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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 #1 Li et al investigated particle-attached (PA) and free-living (FL) bacterial and archaeal community structures in South China Sea. They quantified the abundance of bacteria and archaea by using qPCR and surveyed the community structure with pyrosequencing. High abundance and diversity of FL than PA were observed. They tried to related microbial community composition, life styles and environmental adaption to organic and inorganic substrate availability from surface to deep ocean.

Major concern: The present MS is a little bit "microbial", not "biogeochemical". It will be great to include organic chemical analysis of particles and waters if any. At least,

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discuss this based on data available in previous studies (1). I suggest to discuss possible technique bias including 1) filtration with 3 um to collect particles, especially for deep sea samples which is very fragile (2). 2) qPCR data which showed relatively low "cell abundance" compared to microscopy (3). Having age data of particles is very interesting. I encourage the authors discuss more about this and its relationship, and biogeochemical implicates, with microbial data. A logic is needed to explain the sinking rate and age of particles as well as microbes attached (3).

Response to comment (1): Thanks for this suggestion.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. 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 (2): Yes, the reviewer is right and we agree. About the criteria to distinguish the PA and FL microbial assemblages, there are different standards about pore-size of filtering membrane such as 3 μm, 1μm and 0.8/0.7 μm. By now, the 3.0 μm nominal pore size is most commonly used. In addition, as pointed out by the reviewer, particles including organic detritus and meiofauna such as metazoans and protists seem to be very fragile and precarious (Lecroq et al., 2011; Bochdansky et al., 2017) and it is inevitable to break them if the filtering process is intensive. Therefore, to avoid damaging fragile particles (and membrane) in our experiment, we use a relatively low vacuum pressure of <âĂL'10 mm Hg, and at the same time, the filtration time was less than 40âĂL'minutes, which has been confirmed as an effective way. In the M&M section of our manuscript, we added one more sentence to provide this method detail: "To avoid damaging the membrane and fragile particles, a relatively low vacuum pressure of <âĂL'10 mm Hg was used, and at the same time, the filtration time was less than 40âĂL'minutes."

Response to comment (3): We respectfully disagree. The microbial abundances estimated by qPCR of 16S rRNA gene approximately equal to the results of staining under microscope (for example, see the results in Zhang et al., 2020, Marine Pollution Bulletin). As we described in M&M section and our response to the last comment (below), although there are some biases in converting 16S rRNA gene copy numbers into bacterial and archaeal cell abundances which mainly results from the significantly different copy numbers of 16S rRNA gene in different taxa, the estimation of cell abundances based on qPCR results of 16S rRNA gene can reflect the approximate biomass of cell abundances and have been widely used. During our sampling, because we did not fix the samples with PFA, so, we used the qPCR to roughly estimate the cell abundances of different size fractions. However, as suggested by the reviewer, we added a few

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sentences to point out this potential biases of this kind of estimation: “Although the cell abundances inferred from the 16S rRNA gene copy number quantified by qPCR may be potentially biased, the estimation of cell abundances based on the qPCR of 16S rRNA gene has been confirmed as an effective method to reflect the approximate cell abundances in previous studies.”

Response to comment (4): Thanks for this advice. However, we think the reviewer misunderstood our dataset. The age dataset in our manuscript is the ages of seawater at different depths rather than ages of organic particles. The age of seawater was determined based on the radiocarbon dating of DIC instead of organic carbon from particles.

Specific comments: Sometimes the “recently” is not appropriate since the references are not recent at all (e.g. Line 59, Line 460).

Our response: Yes, we agree. We have corrected these points by deleting “recently”.

Provide methods for particle age measurement.

Our response: Thanks for pointing out this. The dating was performed in Beta Analytic (Miami, United States). We provided this method in our M&M section as below: “1 L of seawater for each sample were sent to Beta Analytic, Inc. in Miami, Florida, for 14C radiocarbon dating with the Accelerator Mass Spectrometry (AMS) methods as described in their website (https://www.radiocarbon.com/beta-lab.htm). When CTD rosette sampler came back on board, seawater for 14C dating was taken from Niskin bottles with first priority. During the sampling, to avoid the disturbance of air, glass bottles were fully filled with seawater with no headspace. In addition, mercury chloride was added to prevent any microbiological influence.”

Salinity does not have unit (e.g. Line 200).

Our response: Thanks. We added the “PSU” as the salinity unit.

Include statistical analysis (e.g. Line 219).

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Our response: Thanks for pointing out this. In our Fig. 1, the standard deviations (SD) were actually provided, but because most SD values are too small that they are not shown up clearly on the graph. In our maintext, we provided these related information of SD in the subsection of “3.2 Microbial cell abundances”.

Line 240, seems meaningless to point out the number of sequences per depth.

Our response: We agree, and therefore, we deleted these numbers of bacterial and archaeal sequences.

Line 365, any evidence or previous study to support the different origins of organic matter of G3 and J5?

Our response: As we stated in the manuscript, it is our hypothesis to speculate the potential influence from POC quality, but without direct evidence in our study. Geographically, G3 site was close to the northern South China Sea, i.e., near the continent, while J5 was in the southern South China Sea, although they both were located in the central basin of the SCS. It has been shown that the Pearl River plume could reach nearby area of the G3 site (He et al., 2016), and moreover, there are more eddy activities around the northern SCS basin (Xiu et al., 2010). Additional allochthonous nutrient inputs from river discharge and eddy pumping could bring multifarious organic particles with different compositional characteristics. In addition, the enhancement of additional nutrient supplies can further irritate the growth (even the blooming) of phytoplanktons at G3 station and shape their community compositions which dominate the organic composition (quality) of POM in seawaters, especially in the surface water. Several researches have revealed significant differences in phytoplankton size structure (Chen et al., 2015; Lian et al., 2018) and community composition (Ke et al., 2009, 2012) between the southern and northern South China Sea. All these indicate a possibility that there may be some differences in the quality of POC between G3 and J5 sites. Therefore, we cited a couple of these references to support our hypothesis.

Line 404, I understand that POM remineralization is oxygen dependent, but the cause

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and effect relationship between DO concentration and particle flux is not clear to me.

Our response: As shown in a couple of studies, DO is an important environmental variable that impacts organic particle flux by affecting respiration rates of particle-associated microbes (Kalvelage et al., 2015), and thus, the remineralization rate of organic particles and transfer efficiency and flux of sinking POM (Marsay et al., 2015; Cram et al., 2018).

Line 462: li?

Our response: It is a typo and here should be a reference, Gong et al., 1992. We had corrected this mistake.

Maybe use copy number, not cell abundance, throughout the MS.

Our response: Thanks for this advice but we respectfully disagree. To provide a direct comparison of cell abundances, we converted the copy number of 16S rRNA gene into cell abundance based on the average values of 16S rRNA gene copy number in bacteria and archaea. In such case, it is relatively easy and intuitive to compare the abundances of bacteria and archaea among different size fractions. Therefore, we keep this conversion about cell abundances.

Please also note the supplement to this comment:
https://bg.copernicus.org/preprints/bg-2020-115/bg-2020-115-AC5-supplement.zip

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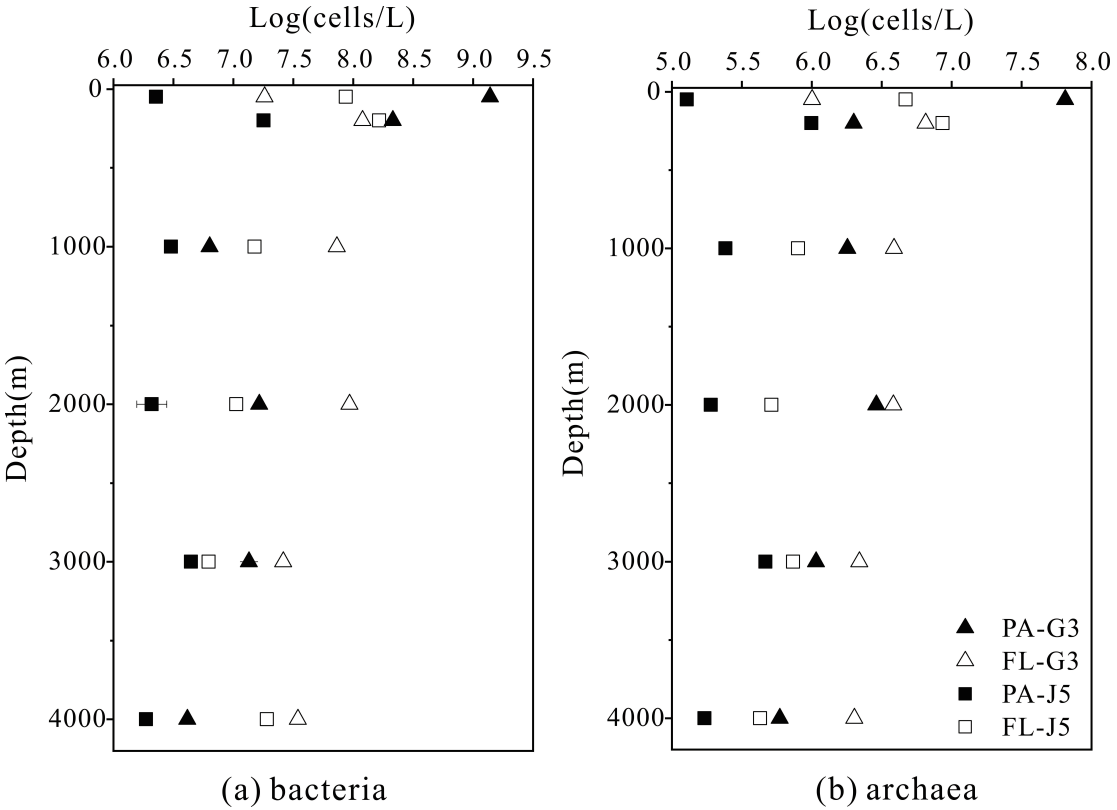

Fig. 1. Bacterial and archaeal cell abundances in seawaters at different depths from G3 station and J5 station in the South China Sea, estimated from 16S rRNA gene copy abundances.

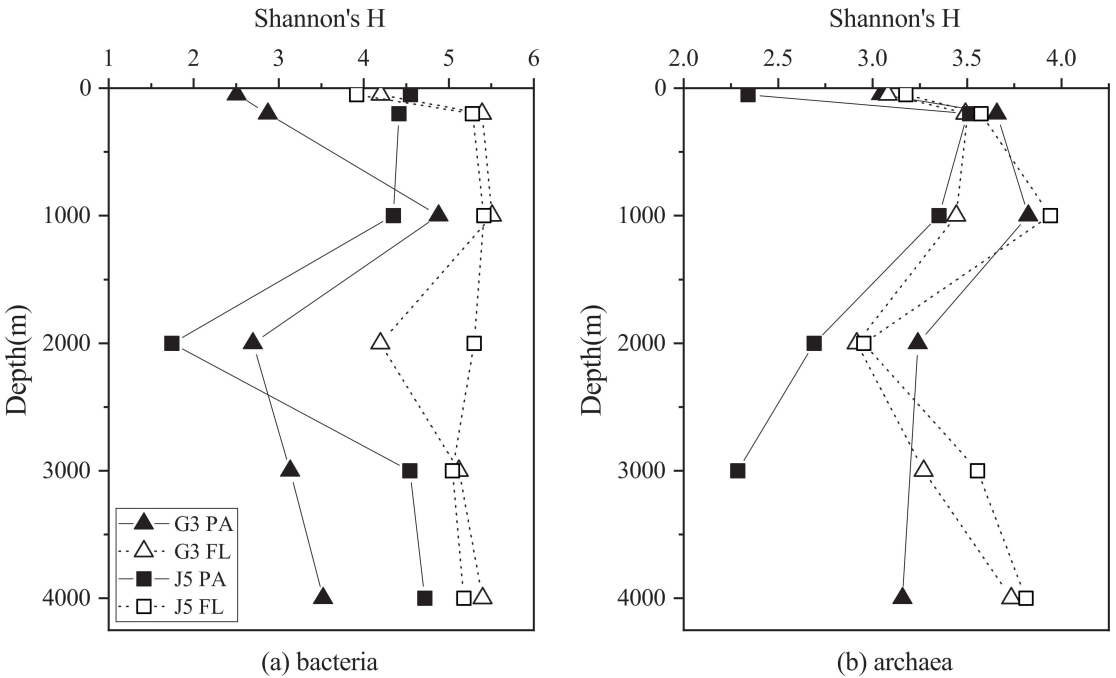

Fig. 2. Shannon's diversity index calculated for all bacterial and archaeal communities of seawaters collected from G3 station and J5 station in the South China Sea.

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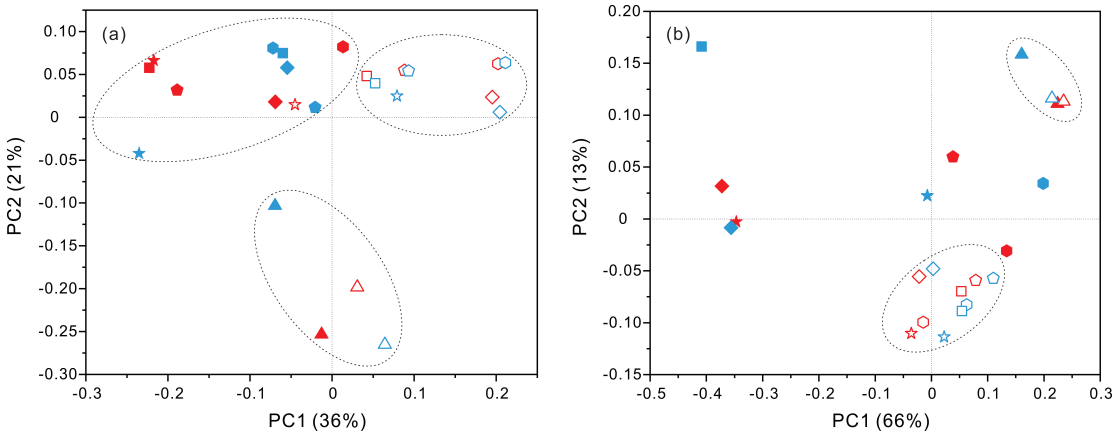

Fig. 3. Results of PCoA analysis for particle-attached and free-living microbial fractions collected from seawater columns of the South China Sea. (a) PA and FL bacteria; (b) PA and FL archaea.

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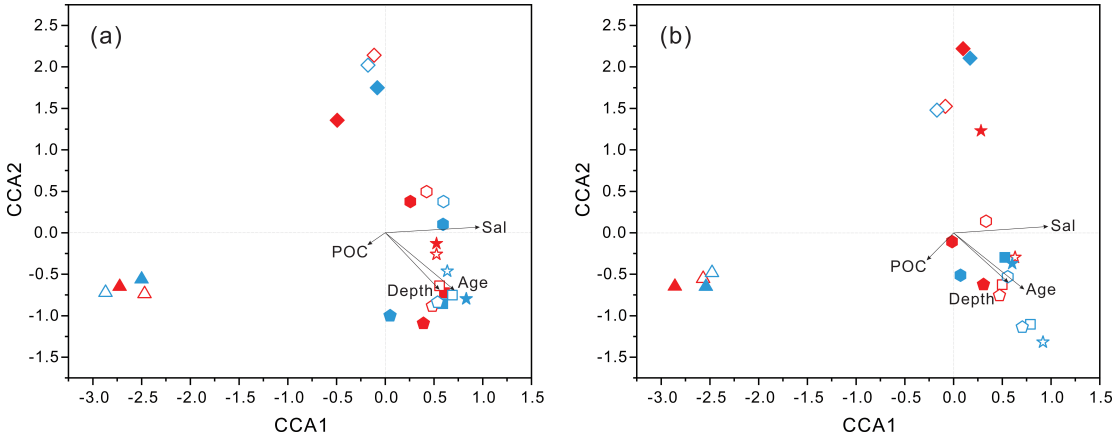

Fig. 4. Results of CCA analysis to correlate several environmental factors including POC, seawater age, salinity and depth to PA and FL microbial communities collected from seawater columns of the SCS.

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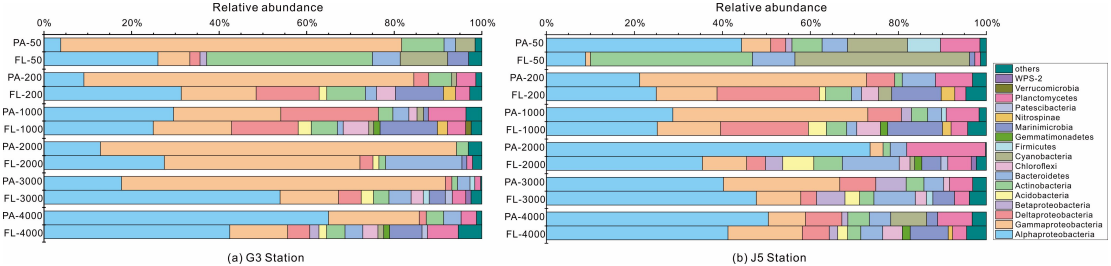

Fig. 5. Taxonomic compositions of particle-attached and free-living bacterial communities of seawaters at different depths along two different water columns in the South China Sea. (a) G3 ; (b) J5.

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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.

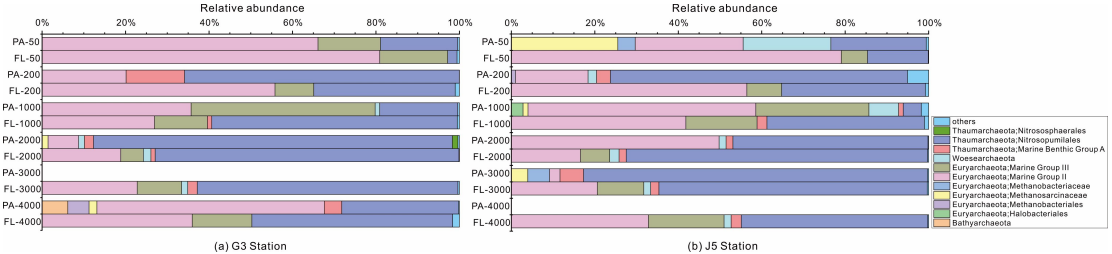

Fig. 7. Taxonomic compositions of particle-attached and free-living archaeal communities of seawaters at different depths along two different water columns in the South China Sea. (a) G3 ; (b) J5.

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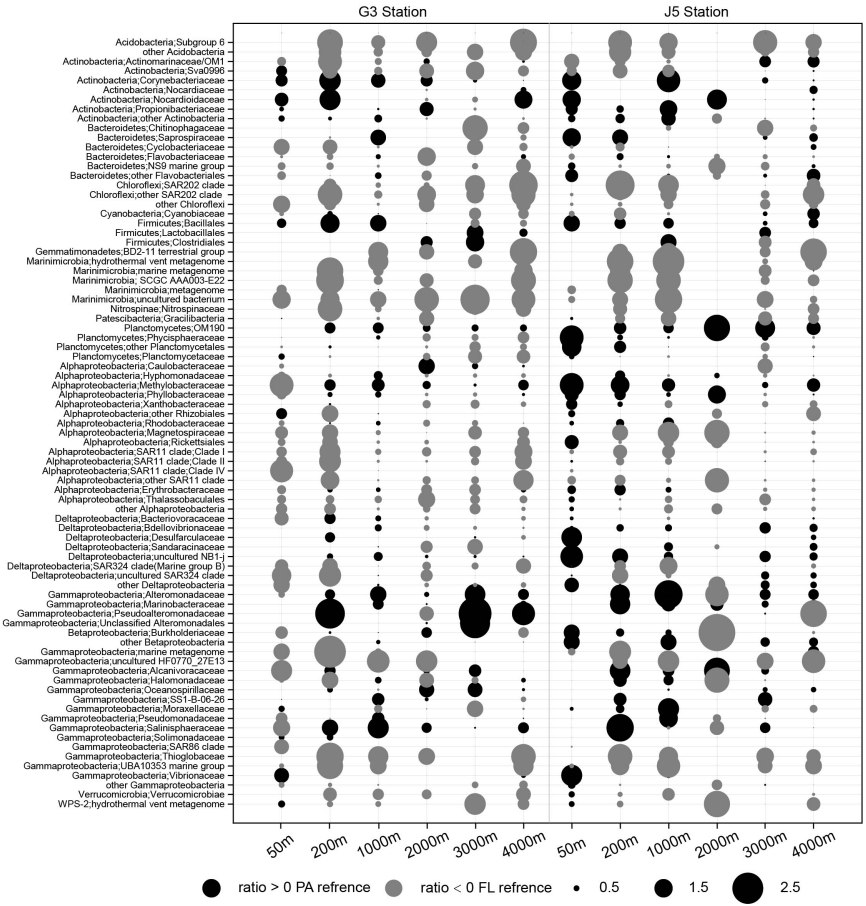

Fig. 8. Odds ratio for each of the families with relatively abundant proportions in each sample.

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