

Anonymous Referee #2

In this study, Wang et al. measured archaeal tetraether lipid concentrations in suspended particulate matter and surface sediments along a salinity gradient at three sites (river water, mixing water, and seawater) from the Pearl River out into the South China Sea and compared these data primarily to Marine Group II Euryarchaeota 16S rRNA gene abundances as determined by qPCR. Lipid data included both core lipids and intact polar lipids and was used to calculate TEX₈₆ and Ring Index values for comparison to sea surface temperature. Additionally, the archaeal community composition was determined through 16S rRNA gene sequencing.

Major comments:

My primary concern with the manuscript is the strong language that is used to suggest that MG II are the source of the measured lipids. The title is already misleading by stating “contribution of MG II” to lipids, and the abstract claims the authors “characterize MG II Euryarchaeota-produced GDGTs” when they have done nothing of the sort. This work merely shows correlations between tetraether lipid concentrations and MG II 16S rRNA gene abundances. Thus the statements and conclusions found throughout the paper that claim MG II produce GDGTs are truly overstated as there is no direct evidence of this here.

We thank the Referee for these comments, and agree that the correlation between archaeal lipid distribution (%) and the relative abundance of MG II 16S rDNA genes (ratio of MG-II 16S to total archaea 16S) does not prove that the MG II are significantly contributing to the tetraether pool. Only the pure cultures of MG II will pin down their lipid profile and enable us to quantitatively evaluate their contributions to the GDGTs in the environment. However, to date, no pure cultures or even enrichments are available for MG-II. We therefore believe that our environmental study is helpful to assess the potential role of MG II in the environment, given that the GDGTs have been extensively applied to reconstruct paleo-environments.

The title, abstract and conclusion have been improved to better reflect the revised content of this paper based on the reviewer’s comments. In particular, the title is now “Evaluating Marine Group-II *Euryarchaeota*’s contribution to cyclopentyl tetraethers in the Pearl River estuary and coastal South China Sea: Potential impact on the TEX₈₆ paleothermometer”. The sentence “we characterize MG II *Euryarchaeota*-produced GDGTs...” was changed to “we assessed the relationship between MG-II *Euryarchaeota* and GDGTs...”.

That said, there is a positive relationship between the MG II 16S rRNA gene abundances as determined by qPCR and GDGTs 1-4. And if this correlation is actually driven by MG II producing GDGTs, then the authors nicely explain the potential implications for TEX₈₆. However, the qPCR results are not in agreement with the sequencing data in Figure 2 which shows relatively more MG II at the seawater site compared to mixing water, which calls into question the accuracy of either method in determining the number of MG II archaea in the total archaeal community. The discrepancies in the results between these two methods must be addressed in the discussion. If one was using Figure 2 in combination with the lipid data, they could conclude that the increase in MG I could be contributing the changes in GDGTs from mixing water to seawater. Additionally, the results of both methods show that MG II are at most on the order of 30

We do recognize the difference of quantification between qPCR and pyrosequencing. As far as we know, almost all of the environmental survey have showed inconsistency of quantification between the two methods, especially in those samples having similar taxonomic compositions. Considering result from qPCR is more straightforward than pyrosequencing to reflect the abundance of archaeal 16S genes, it is reasonable to apply qPCR data making a comparison with the lipid data in this study. On the other hand, the pyrosequencing results were merely from three samples; whereas, qPCR results were based on 12 samples, which is more convincing to reflect the variation of archaeal 16S gene abundance

The original purpose of utilizing pyrosequencing data was to indicate the change of archaeal community composition. In order to make it more clear, the Figure 2 was changed to a phylogenetic tree coupled with the distribution of OTUs, which can not only indicate the change of archaeal community composition, but also display the diverse distribution of OTUs along the salinity gradient.

To better quantify the abundance of MG-I (AOA) and total DNA, makeup experiments based on the same filter samples were performed. The qPCR data were added into the Table 1 and Figure 5. The results exhibited that MG II was statistically higher (duplicate experiments) than MG I in the mixing water and seawater, which suggests that MG II predominantly occurred in the water column of sampling stations at the Pearl River Estuary and coastal South China Sea. On the other hand, linear regression analysis showed that there is no correlation between the ratio of MG I/total Archaea (%MG I) and the fractional abundance of GDGTs (%GDGTs) (data not shown); however, a significant correlation existed between the ratio of MG II/total Archaea (%MG II) and %ringed-GDGTs (Fig. 6), which suggests that MG II may be a significant source of GDGT-1, -2 and -3 in the PR estuary and coastal SCS.

In the methods section, it appears that the suspended particulate matter samples were collected on glass fiber filters with nominal pore size of 0.7- μ m. From Thaumarchaeota cultures, we know that at least some of these MG I cells are smaller than this (occasionally they pass through 0.2- μ m), while some evidence exists that the MG II are particle-attached. Thus this sample collection technique may be biased in favor of the MG II and not giving a full picture of the archaeal community. Additionally, the methods indicate that no lysis step was performed on these filters for DNA extraction but just a simple washing with buffer. While little is known about the MG II archaea, this again could be biasing the results.

This is an important criticism. 0.2 μ m filters were collected during the cruise. Unfortunately, the whole 0.2- μ m filters were extracted for lipids to ensure enough lipids were collected for analysis and no extra samples left for DNA quantification. Nonetheless, we compared the GDGTs yields from 0.2 μ m and 0.7 μ m filters collected at the same time, and found that 0.7 μ m filter collected significant more GDGTs with phosphate head groups (90% ~ 95% of the total phospho IPL-GDGTs), which is thought to be the mostly closely tied to the living organisms among all IPLs. This result indicates that the 0.7 μ m filter might collect significant abundance of active archaeal biomass. Therefore, based on the lipid results, we conclude that the 0.7 μ m filter can be applied to evaluate the relationship between lipids and DNA.

Despite the co-existence of MG-I and MG II in the water column of the studying area, the linear relationship between %MG II and %phospho IPL-GDGTs is able to at least suggest that MG II

(rather than MG I) have the potential to produce ringed-GDGTs *in situ* in the water column of the study area.

There was no lysis step on the filters for DNA extraction. We recognize the differences in the extraction efficiencies for DNA. Although the absolute quantification might be affected by the extraction method, the ratio of target gene to the total, such as the ratio of MG II/Archaea, could avoid systematic error and reflect the relative distribution of MG II.

There are many missing articles (i.e., “the” or “a”) throughout the manuscript but primarily in the introduction, to the point that it is distracting for the reader. The entire manuscript needs to be checked and corrected thoroughly for these errors. For example, “the” should be added before “marine sediment record” in line 8 of the abstract and again in line 25.

Thank you. All the missing articles have been added throughout the text.

Minor comments:

- Wuchter et al., 2006 is listed in the references but not cited in the text

Wuchter et al., 2006 has been deleted from the references. Thanks.

- Tierney and Tingley, 2014 is cited on pg 12457, line 8 but missing from the reference list

The reference has been added into the reference list.

- Section 2.3.2 in the methods is written in present tense while the rest is past tense, please correct for consistency

Done.

- Figure 4 caption notes colors (blue and black) but everything appears black in my copy of the figure

Figure 4 with only black and white is right. The caption notes of Figure 4 were changed.

- Figure 5 caption should include “MG II” for the purple line description (“the abundance of MG II 16S rRNA genes”)

Done. Since qPCR data of MG-I thaumarchaeota *amoA* gene were added into the Figure 5, the caption notes have been re-written.

- Table 1 caption should include “abundances” after 16S rRNA gene and matter should have no s (“... 16S rRNA gene abundances for suspended particulate matter. . .”)

Changes were made. Thank you.

- pg 12465, line 17: “. . . has been suggested to attribute to . . .” does not make sense, please correct

This sentence was changed to “wang et al (2015) suggested that the variation in TEX₈₆ was due to the change in archaeal community composition in the water column”.

- pg 12468, line 16 and pg 12469, line 9: Euryarchaeota appears twice in a row (and is misspelled the second time)

Chang was made.

- pg 12468, line 9: “since no more samples to quantify. . .” does not make sense, please correct C8265

This sentence was changed to “Unfortunately, samples from this study did not allow us to estimate the contribution of MG-I Thaumarchaeota to the ringed GDGT pool”.

- pg 12468, line 18: “In respect to. . .” should be “With respect to. . .”

Chang was made. Thank you.