Ia and GDGT-IIa, both of which were strongly related to MAP (Peterse et al., 2012). As the latter is largely similar at adjacent sites, we consider the paddy soil specific management techniques, including periodically flooding of soils, as responsible for the low GDGT-Ia and high GDGT-IIa content in paddy soils compared to upland soils (Table S1). ~~This indicates that moisture is an important environmental variable affecting the distribution of~~ The temperatures inferred from brGDGT ~~in soil. Moisture is also known to affect soil temperature, in particular in surface soils. Indeed, calculated~~patterns, i.e. T_{MC} values, were generally lower in paddy soils compared to the adjacent upland soils (Table 1), ~~indicating~~suggesting that ~~temperature denotes more the~~ T_{MC} reflects mean annual soil temperature~~.~~ rather than air temperature. Vegetation cover and soil moisture affect soil temperature, in particular in surface soils (Seneviratne et al., 2010; 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}.

~~-~~A recently developed method separates the structural isomers of brGDGTs with their methyl groups located ~~in~~at positions 5 and 6 (De Jonge et al., 2013). De Jonge et al. (2014) showed that the new CBT_{5ME}, calculated without 6-methyl brGDGTs, to correlate stronger with soil pH than the regular CBT, which includes both isomers, the 5- and 6-methyl brGDGTs. In addition, ~~they~~these authors found no correlation between pH and the newly developed MBT'_{5ME}, which is calculated without the 6-methyl isomer but a stronger correlation of ~~MBT'_{5ME}~~this index with MAT. De Jonge et al. (2014) thus demonstrated that co-elution of GDGTs can affect estimation of pH values. Conventional methods, such as ~~applied~~the one employed in this study, ~~use a Prevail cyano column upon HPLC-MS analysis, which does~~are not suited to fully separate ~~these~~the different structural isomers~~. Therefore,~~ of brGDGTs and hence it is possible that ~~our CBT based pH reconstruction revealed~~ some scatter observed between our CBT-reconstructed and measured pH may result from the analytical setup (Fig. 6a~~) due to the presence of unresolved 5- and 6-methyl brGDGTs. The~~). However, the overall ~~covariation~~good co-variation of CBT and pH~~, however, was unaffected by this~~ for our sites suggests that the partial co-elution of brGDGT had only a minor effect on the calculation of the lipid-based proxies used in this study.

4.3 Influence of management systems on GDGT ~~distribution~~distributions

The BIT index quantifies the relationship between acyclic brGDGTs and crenarchaeol and has been used previously to determine the input of terrestrially derived organic matter to marine and lake environments (Hopmans et al., 2004; Weijers et al., 2007). The interpretation of BIT values in soil is not that straight forward as ~~crenarchaeol originates from terrestrial *Thaumarchaeota* with less well constrained crenarchaeol abundances.~~all GDGTs are terrestrially derived. Thus variations in BIT values must be governed by a microbial input whose GDGT distribution is currently only incompletely known. Wang et al. (2013) observed a positive correlation between increasing soil water content and BIT values in Chinese marsh soils. In our sample set, the BIT index was slightly higher in paddy soils than in the adjacent upland soils (Fig. 4b). Furthermore, higher values were observed generally in paddy ~~soil~~soils from tropic (1.02-1.04 fold) compared to subtropic (1.07-1.11 fold) locations. In contrast to the general trend, we found highest BIT values (1.27 fold) in the subtropical paddy ~~soil~~soils of the Chinese Cixi location. In this area, the BIT values in marsh and upland ~~soil~~soils (0.61-0.89) were comparatively low, indicating that the latter have a mixed lipid composition with crenarchaeol originating predominantly from the residual parent substrate (tidal wetland sediment) and in smaller quantities also from the current microbial soil community. ~~Comparable~~Similar results were ~~observed~~made in a study of ~~the~~ plant wax lipids, which confirm the mixed ~~lipid~~organic matter composition in these soils (Mueller-Niggemann and Schwark, 2015). ~~Despite~~Except for the higher contribution of crenarchaeol to the marsh soils, our results show that brGDGT ~~producing bacteria~~ clearly dominate over iGDGTs originating from *Thaumarchaeota* in all of the investigated soil types. Interestingly, based on relations of brGDGTs to crenarchaeol ~~producing~~, *Thaumarchaeota* seem to be more abundant in upland soils compared to forest and ~~periodical~~periodically flooded paddy soils (Fig. 4b). ~~This is the opposite to results of an 152 day experimental study, with a higher production rate of crenarchaeol in soils that were incubated with different types of water (river, ocean or distilled water) to simulate the development of an aquatic environment under aerobic conditions (Peterse et al., 2015).~~ Low redox conditions as assumed for paddy soils may thus lead to an enrichment of brGDGTs either by higher production or increased preservation of brGDGTs compared to crenarchaeol in wetland soils. Our results thus contradict those of Peterse et al. (2015), who 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, lower BIT values were measured in flooded soils, potentially due to a higher contribution of crenarchaeol while brGDGTs remained unchanged until the end of the experiment.

PCA was performed to obtain information on the major factors that control the variability of the distribution of iGDGTs and brGDGTs. Results of this analysis indicate that crenarchaeol exerts a major control on the iGDGT composition in upland soils (Fig. 7a). The component loading score of GDGT-0 is opposite to crenarchaeol and has the highest negative score in PC1. In general, soils can be sorted into two groups on the basis of their scores on the first component. Paddy soils load negatively and all other soils load positively on PC1. Paddy soils that plot in the quadrant of upland soils are characterized by a higher intensity of crop-rotation with upland crops on the fields. The iGDGT composition of periodically flooded paddy soils is mainly controlled by GDGT-0 and that of non-paddy upland soils by crenarchaeol derived from *Thaumarchaeota*. In flooded rice paddy soils, oxygen availability determines the development of microbial consortia adapted to more anoxic conditions such as GDGT-0 synthesizing methanogenic archaea (Koga et al., 1998; Koga and Morii, 2005). The variance on PC2 is mainly associated with the relative abundance of GDGT-2 and separating forest and bushland soils from all other soils. The larger scatter of paddy soils on PC2 is explained by the number of rice cultivation cycles per year, which apparently influence the GDGT-2 ~~contents~~content significantly (Fig. 7b). Methanogenic archaea were found to be phylogenetically related to ANME ~~living~~ archaea (Krüger et al., 2003; Shima et al., 2012). ANME archaea are a well known source of iGDGTs (including GDGT-2) in natural environments (Pancost et al., 2001; Blumenberg et al. 2004). Both, the interaction of methanogenic and methanotrophic archaea as well as the fact that ANME are an abundant source of GDGT-2, could explain the relationship between higher numbers of rice cultivation cycles, which induce increased methanogenesis through abundant redox cycling, and the presence of GDGT-2. MAT and MAP had no obvious influence on discrimination of agricultural soil via iGDGT distribution (Fig. S3).

PCA analysis on the relative abundances of brGDGT shows an opposite relation of GDGT-Ia to all other brGDGTs, with the highest component loading score on PC1 for GDGT-Ia (Fig. 8). The cyclopentane ring containing GDGT-IIb and -IIIb plot negatively on PC1. Higher contents of GDGT-Ia in upland soils compared to adjacent paddy soils (Table S1) confirm that tetra-methylated brGDGTs may be useful in separating different agricultural soils.

GDGT-IIa has the lowest loading score on PC1 but the highest on PC2. Upland soils load separately from paddy soils along the PC2 with variation of relative abundance of the cyclic GDGT-Ib and GDGT-Ic playing the most important role. In contrast, paddy soils are mainly influenced by the abundance of GDGT-IIa and GDGT-IIIa, which both show only a low correlation with pH (Table S2). ~~We rather, assume their dependency on soil moisture.~~We rather assume their dependency on soil moisture, due to the lack of correlation between the GDGT distribution and soil properties (e.g. pH) as well as climate factors (e.g. precipitation, air temperature) in adjacently located paddy and upland soils. 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. The first PC, explaining 69.11% of the variance, indicates a separation between locations, with a strong negative score in subtropical Italian and Chinese soils and more positive scores in soils originating from the tropics (Fig. 8a). The MAP (Fig. 8b) and MAT (Fig. S4) gradients of sampling locations on PC1, confirms a relation of climatic parameters to the variation of acyclic brGDGTs.

PCA analysis on environmental parameters as well as on indices of bacterial and archaeal GDGTs indicated that separation of paddy and upland soil is mainly controlled by the intensity of methanogenesis (Fig. 9a). The GDGT-0/crenarchaeol ratio and the BIT index had the highest positive loading score on PC2. The SOC and TN loaded in the same quadrant as the BIT index, suggesting that a positive correlation between the amount of organic matter and acyclic brGDGT, especially in paddy soils, prevailed. Alternating anoxic conditions in paddy soils are known to favour the preservation and therefore the accumulation of organic matter (Lal et al., 2002), which could lead to an increase of heterotrophic and brGDGT producing bacteria. In general, the CBT loaded opposite of the soil pH on PC1, indicating their negative relation to each other. The internal separation of paddy soils via the number of rice cultivation cycles is evident by high loading scores of the CBT and MBT' (Fig. 9b). Apparently, the increase of the MBT' is linked ~~with~~to the number of rice cycles, and therefore with lowering of penta- and hexa-methylated brGDGT during increasing redox cycles. Similar loading scores as well as similar directions of climatic parameters, such as MAP and MAT, and of CBT and MBT' also indicated a linkage to each other. In addition to methanogenesis, differences in MAT and soil water content seemed to be secondary factors controlling the distribution of brGDGT in soils, which also allowed a separation between upland and paddy management. It should be considered though that MAT is not identical to

MST as the latter was also affected by e.g. the albedo and soil management, which can be different in the adjacent soils (Liu et al., 2014; Awe et al., 2015 and references therein). The reflection coefficient of the surface differs in agricultural soils as a consequence of management practises, which influence the soil bulk density (via tillage), the plant cover (function of the crop leaf area index) and the soil water content. For example, Awe et al. (2015) found differences in soil temperature as a consequence of management practises with lower temperatures in soils under chiselling and conventional tillage compared to no-tillage.

4.4 Effects of long-term management on GDGT distributions

Changes in GDGT distribution within two Cixi chronosequences with different cropping systems, one under continuous non-flooded upland and the other under paddy management, indicated specific adaption processes during the long-term usage at each site. Marsh soils were the first soils to develop after the construction of dykes on tidal wetland sediments and therefore represent the starting point of the subsequent soil development. We observed high BIT values (~0.77) already in the surface horizon of the marsh soils, indicating the rapid adaption of the microbial community to more terrestrial conditions. A plot of the brGDGT/iGDGT ratio over time provides evidence for a dominance of brGDGT over iGDGT in all soils, with values of this ratio varying between 2 and 6 in upland soils (Fig. 10a). In contrast to paddy soils, which had a fourfold increase of the ratio after 2000 yr rice cultivation, this suggests an influence of long-term processes on the proportion of archaeal and bacterial soil microorganism. These processes may include desalinization, decalcification through leaching as shown in changes of soil pH values (Fig. S5a), fertilization activities, organic matter input and accumulation (Fig. S5b). Paddy soil management is known to strongly affect the accumulation of organic matter (Wu, 2011; Mueller-Niggemann et al., 2012; Kölbl et al., 2014) as the periodically anaerobic conditions result in a slower degradation of organic matter (Lal et al., 2002). Kölbl et al. (2014) investigated the response of redox dynamics to changing water conditions over a one year time period in 100, 700 and 2000 yr old paddy soils. They noted a change of the redox potential towards anoxic conditions, already after 5 days of flooding. After stabilization, the redox potential was in the same range in all soils (-170 to -200 mV), independent of the duration of paddy management. In upland soils, permanent oxic conditions were persistent throughout the time period investigated. Results of Kölbl et al. (2014) demonstrate that the rapid establishment of anoxic

conditions and the long-term usage of paddy soils may lead to an increase of organic carbon concentrations over time.

Within the upland soil chronosequence, the TEX_{86} does not change significantly over the 700 yr cultivation time and averages 0.7 (Fig. 10b). In paddy soils, on the contrary, the TEX_{86} decreased from the initial marsh soil value of 0.7 to values of 0.3 within only 50 yr of paddy management. Rotation between paddy- and upland-type of cultivation resulted in a comparatively high TEX_{86} value of 0.5 in the 2000 yr-old paddy soils (Fig. 10b). Our results thus suggest that management systems significantly affect the microbial soil community. Long-time paddy management also led to the successive increase of ammonia-oxidizing *Thaumarchaeota* based on high relative abundances of crenarchaeol, indicating either a recovering process of water-stressed soil *Thaumarchaeota* or the enrichment of fossil crenarchaeol. The latter is potentially ~~explainable~~explained by the management type used in the Cixi area, with one wetland rice season and one dry inter-crop season per year that influence the presence of aerobic and anaerobic microbes in these paddy soils. In particular, the periodically anaerobic conditions may result in a slower degradation of organic matter (Lal et al., 2002). GDGTs may originate from a mixed source of microbial membrane lipids that were recently deposited (during the oxic as well as in the anoxic period) additionally to the previously preserved ones. Thus, higher proportions of crenarchaeol e.g. as marker for terrestrial ammonia oxidizers, being active during the oxic inter-crop period, were detected but in lower amounts as commonly observed in upland soils (Table S1). At the same time, the proportion of methanogenic archaea, which was estimated by using the GDGT-0/crenarchaeol ratio, decreased during the long-term paddy management from 5.0 in the 50 yr to 2.8 in the 2000 yr old paddy soil.

The pH values ranged between 8.0 in marsh soil and 5.5 in the 2000 yr paddy soil. The paddy management (including flooding practises) thus leads to enhanced decalcification of soils compared to the non-flooded upland management. However, most soils have an alkaline or neutral pH with exceptions of the 700 yr upland soil and the 2000 yr paddy soils, which all had pH values < 6.5 (Fig. S5a). It has previously been demonstrated that the CBT is negatively correlated with increasing pH values (Weijers et al., 2007; Peterse et al., 2012). In the ~~alkaline~~ soils of the Cixi chronosequences a negative correlation was also observed, which was higher for paddy soils (r = -0.94, r^2 = 0.88, n = 4, p < 0.001) than for upland soils (r = -0.69, r^2 = 0.47, n = 5, p < 0.001). Interestingly, an offset of CBT values between paddy and

upland soils with no apparent changes during cultivation time was noted (Fig. 10c). In addition, the CBT was higher in the younger of both marsh soils, probably because of the greater soil water content in the ~10 yr old compared to the ~35 yr old marsh soil as a result of the progressive dewatering during marsh soil pedogenesis. The observation ~~for~~regarding the CBT values supports the idea that soil moisture in addition to pH controls the degree of cyclization of brGDGTs under alkaline conditions; possibly as a reaction to water stress or oxygen deprivation on ~~microorganism.~~microorganisms. The increase of CBT values in acidic soils (Fig. 10c) also suggests that low soil pH results in the increased synthesis of brGDGTs with no cyclopentyl moieties.

Except for the youngest paddy soils (50 yr), the MBT' was slightly lower in Cixi upland soils compared to their corresponding paddy soils with identical cultivation time (Fig. 10d). This is in contrast to the observations that paddy soils in general showed a lower MBT' compared to the adjacent upland soils (Fig. 6b). This may indicate that soil bacteria living under contrasting pH regimes adapt the composition of their membrane lipids in a different fashion, even if the agricultural management is comparable.

The CBT and MBT' are both considered to be strongly related to MAT (Weijers et al., 2007; Peterse et al., 2012), which is largely similar for paddy and upland soils from the same sampling region. However, the calculated T_{MC} was different in adjacent paddy and upland soils (Table S1) and gradually increased during long-term management in both chronosequences (Fig. 11) from 14.4 °C to 17.8 °C in paddy soils and from 17.1 °C to 19.3 °C in upland soils, respectively. In general, temperatures were approximately 1.4°C higher in upland soils compared to soils under paddy management with the same cultivation time. This implies that the management type affects the MST, which in turn controls the membrane lipid composition of brGDGT producing bacteria.

5 Conclusions

Our results show that archaeal and bacterial GDGTs were ubiquitously distributed in paddy, upland, forest, bushland and marsh soils of tropical and subtropical climate regimes. Independent of ~~the~~ soil usage, ~~the~~ brGDGTs predominated over iGDGTs in all soils, but had lower relative proportions in soils located in the subtropics compared to soils ~~in~~at tropical latitudes. This implies that warm and humid environments favour ~~the growth~~an increased

occurrence of ~~bacteria that produce~~ brGDGT in the GDGT pool. The distribution patterns of iGDGTs indicate no differences in archaeal/thaumarchaeal composition in dependence on climatic exposition.

Agricultural management was a major factor that controlled the distribution of the archaeal community in soils. ~~Biomarker for methanogens were enhanced in~~ In subaquatic paddy soils, the lower proportion of crenarchaeol compared to ~~predominantly thaumarchaeal~~other iGDGTs indicates an enhanced presence of methanogenic archaea compared to ammonia ~~oxidation~~oxidizing *Thaumarchaeota*, which were more abundant in dry upland soils. In addition, the ~~number of - or a long-term~~intensity and duration of~~-~~ rice cultivation ~~cycles per year~~ significantly affected the composition of iGDGT with an increase of the GDGT-0/crenarchaeol ratio in soils with a higher number of rice cultivation cycles per year.

CBT values were correlated with soil pH and were controlled by a predominance of acyclic brGDGT in acidic soils. In alkaline soils, CBT values were rather invariant but the offset between soils under periodical flooding (paddy soils) and soils under non-flooded upland management suggests that parameters other than pH affected the distribution of brGDGTs as well (e.g. soil moisture that in addition to soil pH and MAT exerts a control on the degree of cyclization of brGDGTs). MBT' values differed in adjacent paddy and upland soils, confirming that other factors than MAT and MAP affect the degree of methylation of brGDGT on a regional scale. brGDGT-based temperatures (T_{MC}) were higher in soils under upland management than under paddy management and these differences in T_{MC} suggest that the specific management influenced the soil moisture, which in turn affects MST. The results of the Cixi chronosequence covering 2000 yr soil development confirm that the SOC, the pH value and the soil moisture controlled the distribution of brGDGT during long-term paddy ~~soils~~soil usage.

Appendix

Chemical structures of branched GDGTs (brGDGTs) and isoprenoid GDGTs (iGDGTs) investigated in this study (Fig. A1).

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Table 1. List of sampling areas, environmental characteristics [mean annual air temperature (MAT), mean annual precipitation (MAP), soil organic carbon (SOC)] and minimum as well as maximum of GDGT proportions (expressed as a percentage of total GDGTs or as indices).

Country	Sampling area	Soil type	Dataset code	N	MAT (°C)	MAP (mm)	SOC (%)		pH		iGDGTs (%)		brGDGTs (%)		GDGT-0/cren		$Tex_{86'}$		CBT		MBT'		T_{MC} (°C)
							Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	mean
Italy	Zeme	Upland	IT-NP	1	12.5	954	0.73		4.1		25.1		74.9		0.42		0.66		1.41		0.52		8,8
		Paddy	IT-P	1	12.5	954	1.15		4.9		9.6		90.4		2.67		0.44		0.90		0.51		11,6
	Vercelli	Paddy	IT-P	4	12.1	923			6.1	7.0	5.5	11.5	88.5	94.5	0.37	1.53	0.54	0.71	0.14	0.65	0.33	0.49	11,6
China	Cixi	Marsh	C-Marsh	3	16.6	1266	0.43	0.63	8.0	8.0	12.4	29.8	70.2	87.6	0.22	0.57	0.64	0.72	-0.03	0.38	0.47	0.50	14,7
		Upland	C-NP	5	16.6	1266	0.72	1.10	6.0	8.2	15.2	35.0	65.0	84.8	0.14	0.37	0.62	0.72	-0.02	0.19	0.53	0.63	18,2
		Paddy	C-P	21	16.6	1266	0.92	2.88	5.2	7.5	7.7	22.5	77.5	92.3	0.29	5.77	0.30	0.68	0.26	0.67	0.49	0.70	16,8
	Red Soil Station	Upland	C-NP	3	18.5	1731	0.70	0.85	4.1	5.1	15.5	16.0	84.0	84.5	0.32	0.48	0.76	0.78	1.56	2.13	0.72	0.77	13,3
		Paddy	C-P	5	18.5	1731	2.04	2.75	4.2	4.5	6.6	11.4	88.6	93.4	2.07	3.51	0.49	0.68	0.99	1.21	0.69	0.76	17,1
Indonesia	Jasinga	Upland	JAV-NP	3	26.9	3252	2.08	3.22	3.8	5.6	5.6	9.1	90.9	94.4	0.20	0.89	0.72	0.84	0.64	1.86	0.92	0.96	22,0
		Paddy	JAV-P	4	26.9	3252	1.97	2.30	4.2	4.4	0.9	2.0	98.0	99.1	2.01	2.26	0.61	0.68	1.60	1.83	0.91	0.92	19,3
	Ngawi	Upland	JAV-NP	3	27.0	2034	1.46	1.74	4.7	5.4	6.9	14.2	85.8	93.1	0.12	0.16	0.72	0.74	0.84	1.15	0.92	0.94	24,0
		Paddy	JAV-P	3	27.0	2034	1.40	1.81	6.4	7.2	6.8	9.5	90.5	93.2	0.58	1.20	0.68	0.71	0.34	0.65	0.72	0.80	21,8
	Padas	Paddy	JAV-P	1	26.7	2162	1.73		6.8		15.3		84.7		0.40		0.70		0.42		0.83		24,1
	Simo village	Paddy	JAV-P	3	26.9	2100	1.52	1.86	6.9	7.5	15.4	23.2	76.8	84.6	0.38	1.24	0.71	0.75	0.29	0.38	0.67	0.82	21,8
	Sukabumi	Upland	JAV-NP	3	23.5	2806	3.50	4.34	4.4	4.8	13.6	22.9	77.1	86.4	0.36	1.28	0.66	0.72	0.90	1.48	0.88	0.90	21,3
		Paddy	JAV-P	3	23.5	2806	4.02	4.41	5.1	5.3	5.5	6.1	93.9	94.5	0.38	0.45	0.68	0.71	1.16	1.24	0.77	0.80	18,4
	Sumbermujer	Paddy	JAV-P	1	17.8	2693	2.49		5.2		11.5		88.5		2.73		0.42		0.82		0.79		20,6
		Bamboo	JAV-Bamb	1	17.8	2693	3.57		5.2		3.1		96.9		1.80		0.63		1.10		0.95		23,9
	Sumatra	Paddy	SUM-P	4	21.8	2170	1.39	2.54	4.7	5.4	6.5	10.2	89.8	93.5	0.49	5.78	0.46	0.71	0.94	1.34	0.75	0.82	19,1
Philippines	Ifugao	Forest	PH-For	3	21.4	2376	2.38	3.22	4.8	5.2	1.8	3.5	96.5	98.2	0.32	1.05	0.59	0.69	0.74	0.88	0.80	0.87	22,3
		Upland	PH-NP	5	21.4	2376	1.21	2.09	4.4	5.6	2.7	7.3	92.7	97.3	0.39	2.02	0.59	0.70	0.78	1.27	0.81	0.90	22,1
		Paddy	PH-P	10	21.4	2376	1.16	5.04	4.3	5.5	3.6	17.6	82.4	96.4	3.67	121.6	0.45	0.58	0.70	1.23	0.63	0.80	18,1
	Laguna	Upland	PH-NP	5	27.1	2064	1.77	2.17	5.1	5.7	4.0	10.0	90.0	96.0	0.14	2.48	0.68	0.85	0.56	1.39	0.87	0.94	23,8
		Paddy	PH-P	10	27.1	2064	1.59	4.01	4.7	6.2	7.8	13.9	86.1	92.2	0.19	5.65	0.50	0.86	0.70	1.08	0.77	0.89	21,2
	Nueva Ecija	Upland	PH-NP	4	27.1	1821	0.54	1.30	4.6	6.5	6.7	25.7	74.3	93.3	0.17	0.92	0.74	0.83	0.51	1.33	0.85	0.91	23,0
		Paddy	PH-P	10	27.1	1821	0.83	1.95	4.3	6.2	5.7	14.4	85.6	94.3	0.15	9.66	0.48	0.81	0.52	1.65	0.73	0.86	19,2
Vietnam	Hai Duong	Upland	VN-NP	2	24.1	1608	0.79	1.17	4.9	7.4	7.7	10.4	89.6	92.3	0.40	1.66	0.59	0.76	-0.04	0.91	0.71	0.73	20,6
		Paddy	VN-P	8	24.1	1608	1.13	1.68	4.8	5.7	4.6	9.0	91.0	95.4	1.42	5.63	0.45	0.59	0.45	0.81	0.65	0.72	18,3
	Lào Cai	Bamboo	VN-Bamb	1	16.2	2223	2.97		4.2		2.3		97.7		0.95		0.66		1.26		0.89		21,2
		Bushland	VN-Bush	2	16.2	2223	2.56	3.32	4.1	4.4	4.1	4.4	95.6	95.9	1.31	3.08	0.65	0.73	1.36	1.61	0.90	0.90	20,3
		Forest	VN-For	2	16.2	2223	2.77	3.88	4.1	4.1	3.0	3.6	96.4	97.0	0.83	1.10	0.63	0.72	1.23	1.60	0.87	0.89	20,1
		Paddy	VN-P	10	16.2	2223	0.83	2.48	4.3	5.2	4.8	10.7	89.3	95.2	0.79	20.73	0.35	0.62	0.80	1.44	0.59	0.86	15,7
	Tien Giang	Paddy	VN-P	13	27.4	1450	2.06	4.43	3.7	4.8	7.6	10.9	89.1	92.4	0.72	17.39	0.54	0.61	0.99	1.14	0.79	0.85	20,4
	Vinh Phúc	Bamboo	VN-Bamb	1	23.6	1687	0.69		4.3		4.4		95.6		0.66		0.75		1.83		0.95		19,8
		Forest	VN-For	1	23.6	1687	1.30		3.8		8.1		91.9		0.55		0.79		2.00		0.86		16,1
		Upland	VN-NP	3	23.6	1687	0.58	1.64	4.0	6.1	5.0	18.8	81.2	95.0	0.57	1.30	0.75	0.77	0.88	1.77	0.87	0.93	20,7
		Paddy	VN-P	8	23.6	1687	1.12	2.41	4.3	4.8	9.1	16.1	83.9	90.9	0.88	8.19	0.50	0.70	0.88	1.60	0.75	0.85	18,4

Figure captions

Fig. 1. Map of sampling locations. Blue coloured area denotes subtropical sampling locations and green denotes tropical sampling locations.

Fig. 2. Box-plot diagrams of **(a)** crenarchaeol, **(b)** GDGT-0, **(c)** GDGT-0/crenarchaeol ratio and **(d)** TEX_{86} in upland (NP, brown), paddy (P, blue), marsh (grey), forest (For), bamboo cultivated (Bamb, red) and bushland (Bush, violet) soils. Abbreviations refer to different sampling locations: Italy (IT), China (C), Philippines (PH), Vietnam (VN), Sumatra (SUM) and Java (JAV). The vertical line separates subtropical from tropical locations. Numbers in all plots indicate samples listed in Table S1. The dotted line in **(c)** marks the GDGT-0/crenarchaeol value of 2 that is the boundary to higher proportions of methanogens, which reveal values > 2. Note the logarithmic scale for GDGT-0/crenarchaeol ratios. Note different symbols (circle or asterisk) for outliers that are more than 1.5 (or 3) box lengths from one hinge of the box.

Fig. 3. Cross-plots showing **(a)** the relative abundance (% of the sum of GDGT-1, -2, -3 and crenarchaeol regioisomer) vs. TEX_{86} and **(b)** the relationship between the most abundant iGDGTs (GDGT-0 and crenarchaeol) and lower concentrated iGDGTs ~~as TEX_{86} and lower concentrated iGDGTs~~ (GDGT-1, -2, -3, and crenarchaeol regioisomer) as TEX_{86}. GDGT-0/crenarchaeol > 2 and TEX_{86} < 0.6 are diagnostic for methanogens. Two outliers from the Ifugao site (Philippines) with GDGT-0/crenarchaeol ratio > 69 were excluded from the figure. Note the logarithmic scale for GDGT-0/crenarchaeol ratios. The filled circles in **(a)** denote paddy soils and the non-filled circles denote upland, marsh, forest, bamboo and bushland soils.

Fig. 4. Box-plot diagrams of **(a)** relative proportion of brGDGT in the total GDGT pool and **(b)** the BIT index in soil. Note different symbols (circle or asterisk) for outliers that are more than 1.5 (or 3) box lengths from one hinge of the box. Abbreviations and subdivisions as in Fig. 2.

Fig. 5. Relative abundance of brGDGT plotted versus measured soil pH. Note logarithmic scale for relative abundance. Dotted ~~line separates acidic from~~lines indicate neutral~~/alkaline~~ soil conditions, which delimitate the interval between 6.6 to 7.3 pH units.

Fig. 6. Plot of **(a)** the cyclization ratio of branched tetraethers (CBT) versus soil pH and of **(b)** the revised methylation index of branched tetraethers (MBT') versus soil pH. Dotted ~~line separates acidic from~~lines indicate neutral~~/alkaline~~ soil conditions, which delimitate the interval between 6.6 to 7.3 pH units. Regressions line of all soils is coloured in black, the line of upland, marsh, forest, bamboo and bushland soils is brown and the line for paddy soils is blue. Abbreviations as in Fig. 2. Red lines in **(a)** show the offset between paddy and upland soil, which have > 6.2 pH values.

Fig. 7. Principal component analysis (PCA) based on standardized relative abundances of six iGDGTs in 170 investigated soils. The first principal component (PC1) accounted for 53.9% of the total variance and the second (PC2) for 29.9%. **(a)** Symbols and colours denote different management forms. Abbreviations as in Fig. 2. **(b)** The sample site symbols are indicative of the number of rice cultivation cycles per year.

Fig. 8. Principal component analysis (PCA) based on standardized relative abundances of nine brGDGTs in 170 investigated soils. The first principal component (PC1) accounts for 69.1% of the variance and the second (PC2) for 14.3%. **(a)** Symbols and colours denote different management forms. Abbreviations as in Fig. 2. **(b)** The sample site symbols are indicative of the mean annual precipitation.

Fig. 9. Principal component analysis (PCA) based on commonly used indices and ratios for the 170 investigated soils. The first principal component (PC1) accounts for 33.5% of the variance and the second (PC2) for 21.4%. **(a)** Symbols and colours denote different management forms. Abbreviations as in Fig. 2. **(b)** The sample site symbols are indicative of the number of rice cultivation cycles per year.

Fig. 10. Time plots of various GDGT ratios and indices in soils of the Chinese Cixi region: **(a)** ratio of branched vs. isoprenoid GDGTs, **(b)** the TEX_{86}, **(c)** the CBT and **(d)** MBT'. Note logarithmic scale for the cultivation time. Numbers in plot **(c)** reflect soil pH values.

Fig. 11. Time plot of MBT'-CBT derived temperatures (T_{MC}) in soils of the Chinese Cixi. Note logarithmic scale for cultivation time.