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Interactive comment on "Contribution of Marine Group II *Euryarchaeota* to cyclopentyl tetraethers in the Pearl River estuary and coastal South China Sea: impact on the TEX_{86} paleothermometer" *by* J. X. Wang et al.

Anonymous Referee #1

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In the manuscript by Wang et al., the authors claim that they have characterized the archaeal tetraether membrane lipids (GDGTs) produced by the marine euryarchaeota group II (MGII) and assess the effect of their synthesized lipids on the GDGT-based paleotemperature proxy TEX86. For that purposes, they analyzed core lipids (CL) and intact polar lipids (IPL) GDGTs, they performed 454 pyrosequencing analyses to address the archaeal diversity and quantified the abundance of the MGII by quantitative PCR (qPCR) in suspended particulate matter (SPM) and surface sediments collected along the salinity gradient of the lower Pearl river and its estuary to the coastal South

China Sea.

I have great concerns about this manuscript. First, the authors do not characterize the GDGTs of the MGII but rather analyze the GDGT content of samples in which MGII is present (but far from being dominant). I do not say that MGII could not produce GDGTs, which is possible (maybe the authors are right that they synthesize GDGT-1, 2 and 3) but this study does not prove that at all (this could only be proven by cultures). I do have to recognize though that the authors based their assumptions in the change in the relative abundance of phospho IPLs (more labile) observed in the Pearl River Estuary (mixed water) site respect to the seawater location, which is the only way to connect lipid biomarkers to their sources avoiding potential preservation issues of CL GDGTs and IPL-GDGTs with glycolipid headgroups. Unfortunately, the samples chosen to make these assumptions are far from being the most ideal as in the best of the cases the MGII was only 30% of the total archaeal population. Even if the samples analyzed would contain 90% of MGII according to genetic analyses it wouldn't be possible to make such an assumption as we also have to take into account other factors such as differences in the extractions efficiencies in the pool of lipid and DNA extracted. This manuscript is also not novel if we compared it with the manuscript recently published by the same authors (Wang et al., Chemical Geology 2015), in which they already suggested that a change in the archaeal community composition (contribution by MGII) could be responsible for the differences in GDGT distribution and thus of the unusually low TEX86 values in the same system described by the current manuscript. Therefore, the only novelties in the present manuscript are the correlations between the different phosphor IPLs GDGTs and the ratio of MGII vs total archaea. However, these are mere correlations. For all we know the Thaumarchaeota Marine Group I (which makes more than 30% of the total archaeal population in the river estuary (mixing water station) could also be responsible for the change in the GDGT distribution detected in this sample (MGII are barely 17% of the total archaea in this sample as seen in Figure 2). It is even possible that the Thaumarchaeota population in the river estuary are phylogenetically different from the seawater station (which actually would make sense

as the ecosystems are quite different) and synthesize GDGTs in different proportions accounting for the differences seen here. In any case, we can't conclude neither of these hypotheses with the experimental data provided in this manuscript. The tone of the title and abstract makes you assume that the contribution of MGII to the GDGT pool has been demonstrated in this study, which is far from being the case and is very misleading. Besides, I have other concerns regarding the design of the experiment (especially regarding the molecular data) that I will list below, which discourage the publication of the manuscript in its current form.

Specific comments: Abstract - Line 8: as mentioned above, in this manuscript you don't characterize the GDGTs produced by MGII - Line 10: would be better to talk about 16S rRNA gene pyrosequencing rather than 454 (which is merely the machine) - Line 15: "MGII euryarchaeota as the second dominant group": fine, MGII are the second dominant group (*17% of the total) but Thaumarchaeota make up more than 30% of the reads (as seen in Figure 2 (PR estuary mixed water). This sentence (and the whole study) is biased towards what the authors want to demonstrate but the rest of the archaeal community (which we know they make GDGTs) are excluded from the conclusions!. - Line 16: "qPCR data indicated that the abundance of MGII euryarchaeota in the mixing water was three to four orders of magnitude higher than in the river water and in the seawater": Yes, this is correct, but still taking the data of the qPCR analysis in Table 1, MGII range from 0.2-30% of the total archaeal population in the mixing water. Why is not the impact of other "more dominant" archaeal populations in this sample being discussed here or in the rest of the manuscript? - Line 18-line 22: For the reasons mentioned above I strongly disagree to this statement: the existence of correlation does not does not suggest that MGII produce GDGT in the water column... Introduction - Lines 20-21: "However the lack of direct link between archaeal lipids and DNA prevented the drawing of a more concrete conclusion"....which the current manuscript has not been able to address neither Material and Methods - Line 25 (page 5): The range of liters filtered is quite broad. It is essential that the authors report the total GDGT and DNA content that was extracted from these filters. Otherwise it's

impossible to asses if enough material was extracted and analyzed - Line 26 (page 5): The filters used were GF/F 0.7 um. This is always an issue for this kind of studies as we don't know if the archaeal population is biased by the diameter of the filter pore. MGII have been seen to be prevalent in particles (Galand et al., 2010) and genome analyses suggest that they have a particle-attached lifestyle (Iversson et al., 2012). Considering this, the 0.7um could potentially select for MGII rather than Thaumarchaeota and completely invalidating the results. The authors cannot assess this point with the data presented here but at least they should account for this possibility. - Lines 8-9 (page 8): I am puzzled with the idea that the authors extracting the DNA contained in the filters by washing frozen filters 3x with PBS filter and centrifuge the supernatants to continue with the DNA extraction. This is insufficient. We regularly extract DNA from glass fiber filters and the DNA is way more attached to the filter than in the case of polycarbonate filters therefore a bed beating step in lysis buffer is essential to get the DNA from the cells (needless to say that this is extremely important for a proper extraction of DNA from archaeal cells). I just can't imagine that you can get representative DNA extracts by washing frozen filters. Besides, the range of extracted DNA is not provided anywhere (not even in the supplementary material), I would be curious to see how much you managed you extract. - Line 18 (page 8): Where the qPCR conditions tested by the authors or previously tested? If these primers have not been tested before the authors should demonstrate with supplementary data how specific these qPCR reactions are (especially the one for the MGII). Besides, no efficiency nor R2 values of the qPCR assays are provided. - Line 21 (page 8): 454 sequencing (as mentioned above would be better to say 16S rRNA gene pyrosequencing), was only done in n=3 SPM samples, no replicates. Dangerous to make such assumptions based in such limited dataset. Also the authors don't report the number of sequences that were recovered from each sample. These should be comparable to make proper comparisons between the samples as seen in Figure 2. - Line 5-8 (page 9): The taxonomy assignation of archaeal 16S rRNA gene reads can be problematic depending on the classifier used. It is recommended that the authors provide further prove of the identity of the archaeal

sequences (such a phylogenetic tree of representative sequences). Results and Discussion - Lines 1 (page 14): "...were produced in situ in the PR estuary by the source microorganisms": Which microorganisms? According to Figure 2 only 17% of the sequences are affiliated to MGII and more than 30% to Thaumarchaeota so the GDGT in situ production could also well be attributed to MGI, right? - Lines 12-24 (page 15): The increased ratio of GDGT-2/3 ratio in deep water column responsible to the warm bias of TEX86-derived temperature has been recently suggested to be related to differences in the GDGT produced by deep water Thaumarchaeota MGI (Villanueva et al., Environmental Microbiology in press doi: 10.1111/1462-2920.12508). As this paragraph is phrased it seems that the authors suggest that the GDGT-2/3 ratio variation in deep waters could be attributed to MGII as suggested for the authors in this study. Rewrite to make this part clearer.

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