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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.

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Reply to general comments: We acknowledge that this reviewer's comments are important and thoughtful. We have done makeup experiments to quantify the abundances of MG I (AOA) and total DNA from the same filter samples (Table 1 and Fig. 5). The qPCR date 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. These results yet seem to be different from the 454 sequencing data (Fig.

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2), which showed that MG I were dominated in both mixing water (estuary) station and seawater (coastal SCS) station. This might be due to the different amplification efficiencies for the two groups (97% for MG II and 87% for MG I). Since result from qPCR is more straightforward than 454 sequencing to reflect the abundance of archaeal 16S genes, it is reasonable to indicate that the samples chosen to make these comparisons are appropriate. On the other hand, linear regression analysis showed that there is no correlation between the ration of MG I/total Archaea (%MG I) and the fractional abundance of GDGTs (%GDGTs) (data no 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.

We do recognize the differences in the extraction efficiencies for lipids and DNA. Although the absolute quantification might be affected by the extraction method, the ratio of individual parameter to the total, such as the ratio of MG II/Archaea and fractional abundance of GDGTs, could avoid systematic error and reflect the relative distribution of MG II and GDGTs.

This study is indeed a follow-up work after Wang et al. (2015, Chemical Geology), but represents an important increment toward a better understanding of sources of GDGTs in marine environment. The two papers also have different focuses. In Wang et al. (2015), the main propose was to evaluate the factor(s) causing the unusually low TEX86 in the coastal area; MG II was tentatively hypothesized to be a factor since this group of archaea did exist in the research region. But we didn't provide any evidence to support the hypothesis. Therefore, emphasis of this study was trying to explore MG II-produced GDGTs and to further evaluate how these GDGTs influence the TEX86. So it is an important and valuable step forward from the Wang et al. (2015) paper. Since no MG II culture exists to show the profile of its membrane lipid, a comparison between lipid and DNA would be the best way to evaluate the relationship between MG II and GDGTs. In order to have a convincing comparison, we chose lipid data

with only phosphate-head groups as they can represent living biomass. 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.

The title and abstract have been improved to better reflect the revised content of this paper based on the reviewer's comment.

Reply to specific comments:

Abstract

-Line 8: as mentioned above, in this manuscript you don't characterize the GDGTs produced by MGII.

It was changed to " we assessed the relationship between MG II Euryarchaeota and GDGTs. . .".

-Line 10: would be better to talk about 16S rRNA gene pyrosequencing rather than 454 (which is merely the machine).

Changed. Thank you.

-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!

Thank you for the comment. Based on the qPCR results, the abundance of MG II was significantly higher than MG I, so this sentence is changed to "with MG-II Euryarchaeota being one of the dominant groups of archaea in the mixing water and

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seawater stations".

-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?

The identified groups of Archaea with notable proportions are MG I, MG II, Methanogens, MBGB, MCG and YLA114. According to the above analysis, MG I was not the significant GDGT-producers in the studying area. Methanogens produce GDGTs without rings. MBGB, MCG and YLA 114 kept a significantly smaller abundance than MG I/II in this area. We cannot exclude the possible contribution of GDGTs from other groups except for MG II; however, the linear relationship between phospho IPL-GDGTs and MG II DNA data is able to strongly indicate a significant contribution of ringed GDGTs from MG II.

-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. . .

The original sentence has been changed to "Significant linear correlations were observed between the gene abundance ratio of MG-II Euryarchaeota vs. total archaea and the fractional abundance (%) of GDGTs-1, -2, -3, or -4 as well as the ring index based on these compounds, whereas no relationship was observed between the ratio of MG-I/total archaea and %GDGTs, which collectively suggest that MG-II Euryarchaeota may actively produce GDGTs in the water column."

Introduction

-Lines 20-21: "However the lack of direct link between archaeal lipids and DNA pre-

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vented the drawing of a more concrete conclusion". . ..which the current manuscript has not been able to address neither Material and Methods.

The materials (filters with dominant group of MG II) and methods (total phospho IPL-GDGTs) have the potential to address this issue. Please see the general response above for more details.

-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.

The total GDGTs and DNA content are listed in Table 1. The results suggest that the materials (lipid and DNA) extracted from the samples are enough.

-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.

Thank you for the comments. We do have a comparison experiment showing the different yield of GDGTs from 0.2 um and 0.7 um filters. The results showed that phospho IPL-GDGTs are predominantly collected from the 0.7 um filters. Therefore, even though 0.7 um filters could not collect enough MG I, the regression analysis between lipid and DNA indicated that the source organisms of phospho IPL-GDGTs were from the 0.7 um filters.

-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

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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.

Thanks for your comments. The protocol of FastDNA SPIN Kit has a bead beating step in lysis buffer. We agree with the comments that the DNA extractions for the filters are not sufficient. That's the reason why we didn't make the comparison based on the absolute abundance of DNA and lipids. However, since the DNA extraction efficiencies for different groups of Archaea are hypothetically identical, the ratio of MGII/Archaea could reflect the relative variation of MGII along the Pearl River and its estuary.

The total abundance of DNA is listed in Table 1.

-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.

The primers targeting on archaeal 16S (Bano et al., 2004), MGII 16S (Massana et al., 1997; Teira et al., 2004) and archaeal amoA gene (Francis et al., 2005) are proven to be specific in published research.

We add the following paragraph into the manuscript section 2.3.1 –

PCR targeting the different genes were conducted before the qPCR. The PCR bands were recovered by Gel Extraction Kit (omega) and sequenced on the 3730 sequencing platform. The sequences were annotated as the corresponding target genes, which

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demonstrated the specificity of those qPCR primers. The melting curve analysis was performed to demonstrate that the fluorescent signal obtained in a given reaction was consistent with the expected profile for specific PCR products on the basis of comparison with standards. The R2 values for standard curve were above 0.99. The efficiency of each qPCR was between 87% and 99%.

-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.

The 454 sequencing data was to show the distribution of the archaeal community composition. It is unfortunate that we did not have replicates in this study. However, the result showed in this study (Fig. 2) was similar to the distribution of archaeal community composition in Wang et al. (2015). The two data sets were collected in the same study area, but different times (samples in this study were collected in 2011; samples in Wang et al., 2015 were from 2010). However, the qPCR data were based on samples from three water columns, i.e. fresh water samples (n=2), mixing water samples (n = 6) and seawater samples (n = 4), and sediments (n =3). The major observation in this study is based upon the regression analysis between qPCR data and lipid data. Therefore, it is reasonable to make the conclusion based on our sample set.

As for the number of sequences, a total of 9,343 effective sequences with an average length of 531 bp were generated. Sequence numbers are 2,751, 2,987 and 3,695 for fresh water, mixing water and seawater samples, respectively (these info was added into the 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

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provide further prove of the identity of the archaeal sequences (such a phylogenetic tree of representative sequences). Results and Discussion.

Thanks for your suggestion. The phylogenetic tree based on the dominated OTUs is showed in Figure S3. Although the taxonomic classify showed they had similar archaeal composition, we further found that the dominated OTUs in the freshwater, mixing zone and seawater were significantly different based on the phylogenetic tree. Comparing with the MGI (only one dominated OTU), the MGII are more diverse in both mixing zone (5 dominated OTUs) and sea water (4 dominated OTUs). This suggested the archaeal composition might account for the variation of GDGTs in different environments.

-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?

We deleted the term "by the source microorganisms". According to Table 1, the abundance of MG II is significantly higher than MG I in the mixing water and seawater station. On the other hand, compared with the linear relationship between %GDGTs and the ratio of MGII/Archaea, there is no relationship exhibited between %GDGTs and the ratio of MGI/Archaea. These results suggest that 1) MG I may not be a significant source of the in situ produced lipids in the studying area, 2) the source organism is more likely to be MG II.

-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.

The change was made.

We greatly appreciate the valuable and insightful comments made by this reviewer.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C7687/2015/bgd-12-C7687-2015-supplement.pdf

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Interactive comment on Biogeosciences Discuss., 12, 12455, 2015.

Fig. 5 (update)

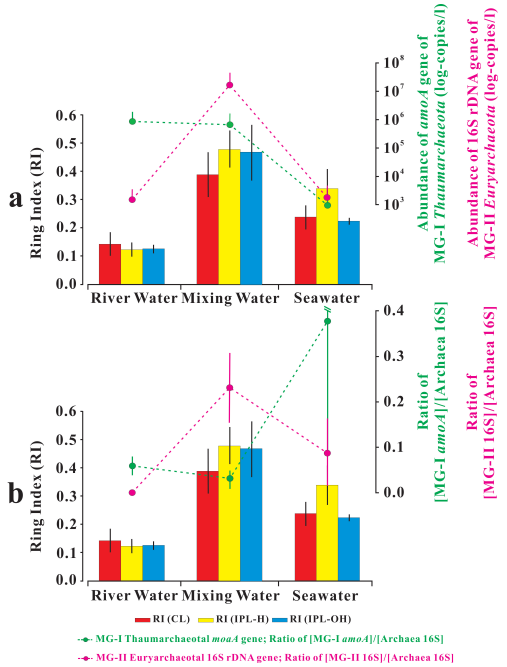

Figure 5. Distribution of the mean values of Ring Index (RI) compared with (a) the

Fig. 1. Fig. 5 (update)

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Fig. S2 (update)

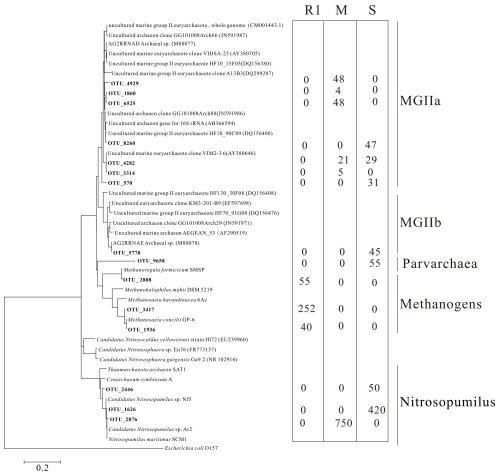

Figure S2. Pylogenetic tree of the SPM samples collected from the lower Pearl River, the PR estuary and coastal SCS. The numbers on the right are the permillages of each OTU in corresponding samples. R1, river water Station R1; M = mixing water Station M; S = seawater Station S (see Figure 1).

Fig. 2. Fig. S2 (update)

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Table 1. Basic information, abundance of isoprenoid GDGTs, TEX86, Ring Index, 16S rDNA gene and total DNA abundance for suspended particulate matters (SPM) in the water column and surface sediments collected from the lower Pearl River, the Pearl River estuary, and coastal northern South China Sea. Basic information includes location, sampling date, water depth, temperature (Temp.), salinity (Sal.) and pH.

Sample no.[a]	Longitude (E)	Latitude (N)	Sampling date (mm/dd/yyyy)	Depth[b] (m)	Temp. (°C)	Sal.	pH	crenArch. conc.[c]			TEX$_{86}$			Ring Index (RI)[d]			Archaeal 16S (copies/l)	MG-II 16S (copies/l)	MG-I 16S (copies/l)	DNA (ng/ul)
								CL	IPL-H	IPL-OH	CL	IPL-H	IPL-OH	CL	IPL-H	IPL-OH				
River water SPM																				
R1_sur	113°34.249′	22°52.647′	06/21/2011	1.5	29.7	0.2	7.25	188.6	201.5	16.7	0.59	0.50	0.57	0.19	0.15	0.12	2.1E+07	3.0E+03	1.6E+06	237.1
R1_bott	113°34.249′	22°52.647′	06/21/2011	6.0	29.6	0.2	7.25	221.3	128.1	8.9	0.60	0.56	0.54	0.16	0.12	0.15	3.6E+06	1.2E+01	1.6E+05	95.3
R2_sur	113°36.680′	22°56.338′	06/21/2011	1.5	29.0	0.1	6.93	64.8	82.5	6.8	0.56	0.42	0.49	0.16	0.15	0.11	-	-	-	-
R2_bott	113°36.680′	22°56.338′	06/21/2011	6.0	29.0	0.1	6.90	266.6	426.5	27.9	0.60	0.45	0.38	0.14	0.10	0.12	-	-	-	-
R3	113°28.726′	23°04.339′	06/22/2011	1.5	29.4	0.1	7.46	79.8	150.2	8.0	0.57	0.41	0.38	0.07	0.09	0.14	-	-	-	-
R4	113°33.507′	22°58.409′	06/22/2011	1.5	29.6	0.2	7.28	19.1	30.6	3.3	0.63	0.57	0.37	0.26	0.13	0.20	-	-	-	-
R5	113°29.941′	22°53.588′	06/22/2011	1.5	28.5	0.1	7.28	20.0	30.4	2.0	0.61	0.48	0.35	0.37	0.40	0.34	-	-	-	-
R6	113°33.088′	22°44.811′	06/22/2011	1.5	27.8	0.1	6.92	49.1	21.8	1.2	0.62	0.68	0.53	0.15	0.19	0.25	-	-	-	-
Mixing water SPM																				
M_h	113°45.098′	22°27.206′	06/18/2011	1.5	-	-	-	25.9	105.0	2.4	0.66	0.55	0.53	0.52	0.47	0.43	2.0E+05	6.1E+04	1.1E+04	40.0
M_st	113°45.098′	22°27.206′	06/18/2011	1.5	-	-	-	35.3	97.3	1.4	0.58	0.65	0.60	0.36	0.61	0.61	1.3E+07	3.1E+06	4.7E+05	564.2
M_hr	113°45.098′	22°27.206′	06/18/2011	1.5	-	-	-	21.9	86.8	2.8	0.59	0.56	0.55	0.43	0.46	0.38	6.9E+05	1.2E+05	4.3E+02	171.1
M_sur	113°45.098′	22°27.206′	06/18/2011	1.5	28.7	11.1	8.03	18.9	67.0	1.9	0.58	0.60	0.63	0.36	0.44	0.65	-	7.2E+05	9.8E+02	51.0
M_mid	113°45.098′	22°27.206′	06/18/2011	5.0	28.3	15.6	7.93	102.6	73.4	5.2	0.61	0.64	0.59	0.35	0.47	0.36	6.0E+07	7.9E+06	2.1E+06	171.1
M_bott	113°45.098′	22°27.206′	06/18/2011	9.0	27.6	23.0	7.89	107.4	76.7	6.0	0.56	0.60	0.58	0.30	0.42	0.38	5.1E+07	1.6E+07	1.4E+06	318.5
Sea water SPM																				
S_sur	113°70.448′	22°05.165′	06/15/2011	1.5	29.6	29.5	8.63	1.1	0.7	0.1	0.52	0.65	0.58	0.28	0.39	0.22	1.0E+03	4.8E+01	5.3E+02	36.0
S_subs	113°70.448′	22°05.165′	06/15/2011	5.0	29.5	29.7	8.64	2.1	1.1	0.1	0.56	0.63	0.58	0.27	0.33	0.24	3.1E+04	5.3E+03	1.8E+03	104.6
S_mid	113°70.448′	22°05.165′	06/15/2011	10.0	28.6	31.7	8.45	12.6	13.5	0.6	0.49	0.55	0.50	0.20	0.24	0.23	9.8E+02	4.4E+01	5.6E+02	35.9
S_bott	113°70.448′	22°05.165′	06/15/2011	18.0	25.4	33.7	7.92	21.4	9.6	0.7	0.53	0.59	0.49	0.20	0.39	0.21	-	-	-	-
Sediment																				
Sedi-R1	113°34.249′	22°52.647′	06/21/2011	8.0	-	-	7.46	461.8	142.8	9.5	0.58	0.37	0.30	0.27	0.38	0.41	2.8E+08	1.2E+04	1.1E+07	39.6
Sedi-R2	113°36.680′	22°56.338′	06/21/2011	7.0	-	-	7.68	687.5	289.2	17.0	0.56	0.35	0.31	0.23	0.22	0.26	-	-	-	-
Sedi-R3	113°28.726′	23°04.339′	06/22/2011	9.0	-	-	7.29	800.3	233.5	19.6	0.57	0.43	0.36	0.15	0.23	0.30	-	-	-	-
Sedi-R4	113°33.507′	22°58.409′	06/22/2011	7.0	-	-	7.50	881.0	149.7	9.7	0.58	0.51	0.38	0.33	0.75	0.62	-	-	-	-
Sedi-R5	113°29.941′	22°53.588′	06/22/2011	8.0	-	-	7.58	621.7	84.2	7.3	0.62	0.52	0.37	0.33	0.66	0.43	-	-	-	-
Sedi-R6	113°33.088′	22°44.811′	06/22/2011	8.0	-	-	7.66	120.2	103.8	6.2	0.67	0.35	0.42	0.42	0.65	0.74	-	-	-	-
Sedi-M	113°45.098′	22°27.206′	06/18/2011	12.0	-	-	7.60	200.4	67.7	2.3	0.62	0.52	0.50	0.38	0.69	0.42	1.1E+08	1.7E+04	1.3E+07	28.3
Sedi-S	113°70.448′	21°95.165′	06/15/2011	20.0	-	-	7.40	5003.6	267.3	20.8	0.50	0.64	0.50	0.19	0.73	0.34	1.3E+08	4.8E+04	8.8E+06	156.6

[a]R, River (the lower Pearl River), which is followed by the station numbers; sur and bott represent surface water and bottom water. M, Mixing water (the Pearl River estuary); h, low tide; st, slack tide; hr, high tide; mid means middle layer water. S, Sea water (northern South China Sea); subs represents subsurface water.
[b]For the SPM samples collected from the water column, the depth is referred to the sampling water depth; For the sediments, the depth indicates the river water depth.
[c]CL, core lipids; IPL-H, intact polar lipid (IPL) derived core lipids upon acid (H) hydrolysis; IPL-OH, IPL-derived core lipids derived upon base (OH) hydrolysis.
[d]RI = (GDGT-1) + 2*(GDGT-2) + 3*(GDGT-3) + 4*(GDGT-4) + 4*(Cren.iso)/(∑GDGTs)*100.
-, data are not available or not examined.

Fig. 3. Table 1 (update)

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