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Interactive comment on "Characterization of particle-associated and free-living bacterial and archaeal communities along the water columns of the South China Sea" *by* Jiangtao Li et al.

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Anonymous Referee #3

This study focused on the depth profiles of free-living (FL) and particle-attached (PA) prokaryotes (Bacteria and Archaea) in two sites in the South China Sea (SCS). As of now, there is a few studies to reveal the particulate-attached prokaryotic community structures (especially, about Archaea). 16S rRNA gene deep-sequencing analyses revealed the shift of bacterial and archaeal community structures among different depths. Also, several environmental factors such as depth, seawater age, salinity, POC, DOC, DO, and silicate could be critical for determining the community structures. Phylo-

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genetic analyses revealed that several lineages including alpha-, gammaproteobacteria, Actinobacteria, Bacteroidetes. . .etc. were overlapped between PA and FL fractions. However, there were differences at family level among them. According to these data, the authors discussed about ecological and biogeochemical roles of FL and PA prokaryotes in the SCS.

Major comments The manuscript is well written. But main limitation is a weak of biogeoscientific discussion of FL and PA (especially PA) fractions (e.g. interaction between chemical composition or degradation of POM, and PA bacteria or archaea) (1). In addition, the critical problem is potential primer biases (especially bacterial primer). The selection of primer set is very important for evaluating the prokaryotic community structure and diversity. Especially, SAR11 clade affiliated with Alphaprotoebacteria seems to be underestimated in this study. This clade is known to be dominant lineage in the oceanic environments, and generally accounted for 15_30% of total prokaryotic cells (Morris et al., 2002 Nature 420: p806-810). Different primer set create different results (e.g. Sanchez et al., 2009, Aquat Microb Ecol, 54: p211-216; Apprill et al., 2015, Aquat Microb Ecol, 75: p129-137) on the community analysis in the ocean (at least Bacterial community analysis). The authors should mention these problems in the discussion section (2). Provide more information on the choice of sites and depths for this work. Moreover, provide more detail profiles of environmental factors collected by a Sea-Bird CTD system (at least seawater temperature, salinity and DO) (3). L152-155: Why did the authors choose these primer set (especially, 27F-533R for Bacteria)? I think the SAR11 clade affiliated with Alphaproteobacteria were underestimated (approximately 15_30% of total 16S rRNA sequences, in general). The selection of primer is one of the most critical factors for evaluating prokaryotic community structures and diversity (4). Provide the data for sequence depth (e.g. rarefaction curves) of 16S rRNA gene used in this study (5).

Response to comment (1): Thanks for this comment. As we responded above (1st reviewer), in-depth discussion on the biogeochemical significance of these finding is

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not waraanted due to lack of chemistry data (e.g., composition of POM and DOM). We agree in that our manuscript is mainly focused on the microbiological part and the role of microbes in marine carbon cycle. On the "biogeochemical" part, we focus our discussion on the role of PAM and FLM in oceanic carbon cycling processes, i.e., decomposition of POM, inter-conversion between POM and DOM, and degradation of DOM. To this end, the present study is an extension of our previous work, focusing on both microbiological and biogeochemical aspects of PAM and FLM and their potentials in mediating carbon cycling processes in the ocean. Therefore, revealing the microbial taxa in PA and FL assemblages and profiling variations of their abundance and diversity along the water column provides a foundation for a better understanding of the coupled microbiological and biogeochemical processes in marine carbon cycle. Conceptually we can make some inferences based on the current dataset and findings from previous studies. In the manuscript, we had/added additional discussion on microbial metabolic potential in utilizing certain organic compounds. For examples: "They often maintain . . .. . ., and are capable of degrading high-molecular-weight (HMW) organic compounds. . .. . ." "It is further revealed that PA microbes . . .. . . metabolic and regulatory capabilities of utilizing compositionally varied organic matter, while . . .. . ." "These γ-proteobacterial members are . . .. . . they are believed to have the abilities to degrade/utilize HMW organic compounds with higher nutrient requirements." "Further phylogenetic assignment revealed . . .. . . belong to the genus Methylobacterium which are strictly aerobic, facultatively methylotrophic bacteria, and can grow on a wide range of carbon compounds." "Genomic information underlines that although these clades have a flexible metabolism utilizing multiple hydrocarbon compounds. . .. . ." "The majority of . . .. . ., and commonly possess the ability to hydrolyze and utilize complex carbon sources. Although their abundance . . .. . . because of their high specificity for organics." "Sva0996 marine group . . .. . . have the ability to assimilate phytoplankton-derived dissolved protein." . . ..

Response to comment (3): As we responed to the 2nd reviewer, this present work is motivated by our early works (Li et al., 2015) in which some preliminary findings indi-

cated that depth probably exert an impact in structuring microbial assemblages. Therefore, in our present research, we selected two stations in the central basin of the SCS with depths >4,000 m to take the samples and test our hypothesis. One sentence was added in "2.1 Sample collection and environmental parameter measurements" subsection to introduce this background: "Both stations have depth > 4,000 m, providing us the bathyal environments to vertically profile the variation of microbial assemblages with depth."

As for the profiles of environmental factors, we also totally agree. As we described in M&M section, a CTD profiler was used to obtain basic environmental parameters of the water column, including depth, salinity, temperature, and dissolved oxygen (DO) were obtained in situ using a DO sensor integrated in the CTD profiler during the sampling. However, unforturnately, it is a pity that we had not the access to get all the continuous datasets of these fundamental environmental parameters at that time. Therefore, as we presented in our manucript, only those data of our sampling depths were provided.

Response to comment (2) and (4):Thanks for this suggestion and we agree. In our manuscript, we selected the primer sets 27F/533R, targeting the hypervariable V1-V3 regions of 16S rRNA gene which is widely used in bacterial community analysis based on the 454 pyrosequencing (for example, Sun et al., 2014, PLOS one; Fonseca et al., 2019, Front Microbiol). As pointed out by the reviewer and previous studies, it has been demonstrated that the relative abundance of SAR11 clade in seawater could be potentially biased by different primer sets. Therefore, we discussed this kind of possibility of the underestimation of SAR11 clade in our samples as below: "In addition, the percentages of SAR11 clade revealed here seem to be relatively lower compared with those reported in previous studies where the SAR11 clade typically makes up 20 to 40% of the bacterioplankton (Morris et al., 2002; Aprill et al., 2015). It may be related to the sequencing primers used which potentially cause underestimation of SAR11 clade and bias the interpretation of their relative abundances (Aprill et al., 2015)."

Response to comment (5): Done. We provided the rarefaction curves in the supple-

mentary materials named as Figure S1.

Specific comments

L96: Marine Group III (MGII)!Marine Group (MGIII)

Our response: Thanks for pointing out this error. It was a typo and has been corrected.

L150: what amount of template DNA (ng) did author used? And provide the information about DNA concentration (or amount) after DNA extraction.

Our response: Thanks for pointing out these questions. For the PCR amplification, $\sim$10 ng DNA template was used. For DNA extraction, we obtained about 4.48 $\sim$ 29.1 ng/μl DNA concentration dissolved in $\sim$ 50 ul sterilized deionized water. We provide these information in this section.

L152-155: provide the references of these primers.

Our response: Done. Ohene-Adjei et al., 2007 and Sun et al., 2014 were provided after these primers.

L161: provide the reference or URL for QIIME 1.9.1 software

Our response: Done. Caporaso et al., 2010 was added here.

L177: provide the reference or URL for R packages.

Our response: Done. The URL (https://CRAN.R-project.org/package=vegan) was provided for R packages.

L251 and other lines: If the author described "significantly differences", provide the information about R or Rho values, and P value. Maybe, the ANOSIM or PERMANOVA analyses should be need to clarify statistical differences among communities.

Our response: Thanks for this suggestion and we agree. So, as suggested by the reviewer, during our revision, the statistical analyses including MRPP, ANOSIM and PERMANOVA were performed to clarify the statistical significances. The statistical

results were provided as supplementary materials (see Table S3). All the P values <0.05, indicating statistically significant difference. We reworded this sentence as below: “PCoA analysis revealed that there were significant differences (P values <0.05, Table S3) in bacteria and archaea community structures over the depth profiles and between the FL and PA fractions.”

L379 “taxonomically” : add information about the taxonomic levels after this word (e.g. taxonomically (at least family or order?? level)).

Our response: Thanks for this comment. Here we just meant to indicate a potential difference in microbial community compositions. The difference can occur at any level of taxonomy. To avoid the unclear statement, we deleted the word of “taxonomically”.

L386 “depth”: I think it is better to correct “hydrological condition (e.g. depth)”.

Our response: Agree and done.

L413: provide the R or Rho value before P value (R or Rho=????, P>0.05).

Our response: Thanks and done. As shown in Table S3, Mantel test was used to test the statistical significance of environmental factors with microbial compositions. In Table S3, R values and P values were listed. Therefore, here we referred this place to Table S3 as following: “However, POC concentration in the present study is not statistically significantly correlated with either bacterial or archaeal community abundances (P values >0.05) (Table S3).”

L417: I can not understand “utilization of refractory POC by microorganisms depends on the quality of POC”. I recognize “refractory” is not usable for microorganisms. “Refractory POC” means “POC in the deep sea”?

Our response: Thanks for pointing out this and the reviewer is right. We now have reworded this “refractory POC” as “POC in the deep sea”.

L442-443: -proteobacterial (change italic to regular)

Our response: Done.

L446-449: Again, primer selection is one of the critical factors for evaluating the community composition and diversity. Thus, the authors should add the discussion about primer biases.

Our response: Totally agree. However, I think it would be better if we disscuss this at the end of this paragraph. Because SAR11 clade mainly contributes to the FL bacterial fraction rather than PA fraction. Therefore, at the end of this paragraph, we added several sentences to discuss the potential underestimation about SAR11 clade caused by the primer sets used in our study: “In addition, the percentages of SAR11 clade revealed here seem to be relatively lower compared with those reported in previous studies where the SAR11 clade typically makes up 20 to 40% of the bacterioplankton (Morris et al., 2002; Aprill et al., 2015). It may be related to the sequencing primers used which potentially cause underestimation of SAR11 clade and bias the interpretation of their relative abundances (Aprill et al., 2015).”

L462: What is (Ii)? Reference?

Our response: Typo and corrected. Here should be a reference, Gong et al., 2012.

L522-523 “statistical analysis”: provide the R or Rho, and P values.

Our response:Thanks and done. We added here a referring to Table S3 in which the R values and P values were listed by three different statistical analysis including MPPR, ANOSIM and PERMANOVA.

Figure 3: the authors circled the points (triangles, rhombus+hexagon+star: : :etc.) for representing different clusters in bacterial and archaeal fractions. Are there statistically significant? Provide the results of statistical analyses (and show R or Rho value, and P value).

Our response: Thanks for this advice. Yes, they are statistically significant with P values <0.05 (Table S3). We added one more sentence in the caption of this figure:

“Statistical analyses supported the groups with statistical significances (Table S3).”

Figure 6: x-axis is confused. It is better to delete minor scale marks (e.g. those between 50m and 200m).

Our response: Done! During our this revision, we redrew this figure based on those of dominant families with >3% proportions and adjusted the x-axis and scale marks.

Figure 7, Figure S5: provide the information about failed samples in the legend.

Our response: Thanks and we did this. We added one sentence in the legends of Figure 7 and Figure S5 like: “PA-3000 at G3 station and PA-4000 at J5 station indicate the samples failed in the sequencing of archaeal 16S rRNA gene.”

Please also note the supplement to this comment:
https://bg.copernicus.org/preprints/bg-2020-115/bg-2020-115-AC4-supplement.zip

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Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2020-115, 2020.

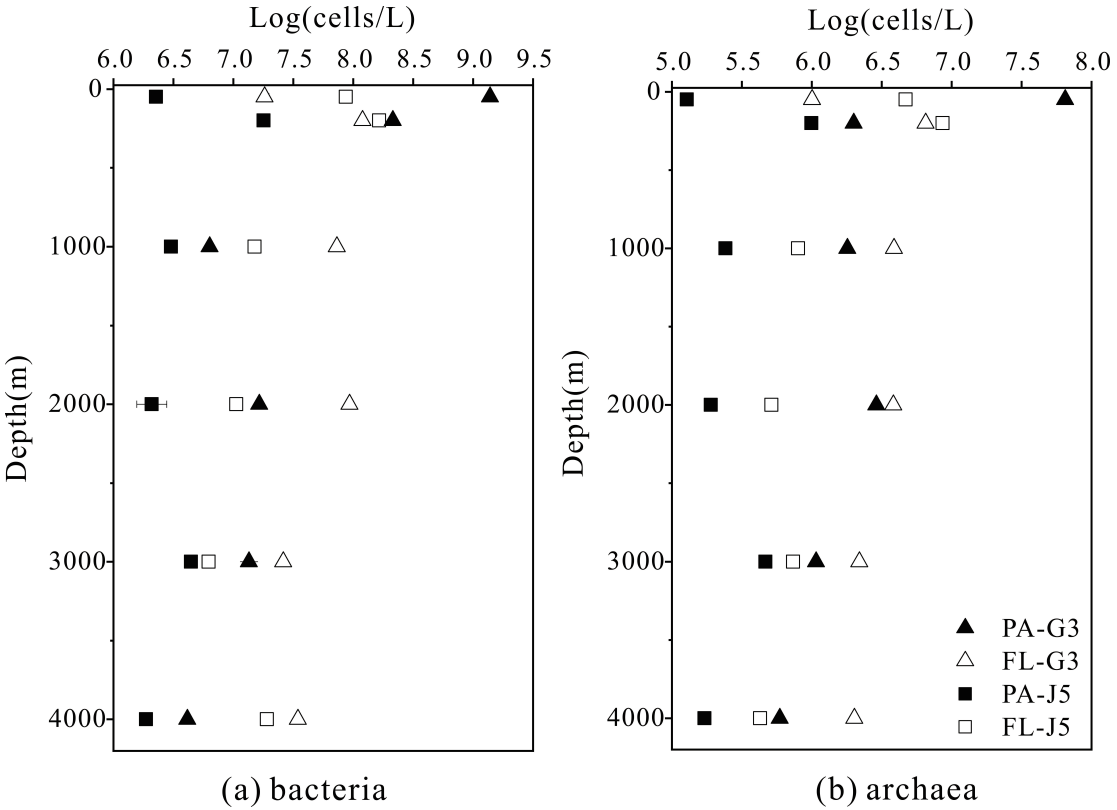

Fig. 1. Bacterial and archaeal cell abundances in seawaters at different depths from G3 station and J5 station in the South China Sea, estimated from 16S rRNA gene copy abundances.

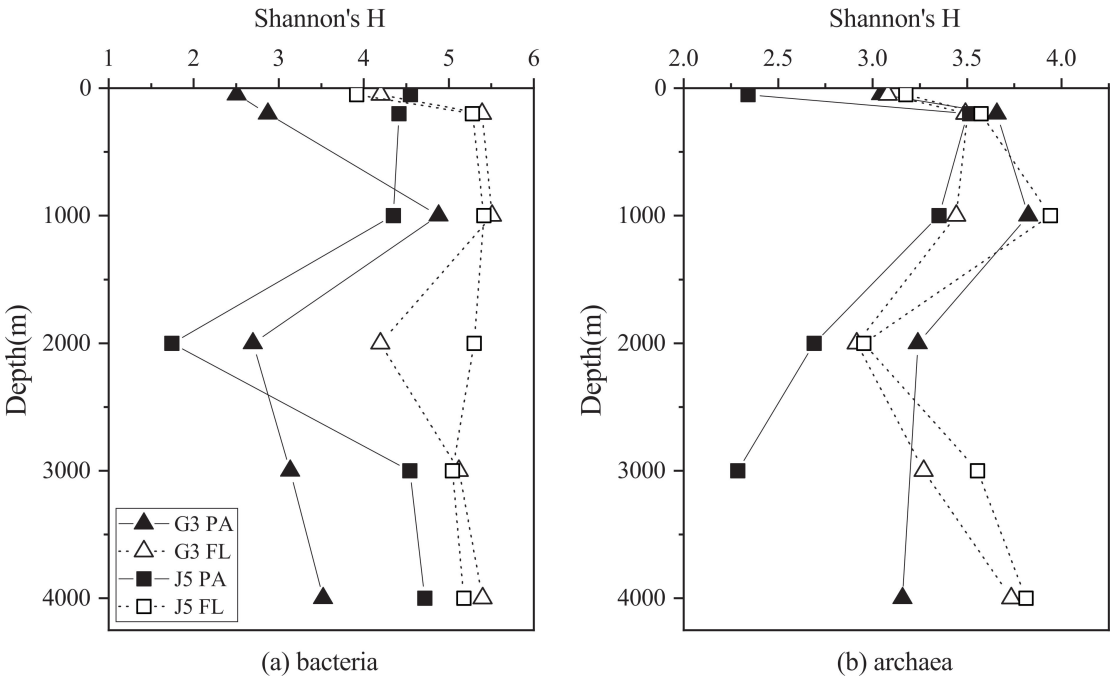

Fig. 2. Shannon's diversity index calculated for all bacterial and archaeal communities of seawaters collected from G3 station and J5 station in the South China Sea.

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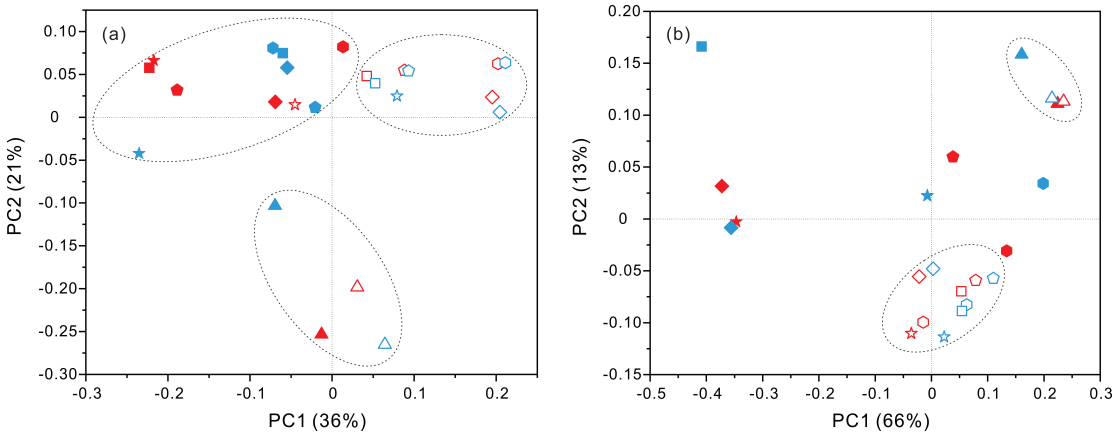

Fig. 3. Results of PCoA analysis for particle-attached and free-living microbial fractions collected from seawater columns of the South China Sea. (a) PA and FL bacteria; (b) PA and FL archaea.

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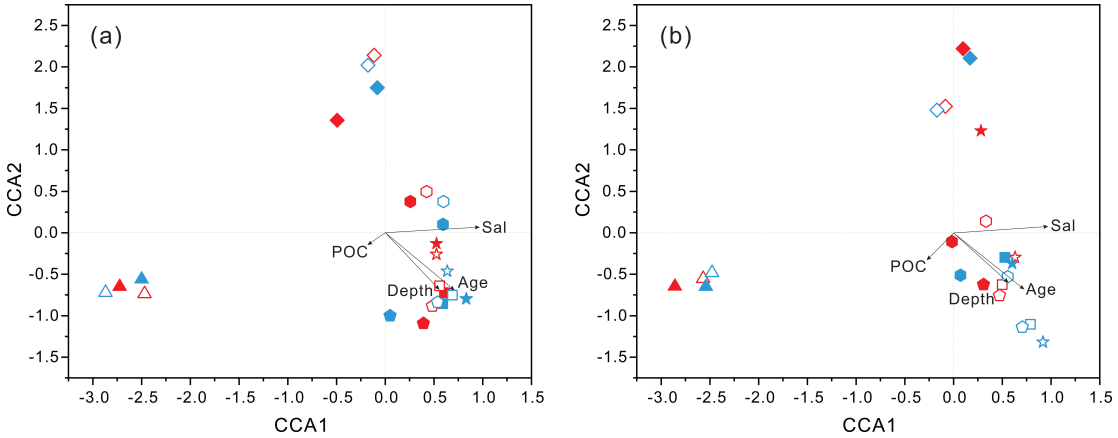

Fig. 4. Results of CCA analysis to correlate several environmental factors including POC, seawater age, salinity and depth to PA and FL microbial communities collected from seawater columns of the SCS.

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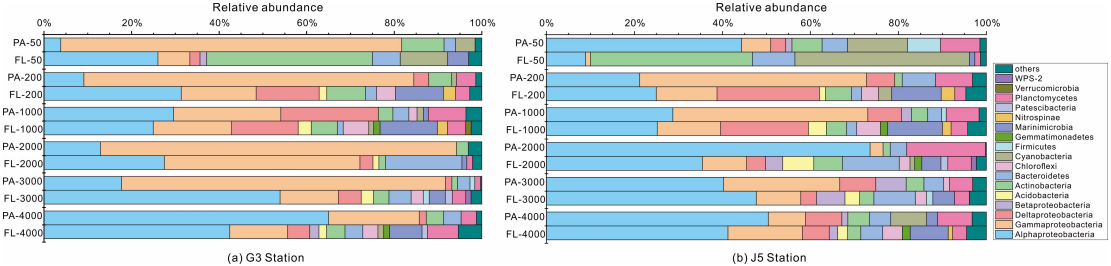

Fig. 5. Taxonomic compositions of particle-attached and free-living bacterial communities of seawaters at different depths along two different water columns in the South China Sea. (a) G3 ; (b) J5.

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Fig. 6. The relative abundances of families in PA and FL bacterial communities. Dark grey bubbles are for the PA fraction, while light grey bubbles are for the FL fraction.

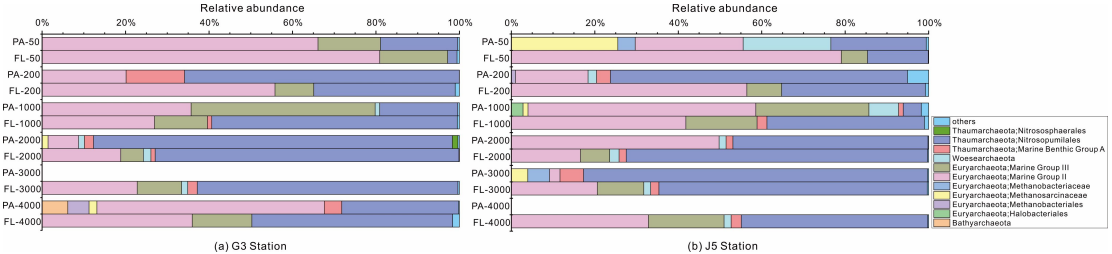

Fig. 7. Taxonomic compositions of particle-attached and free-living archaeal communities of seawaters at different depths along two different water columns in the South China Sea. (a) G3 ; (b) J5.

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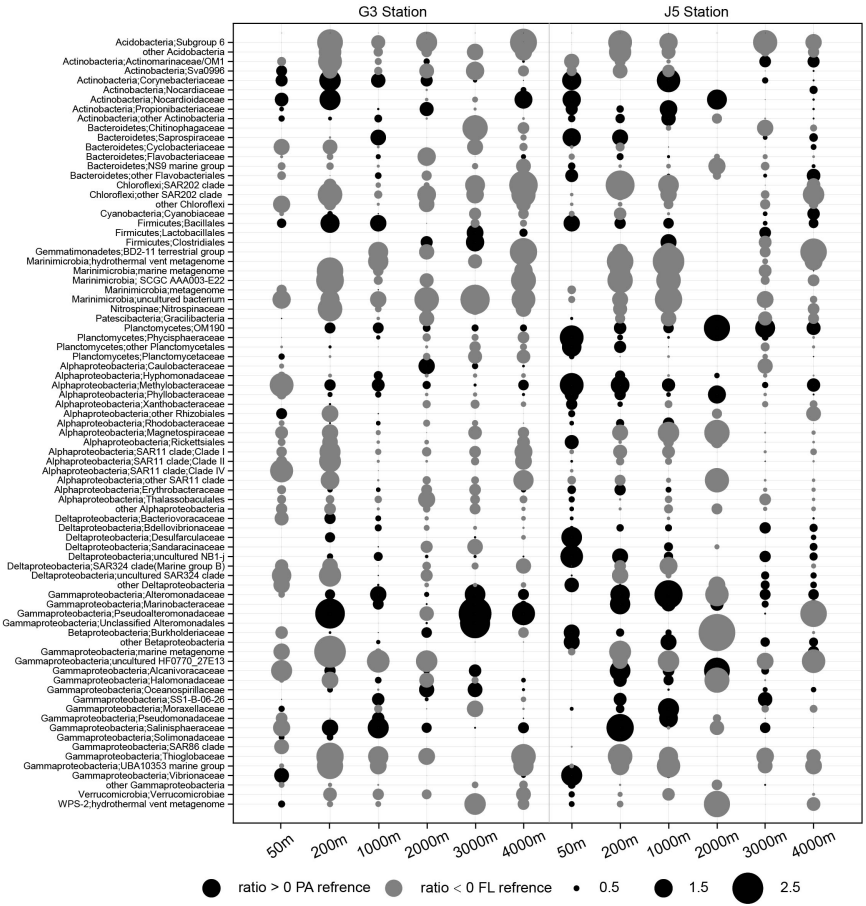

Fig. 8. Odds ratio for each of the families with relatively abundant proportions in each sample.

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