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, 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 (4).

Response to comment (1): Thanks for this suggestion.We agree 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. As suggested by the reviewer, in the revised 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 analysis 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 suggests 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 the 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 used a relatively low vacuum pressure of < 10 mm Hg, and at the same time, the filtration time was less than 40 minutes, which has been confirmed as an effective way. In the M&M section of our revised manuscript, we added one more sentence to provide this method detail:

"To avoid damaging the membrane and the fragile particles, a relatively low vacuum pressure of < 10 mm Hg was used, and at the same time, the filtration time was less than 40 min."

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 other comment (below), although there are some biases in converting 16S rRNA gene copy numbers into bacterial and archaeal cell abundances, resulted mainly 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 this technique has been used widely. 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 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 comment. 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:*

"About 1 L of seawater for each sample was sent to Beta Analytic, Inc. in Miami, Florida, for ^{14}C 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 ^{14}C dating was taken from Niskin bottles with first priority. To avoid the disturbance of air during the sampling, glass bottles were fully filled with flowing 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).
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 "**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, this is our hypothesis. 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. It has been shown that the Pearl River plume could reach the 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; Liang 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 these references to support our hypothesis.*

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

effect relationship between DO concentration and particle flux is not clear to me.
Our response: As shown in several previous 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.

Anonymous Referee #2

General comments

This manuscript by Li et al. is an examination of the PA and FL microbial communities found throughout depth at two stations in the South China Sea, and is an interesting addition to the body of literature on particle association of ocean microbes. The general patterns found in the microbial community composition data are reasonable. However, it is unclear whether the authors performed specific important transformations of the count table data before statistical analyses. Without that point being clarified, I would be very cautious to interpret anything from the ordinations and diversity calculations (1). The authors also included an analysis of seawater age, which is a unique aspect of this dataset. I would like to see a bit more exploration of that in relation to specific microbial taxa (2). Generally, I think this is an interesting and publishable dataset, but some refinement of the statistical methods are necessary (3).

Response to comment (1): Thanks for comment. In our original manuscript, we did not describe the details about our statistical analyses such as PCoA and CCA in the M&M section. During the revision, basic information about these methods were provided. In brief, we first removed all the singletons from our OTU tables. Then, to avoid the variation caused by an unequal sequence number across samples, we normalized the OTUs abundance by resampling the sequences for each sample based on the sample with the least number of sequences. After resampling the sequences to the same number, alpha diversity including Chao 1 and Shannon was calculated and then used to compare diversity between different samples. For the β-diversity such as PCoA and CCA ordinations, we performed the transformation of the resampled OTU abundance by taking the log of the sequence numbers. All the details about these analyses were provided in our revised M&M section:

"To avoid the variation caused by an unequal sequence number across samples, the OTUs abundance was normalized by resampling sequences for each sample based on the sample with the least number of sequences. After resampling the sequences to the same number, diversity estimators including Chao 1 and Shannon's diversity (H) were calculated. Similarities among different microbial communities were determined using similarity matrices generated according to the phylogenetic distance between reads (Unifrac distance), and beta diversity of principal coordinates analysis (PCoA) was computed as components of the QIIME pipeline. The correlation between the microbial community structures and environmental parameters was analyzed by canonical correspondence analysis (CCA). For the PCoA and CCA ordinations, the transformation of the resampled OTU abundance table was performed by taking the log of the sequence numbers. In addition, to test the statistical significance of different groups identified by PCoA ordination, multiple statistical analyses including MRPP, ANOSIM and PERMANOVA were performed based on the resampled and transformed OTU abundance table. Mantel test was also performed to test the statistical significance of environmental factors with microbial community compositions from the results of CCA. All statistical analyses were performed in the R environment (v 3.2.1) using the Vegan package (https://CRAN.R-project.org/package=vegan)."

Response to comment (2): Agree and done. Please see our response to the comment below about line 410-432.

Response to comment (3): Thanks for the informative comments. As we responded above, we reanalyzed our data and also provided the detailed information of statistical analyses.

Specific Comments

L107- A little context on the stations would be nice. They seem to be part of a larger study. What is their significance and why were these two chosen?

Our response: The present work is motivated by our early works (Li et al., 2015) in which some preliminary findings indicated that depth probably exert an impact in structuring microbial assemblages in the water column. Therefore, we selected two stations in the central basin of the SCS with depths >4,000 m to take the samples and test our hypothesis. The following 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."

L172 This is a very outdated version of SILVA. I'm not going to argue that the classification should be redone, but there are likely implications that can be discussed (eg. It may explain the large amount of unidentified archaeal taxa. Also, another example: the Nitrospinaceae are no longer considered part of the delta-proteobacteria, but in their own Nitrospinae phylum, L348).

Our response: Thanks for pointing out this. The version of SILVA database used for our study was actually 128 rather than 119 for the annotation of 16S rRNA gene sequences. However, even in the 128 version, the family Nitrospinaceae was still assigned into the class δ-Proteobacteria which is, as said by the reviewer, outdated. Therefore, during our revision, we reanalyzed all our OTUs based on the latest 132 version of SILVA database. Only a few variations occurred in bacterial and archaeal community compositions at ~ phylum or class level compared with our original results (Fig. 5, Fig. 7 and supplementary Fig. S5 and S6). It should be pointed out that in the latest 132 version, we found some inconsistent annotations with known taxonomic classifications at family level. So, we double-checked all the dominant lineages with manual curation.

Section 2.4- There is no mention of transformation/normalization of count tables or removing singletons. Removing singletons is absolutely vital for analyzing OTU data because 97% clustering introduces lots of singleton artifacts (Edgar RC. 2017. Accuracy of microbial community diversity estimated by closed- and open-reference OTUs. Peer J 5:e3889. DOI: 10.7717/peerj.3889), and this could greatly skew estimates of diversity and ordination results. Removal of singletons will also change the results for Figure 9 and the diversity estimates. Transformation is absolutely necessary for ordinations (see Legendre and Gallagher. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280. DOI: 10.1007/s004420100716 and Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. 2017. Microbiome Datasets Are Compositional: And This Is Not Optional. Front

Microbiol. 8(NOV):1–6. doi:10.3389/fmicb.2017.02224), so it needs to be made clear if this was done or not. Tables S1 and S2 appear to be raw count data with no transformation or normalization.

Our response: *Yes, we totally agree. Firstly, as suggested by the reviewer, we removed all the singletons from our OTUs tables (see supplementary Table S1 and S2) and then reannotated our OTUs based on the latest SILVA database as mentioned above. For the OTU tables, 1,982 singletons were removed from bacterial OTUs, and 329 singletons were deleted from archaeal OTUs. The sequences represented by bacterial singletons only accounted for ~ 0.2-1.4% of bacterial communities, and 0.07-0.3% of archaeal populations. Therefore, the removal of singletons did not affect our results of microbial community compositions (Fig. 5, 7 and Fig. S5, S6). Secondly, after the removal of singletons, we updated the results of statistical analyses such as PCoA and CCA ordinations and diversity estimation. As we responded to comment (1), for these statistical calculations, we performed the transformation or normalization of OTUs abundance tables. We resampled OTUs with sing_rarefaction.py for each sample to make all the samples have the same number of sequences. After resampling, alpha diversity including Chao 1 and Shannon was recalculated. For β-diversity such as PCoA and CCA ordinations, we also performed the transformation of the resampled OTU abundances by taking the log of the sequence numbers. All the details about these analyses were provided in our revised M&M section. Thirdly, supplementary Table S1 and S2 are provided with the original datasets of OTU information including names, abundances, annotating taxonomic classification at different levels, singletons and resampling results.*

Section 2.5- No quality parameters of the qPCR assays are reported (eg. R^2 of the standard curve or efficiency of the reaction). Also what standard was used for qPCR? A PCR product? Genomic DNA from cultured organism with a known 16S rDNA copy number? This should be briefly mentioned.

Our response: *Thanks for pointing out these and we totally agree with the reviewer's opinion. The PCR products of bacterial and archaeal 16S rRNA gene were first cloned into a plasmid vector, and then transformed into E. coli DH5a. The recombinant plasmids were extracted and purified. The obtained plasmid solution was adjusted to a concentration of about 100 ng/μL, and subsequently diluted 10-folds with sterile water as the standards for qPCR reactions. Standard curves were acquired from 10-fold serial dilutions of standards.* R^2 *for our qPCR amplifications varied between 0.994 and 0.996, indicating a strong linear relationship over the concentration ranges used in our study. The conversion between copy number of 16S rDNA and cell abundance is based on the average values of known pure cultures of bacteria and archaea listed from the database as shown by Lee et al., 2009. As suggested by the reviewer, we mentioned all above information in our M&M section as below:*

"The PCR products of bacterial and archaeal 16S rRNA gene were first cloned into a pUC18 plasmid vector (Takara Bio Inc, Japan), and then transformed into E. coli. The recombinant plasmids were extracted and purified, and subsequently diluted 10-folds as the standards for real-time PCR reactions. R^2 *for the standard curves varied between 0.994 and 0.996, indicating a strong linear relationship over the concentration ranges used in our study."*

L249-256 & L377 It's very interesting that diversity decreased mid-water column and then increased again below that. Can the authors speculate what's going on here? Could they relate it to their DOC/ POC data or age of seawater?

Our response: *Thanks for this constructive comment. Yes, we also agree. It is an interesting observation that mid-water around 2000 m depth showed a lower diversity. One possibility is that 1500-2000 m is a rough boundary for different water masses in the deep, central basin of the South China Sea. Generally, the concentrations of POC and DOC gradually decreased with depth, causing a continuous decreasing in microbial diversity. However, the deep water mass (>2600 m) of the central basin comes from the western Pacific Ocean through Bashi Channel which is relatively rich in nutrients than the mid-water masses of SCS at shallow dapth. Therefore, it may cause a relative increase in microbial diversity in deep water masses such as those at 3000 m and 4000 m. In addition, some "old, deep" water from the bottom of the central basin will also rise to the 2000 m depth because of the basin-scale circulation. These old waters are relatively enriched in refractory DOC (RDOC), remained after microbial ultilization of labile OC during their cirlulation, potentially reducing microbial diversity. This hypothesis is supported by the seawater age at J5 station. It is shown that the age of seawater at 2000 m depth of J5 station is 1670 years, roughly equal to those of deep waters at 3000 m and 4000 m (1680 years and 1610 year).*

Therefore, in the 3.3 subsection of Results section, we added one more sentence (Line 276-278) to describe such a result:

"H index of FL and PA bacterial fractions gradually increased from 50 to 1,000 m, decreased in the intermediate water of around 2,000 m depth, and increased again at 3,000 and 4,000 m (Fig. 2a)."

In the 4.1 subsection of Discuss section, we also added some sentences to speculation the possibility (Line408-419):

"It is interesting that the mid-water around 2000 m depth showed the lowest bacterial diversity (Fig. 2, Fig. S3). One possibility is that 1,500-2,000 m is a rough boundary for different water masses in the deep, central basin of the South China Sea. The deep water masses (>2600 m) of the central basin coming from the western Pacific Ocean through Bashi Channel are relatively rich in nutrients than the mid-water masses of SCS. Therefore, it may cause a relative increase in microbial diversity in deep water masses such as those at 3,000 m and 4,000 m. In addition, some "old, deep" water from the bottom of the central basin will also rise to around 2,000 m depth because of the basin-scale circulation. These old waters are relatively enriched in refractory DOC (RDOC), remained after microbial utilization of labile DOC during their circulation, potentially reducing microbial diversity. This hypothesis is partly supported by the seawater age at J5 station. It is shown that the age of seawater at 2,000 m depth of J5 station is 1,670 years, roughly equal to those of deep waters at 3,000 m and 4,000 m (1,680 years and 1,610 year)."

L257- 259 & Fig. 3 I see the separation of the 3 identified groups in the ordination but it is unclear which test was used to statistically distinguish these groups or if the circles were just drawn based on looking at the figure.

Our response: *Yes. As pointed out by the reviewer, the different groups were identified based on the PCoA analysis. To testify whether these groups are statistically disinguished, we*

performed three additional statistical analyses including MPPR, ANOSIM and PERMANOVA analyses. The results of these three analyses were listed in Table S3 of the supplementary materials. They are statistically significant with P values <0.05. To clarify this statistical significance, we added this statistical support in the sentence as:

"PCoA analysis revealed that there were significant differences (P <0.05, Table S3) in bacteria and archaea community structures over the depth profiles and between the FL and PA fractions."

In addition, in the caption of Figure 3, one more sentence was also added: "Statistical analyses supported the grouping of the clusters (Table S3)."

L410-432 Since the authors analyzed the age of seawater, it would be nice to interpret this more directly with respect to DOC/POC quality and microbial community composition. What is the precise impact on microbial community composition based on age of seawater (which groups were important and why?). I like that this part of the discussion begins to interpret the impact of silicate (which is really an indirect correlate and likely a signal of diatom biomass impacting microbial community, as the authors begin to suggest). But I think this can go deeper given the high-resolution community composition data that is available here (similar to the detailed discussion on PA/ FL preference).

Our response: Thanks for the compliment. We agree that age of seawater will affect DOC/POC quality and microbial community compositions. However, it is not easy to directly connect age of seawater with DOC/POC quality and microbial communities, especially in the case of lacking the measurement and analysis of DOC/POC quality. It is well known that the degree of remineralization and degradation of POC increases as seawater ages. In our study, along the vertical depth profiles, the seawater gradually becomes older. Therefore, for POC, older seawater stands for longer sinking distance and higher degradation. To some degree, the impact of age of seawater to microbial community is similar to that of depth. In our original manuscript, we presented our primary hypothesis to describe this kind of influence from depth (Line 416-424). In response to this comment from the reviewer, we added the following text:

"During POC sinking from surface through the water column, and also as seawater ages, the labile organic matter becomes increasingly decomposed, while the more refractory material remains and resists further degradation (Simon et al., 2002). In such cases, utilization of the POC in the deep sea by microorganisms depends on the quality and quantity of the remaining POC. Meanwhile, in older seawater, DOC also become more refractory because free-living microorganisms preferentially utilize labile DOC and the remained refarcotory DOC gradually accumulates, which potentially affect microbial community structures."

Figure 9 is not introduced in the results but heavily discussed in the discussion. The results reported for Fig. 9 in the Discussion should be moved to the Results.

Our response: Thanks for this advice and we agree. As stated above, because of the removal of singletons, we adjusted this figure based on the new bacterial OTU table correspondingly (supplementary Table S1). Meanwhile, as suggested by the reviewer, we also moved this figure into the "Results" section as a supplementary material (newly named as Figure S7).

*Correspondingly, we added some sentences to describe this Figure S7 at the end of the subsection of "**3.5 Bacterial preference to PA or FL lifestyles**" as:*

"Actually, at OTU level, near 1/2 of the total OTU (2005 out of 4338 OTUs) were shared by PA and FL fractions (Fig. S7). Phylogenetically, these PA/FL-shared OTUs were mostly fallen into α-, γ-, δ-Proteobacteria, Planctomycetes, Chloroflexi, Bacteroidetes, Marinimicrobia and Actinobacteria. The taxonomic components of the PA/FL-shared OTUs at different levels are approximately similar to OTUs retrieved exclusively from either the PA fractions or the FL fractions (Table S1, Fig. S7)."

L602- Bchl a is introduced for the first time with no context on what this is or what it is short for.
Our response: *Thanks for pointing out this. We used the full name "bacteriochlorophyll a" to replace the abbreviated "Bchl a".*

Technical Corrections:

L37- A high proportion "of" overlap.
Our response: *Done.*

L140- What is CTAB?
Our response: *CTAB is the abbreviation of "hexadecyl trimethyl ammonium bromide". In our revised manuscript, we provided the full name of CTAB like "1% hexadecyl trimethyl ammonium bromide (CTAB)."*

L151- "each DNA was" should be each "DNA pellet was"?
Our response: *Done.*

L259- I am not sure what is meant by incompact.
Our response: *I am sorry for this unclear statement. We deleted the word of "incompact".*

L388: "were supposed to" is a misleading phase. It sounds like an expectation of a result. Perhaps this would better be "several environmental parameters played a pivotal role…".
Our response: *Done.*

L403 impaction should be impact.
Our response: *Thanks and done.*

L412, "It is considered…" I am not sure what the 'subset' is and I think this can be better phrased.
Our response: *Yes, we agree. We reworded this sentence as following:*

"DO is considered as one of the most crucial environmental variables for shaping the compositions of particle-attached bacterial assemblages (Salazar et al., 2016)."

L414-415- should be 'A recent study' (not 'A most recent study').

Our response: *Done.*

L425 – should be ‘unexpected’ rather than ‘out of our expectation’.
Our response: *Agree and done.*

L425-426 – should be ‘generally exhibits N- or P-limited phytoplankton production’.
Our response: *Done*.

L436- ‘niches’ is not the correct word here. Maybe habitats? Locations?
Our response: *Yes, agree. We replaced “niches” here with “habitats” as suggested by the reviewer.*

L445- The phrase ‘significantly divergent’ implies statistical significance, but no such test was done to prove that PA and FL communities were significantly different (also in lines 641, 27, and 103). I think just ‘divergent’ would be acceptable unless a test is incorporated.
Our response: *Totally agree. During our revision, we performed the MPPR, ANOSIM and PERMANOVA statistical analyses (Table S3). The results confirm the significant differences with P values <0.05. Therefore, we kept these words.*

L463- ‘dominantly govern’ should just be ‘dominate’.
Our response: *Done*.

L498 – I don’t understand the meaning of this phrase: ‘nothing is available to elaborate the selection better PA and…’ I think it needs to be reworded.
Our response: *Yes, we agree. We reworded this sentence as like:*

“However, due to lack of necessary pure culture or their genome information, it is not yet possible to elaborate their preferences for PA and FL lifestyles.”

L580- The phrase ‘intelligibly convinced’ is unclear. Also the entire sentence L580-583 is a run-on sentence with some unclear phrasing and I’m not sure what the intended meaning is.
Our response: We thank the reviewer for pointing out these problems. We reworded our sentences and corrected the grammar errors. The revised sentences are as below:

“Their preference to particle-attached lifestyle in the water column is intelligible. Within normal water column, seawater is usually oxic in spite of low oxygen concentrations. Only on or inside the organic particles where heterotrophic microbes attach and digest organic matter using oxygen as electron acceptor, local anoxic niches are developed with the gradual exhaustion of ambient oxygen, and become suitable for the survival of anaerobic methanogens.”

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. Phylogenetic 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 not waraanted due to lack of chemistry data (e.g., composition of POM and DOM). We agree 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 indicated 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 supplementary materials named as Figure S2.

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, ~10 ng DNA template was used. For DNA extraction, we obtained about 4.48 ~ 29.1 ng/μl DNA concentration dissolved in ~ 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 (li)? 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.”

Characterization of particle-associated and free-living bacterial and archaeal communities along the water columns of the South China Sea

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Abstract

There is a growing recognition of the role of particle-attached (PA) and free-living (FL) microorganisms in marine carbon cycle. However, current understanding of PA and FL microbial communities is largely on those in the upper photic zone, and relatively fewer studies have focused on microbial communities of the deep ocean. Moreover, archaeal populations receive even less attention. In this study, we determined bacterial and archaeal community structures of both the PA and FL assemblages at different depths, from the surface to the bathypelagic zone along two water column profiles in the South China Sea. Our results suggest that environmental parameters including depth, seawater age, salinity, POC, DOC, DO and silicate play a role in structuring these microbial communities. Generally, the PA microbial communities had~~ve~~ relatively low abundance and diversity compared with the FL microbial communities at most depths. Further microbial community analysis revealed that PA and FL fractions generally accommodate significantly ~~significantly~~ divergent microbial compositions at each depth. The PA bacterial communities mainly comprise members of ~~*Actinobacteria* *α-*~~ and *γ-Proteobacteria*, together with some from ~~*Bacteroidetes*, *Planctomycetes*~~ and *δ-Proteobacteria*, while the FL bacterial lineages are also mostly distributed within *α-*~~,~~ and *γ-Proteobacteria*, but ~~*Actinobacteria* and *Bacteroidetes*,~~ along with ~~certain~~ other abundant members chiefly from *Actinobacteria*, *Cyanobacteria*, *Bacteroidetes*, *Marinimicrobia*~~*β-*, *δ-Proteobacteria*, *Planctomycetes*~~ and *δ-Proteobacteria*~~*Firmicutes*~~. Moreover, there wa~~i~~s an obvious shifting in the dominant PA and FL bacterial compositions along the depth profiles from the surface to the bathypelagic deep. By contrast, both PA and FL archaeal communities dominantly consisted of euryarchaeal Marine Group II (MGII) and ~~Marine Group I (MGI)~~thaumarchaeal *Nitrosopumilales*, together with variable amounts of ~~minor~~ Marine Group III (MGIII), *Methanosarcinales*, Marine Benthic Group A (MBG-A) and *Woesearchaeota*. However, the pronounced distinction of archaeal community compositions between PA and FL fractions ~~are~~ were observed at finer taxonomic level. A high proportion of overlap of microbial compositions between PA and FL fractions implies that most microorganisms are potentially generalists with PA and FL dual lifestyle for versatile metabolic flexibility. In addition, microbial distribution along the depth profile indicates a potential vertical connectivity between the surface-specific microbial lineages and those in the deep ocean, likely through microbial attachment to sinking particles.

Keywords: particle-attached, free-living, marine microbe, vertical distribution, sinking particles, deep ocean, the South China Sea.

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1. Introduction

The sinking of particulate organic matter (POM) formed in the photic layer is a fundamental process that transports carbon and nutrient materials from the surface into the usually starved deep ocean, with a significant role in structuring the distributions and activities of marine microorganisms in the dark realm (Azam and Malfatti, 2007; Mestre et al., 2018; Suter et al., 2018). During sinking, the POM is generally colonized and concurrently, decomposed by particle-attached (PA) prokaryotes, releasing dissolved organic matter (DOM) into ambient seawater, fueling the free-living (FL) microbes (Kiorboe and Jackson, 2001; Azam and Malfatti, 2007). It has been revealed that PA and FL microbial populations exhibit different taxonomic composition, physiology and metabolism, corresponding to their lifestyle and ecological behavior. For example, PA bacteria, compared to FL bacteria, are often larger in size (Alldredge et al., 1986; Zhang et al., 2007; Lauro et al., 2009) and metabolically more active (Karner and Herdl, 1992; Grossart et al., 2007). They often maintain higher levels of extracellular enzymes, adhesion proteins and antagonistic compounds, and are capable of degrading high-molecular-weight (HMW) organic compounds (Smith et al., 1992; Crump et al., 1998; Long and Azam, 2001; Mevel et al., 2008; Ganesh et al., 2014). ~~Recently,~~An examination of microbial metagenomes suggests that there are notable differences between PA and FL assemblages in GC content, effective genome size, general taxonomic composition and functional gene categories (Smith et al., 2013). In particularly, some broad key functional gene categories involved in DOM utilization (Poretsky et al., 2010; Rinta-Kanto et al., 2012) and specific functional gene groups linked to successive decomposition of phytoplankton blooms (Teeling et al., 2012) are significantly different, indicating the fundamental differences in survival strategies in relation to potentially available substrates. It is further revealed that PA microbes generally have larger genomes with a variety of metabolic and regulatory capabilities of utilizing compositionally varied organic matter, while the genomes of FL microbes usually are smaller with streamlined metabolic and regulatory functions that enable efficient adaption to oligotrophic conditions (Smith et al., 2013; Yawata et al., 2014; Yung et al., 2016). Phylogenetically, PA and FL lineages generally exhibit different compositions. The PA fraction is relatively enriched in members of *γ-Proteobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Firmicutes* and *Planctomycetes* (Azam and Malfatti, 2007; Milici et al., 2016; Salazar et al., 2016; Suter et al., 2018), while the FL assemblages are often populated by members of *α-Proteobacteria* (SAR11 clade or *Ca.* Pelagibacter) and *Deferribacteres* (DeLong et al., 1993; Crespo et al., 2013; Milici et al., 2017). However, significantly overlapped compositions of PA and FL microbial communities were also reported in a few studies (Hollibaugh et al., 2000; Ghiglione et al., 2007; Ortega-Retuerta et al., 2013; Rieck et al., 2015; Liu et al., 2018a). Actually, most members of the PA and FL clades are generalists which switch their lifestyles via attachment and detachment to particles (Crespo et al., 2013; Li et al., 2015). As revealed in many marine niches, *α-Proteobacteria*, *γ-Proteobacteria* and *Bacteriodetes* are the major overlapped phyla in both PA and FL microbial fractions (Yung et al., 2016).

Our current knowledge of PA and FL microbial populations largely relies on the upper photic ocean, whereas little information is known from the deep dark ocean, which is the largest biome and accommodates more than half of the ocean's microbes (Aristegui et al., 2009; Salazar et al., 2016). Recently, a number of studies have revealed the PA and FL microbial communities in the bathypelagic

waters (Li et al., 2015; Salazar et al., 2015; Milici et al., 2017; Mestre et al., 2018) or the deepest abyssal and hadal environments (Eloe et al., 2011; Tarn et al., 2016; Liu et al., 2018a). It is shown that PA and FL bacterial communities in the deep ocean have clear differences in abundance and composition, in addition to the detection of novel, unknown prokaryotic taxa. Furthermore, although archaea are a major component of the marine ecosystem and play significant roles in the degradation of organic materials (Iverson et al., 2012; Suzuki et al., 2017), PA and FL archaeal communities receive less attention and little is known about them. Previous limited reports have observed controversial results, as several studies showed that no obvious differences in archaeal community structures between PA and FL assemblages (Galand et al., 2008; Eloe et al., 2011; Suzuki et al., 2017), while a clear separation was found in recent reports (Tarn et al., 2016), with PA archaeal fraction dominated by Marine Group II (MGII) and Marine Group III (MGIII), and FL archaeal fraction by Marine Group I (MGI) and anaerobic methane-oxidizing archaea (ANME). In brief, it is not well known about the changes of PA and FL prokaryotes along vertical profiles of water column, from the surface to the deep bathyal, abyssal and hadal depths.

In this study, we analyzed and compared microbial compositions between PA and FL fractions at different depths along the vertical profile in the South China Sea (SCS). The SCS is a marginal sea located in the Northwest Pacific with a maximal depth of approximately 5,380 m (Fig. S1). Our results reveal diverse and significantly divergent microbial compositions in PA and FL fractions, and obvious community stratification at different depths along the vertical profiles.

2. Materials and Methods

2.1 Sample collection and environmental parameter measurements

Seawater samples were collected from two stations, G3 station, depth of 4,039 m at 117° 00.131′ E, 16° 59.947′ N, and J5 station, depth of 4,301 m at 114° 00.209′ E, 13° 59.958′ N, located in the central deep basin of the SCS during the Open Cruise of R/V *Dongfanghong* II from July 3 to 18, 2014 (Fig. S1). Both stations have depth > 4,000 m, providing us the bathyal environments to vertically profile the variation of microbial assemblages with depth. A Sea-Bird CTD rosette sampler (SBE 911 plus) with 12 L Niskin bottles (Seattle, Washington, USA) was used to collect seawater from six different depths (50, 200, 1,000, 2,000, 3,000, and 4,000 m) at each station.

Basic environmental parameters of the water column, including depth, salinity, temperature and dissolved oxygen (DO) were obtained in situ using the conductivity-temperature-depth (CTD) profiler and a DO sensor during the sampling. Once water samples were collected onboard, about 0.1 L of seawater was taken immediately for pH measurement using a pH meter (Mettle Toledo Inc., Switzerland).

Approximately 8 L of seawater was filtered onboard through a Φ142 mm precombusted glass fiber membrane (0.7 μm nominal pore size, Whatman, USA) under a gentle vacuum of <150 mm Hg for particulate organic carbon (POC) collection. The membranes were folded and stored at -20°C until our POC analysis. Then about 30 mL of filtered seawater of each sample was collected into 40 mL

precombusted EPA vials and stored at -20°C immediately for DOC concentration measurement (laboratory on land). ~~—~~About 200 ml filtered seawater at each depth was stored at -20°C for analysis of nutrients (NO_3^-/NO_2^-, dissolved inorganic phosphate and silicate). The remaining seawater was stored at -20°C for other analyses.

At each depth, we collected 4 L of seawater to obtain microorganisms for further analysis. Seawater was filtered first through a Φ47 mm polycarbonate (PC) membrane of 3.0 μm nominal pore size (Millipore, USA) and subsequently, through a Φ47 mm PC membrane of 0.22 μm nominal pore size (Millipore, USA) to collect the PA and FL microorganisms, respectively (Eloe et al., 2011). To avoid damaging the membrane and the fragile particles, a relatively low vacuum pressure of < 10 mm Hg was used, and at the same time, the filtration time was no longer than 40 min~~utes~~ for each membrance. The membranes were then frozen at -80°C until further microbial analysis.

Concentration of POC was determined with a PE2400 Series II CHNS/O analyzer (Perkin Elmer, USA) (Chen et al., 2008). DOC concentration was measured using a Shimadzu TOC-V Analyzer (Shimadzu Inc., Japan) (Meng et al., 2017). Nutrients were determined using a Four-channel Continuous Flow Technicon AA3 Auto-Analyzer (Bran-Lube GmbH, German).

About 1 L of seawater for each sample ~~were~~was sent to Beta Analytic, Inc. in Miami, Florida, for ^{14}C radiocarbon dating with the Accelerator Mass Spectrometry (AMS) method~~s~~ as described in their website (https://www.radiocarbon.com/beta-lab.htm). When CTD rosette sampler came back on board, seawater for ^{14}C dating was taken from Niskin bottles with first priority. ~~During the sampling, t~~To avoid the disturbance of air ~~D~~during the sampling, glass bottles were fully filled with flowing seawater with as little head space as possible. In addition, mercury chloride was added to prevent any microbiological influence.

2.2 DNA extraction

In this study, we used the SDS-based method to extract the total DNA as described by Li et al. (2015) with minor modifications. The PC membranes containing seawater microbes were first cut into small pieces in a sterile petri dish and put into autoclaved 2 ml centrifuge tubes. 800 μL DNA extraction buffer consisting of 100 mM Tris-HCl, 100 mM sodium EDTA, 100 mM sodium phosphate, 1.5 M NaCl and 1% hexadecyl trimethyl ammonium bromide (CTAB) was added into each tube. The centrifuge tubes were frozen in liquid nitrogen and then thawed in a 65°C water bath. This procedure was repeated for 3 times. When the centrifuge tubes cooled down to room temperature proteinase K was added with a final concentration of ~ 0.2 mg mL^{-1}. The tubes were then incubated in a 65°C water bath for 2 h and shaked gently every about 30 min. Then, 800 μL phenol/chloroform/isoamyl alcohol (25:24:1, v/v) was added into the centrifuge tubes and the tubes were shaked gently several times, and centrifuged at 12,000 ×g for 10 min. The supernatant was carefully transferred into new tubes and equal volume of chloroform/isoamyl alcohol (24:1, v/v) was added. The tubes were centrifuged at 12,000 ×g for 10 min. The aqueous layer was pipetted into clean 2 ml tubes, and 0.6 volume of cold isopropanol and 0.1 volume of 3M sodium acetate were added. The centrifuge tubes were incubated at -20°C for 1 h and centrifuged at 12,000 ×g for 10 min. The liquids were carefully discarded and DNA pellets at the bottom were gently rinsed with 70% pre-cooling ethanol. Finally, each DNA pellet was

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suspended into sterile deionized H_2O with a volume of 50 μL.

2.3 Pyrosequencing and analysis of 16S rRNA gene sequence amplicons

Before PCR amplification, we first used the PicoGreen dsDNA Quantitation Kit (Life Technologies, USA) to quantify the concentration of DNA. DNA concentrations obtained varied between 4.48 and 29.1 ng/μl~~L~~ with a volume of ~ 50 μ~~l~~L for each sample. ~~-~~For the PCR amplification of bacterial 16S rRNA gene, the primer set 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 533R (5′-TTA CCG CGG CTG CTG GCA C-3′) with 10-nucleotide barcodes were used, while Arch344F (5′-ACG GGG YGC AGC AGG CGC GA-3′) and Arch915R (5′-GTG CTC CCC CGC CAA TTC CT-3′) containing 8-nucleotide barcodes were used for archaea (Ohene-Adjei et al., 2007; Sun et al., 2014). About 10 ng DNA template was amplified for PCR reaction. The PCR reaction condition ~~for PCR amplification~~ was: firstly, 94°C, 5 min; then, 94°C, 50 s, 53°C, 50 s, and 72°C, 50 s, total 25 cycles; 72°C, 6 min. The products after PCR amplification were purified with the MiniBEST DNA Fragment Purification Kit (Takara Bio Inc, Japan) and then quantified using the NanoDrop 2000 (Thermo Scientific, USA). The pyrosequencing was carried out at the Majorbio Bio-Pharm Technology, Co., Ltd. (Shanghai, China) with the 454 GS-FLX Titanium system (Roche, Switzerland).

QIIME 1.9.1 was used to perform the following phylogenetic analysis of pyrosequenced amplicons (Caporaso et al., 2010). As described in our previous study (Li et al., 2017), the low-quality reads were first filtered with the following quantity control (QC) criteria: (1) the reads with ambiguous nucleotides; (2) the length of reads < 200 bp; (3) the reads containing > 5 bp homopolymers; (4) the reads with an average flowgram score < 25 in a quality window of 50 bp. The Operational Taxonomic Units (OTUs) were generated based on 3% cutoff of sequence similarity, and the longest sequence was picked as the representative sequence of each OTU for downstream analysis. The RDP classifier was used for the taxonomy assignment by against the SILVA 16S rRNA gene database (Version ~~119~~132). The ChimeraSlayer in the QIIME package was used to identify and exclude those of potential chimeras after alignment with PyNAST. In addition, t~~T~~he singletons were removed from the final OTU tables.

2.4 Diversity estimators and statistical analyses of microbial communities

To avoid the variation caused by an unequal sequence number across samples, the OTUs~~s~~ abundance ~~information~~ was normalized by resampling of sequences for each sample based on the sample with the least number of sequences. After resampling the sequences to the same number, diversity estimators including Chao 1 and Shannon's diversity (H) were calculated. Similarities among different microbial communities were determined using similarity matrices generated according to the phylogenetic distance between reads (Unifrac distance), and beta diversity of principal coordinates analysis (PCoA) was computed as components of the QIIME pipeline. The correlation between the microbial community structures and environmental parameters was analyzed by canonical correspondence analysis (CCA) ~~and Mantel test~~. For the PCoA and CCA ordinations, the transformation of the resampled OTU abundance table was performed by taking the log of the sequence numbers. In addition, to testify the statistical significance of different groups identified by PCoA ordination,

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multiple statistical analyses including MRPP, ANOSIM and PERMANOVA were ~~calculated~~performed ~~at the same time~~ based on ~~a z-score transformation of~~ the resampled and transformed OTU abundance table. ~~The correlation between the microbial community structures and environmental parameters was analyzed by canonical correspondence analysis (CCA) and Mantel test.~~ Mantel test was also performed to testify the statistical significance of environmental factors with microbial community compositions from the results of CCA. All statistical analyses were performed ~~by~~ in the R ~~project~~ environment (v 3.2.1) using the Vegan ~~and Agricolae~~ package~~s~~ (https://CRAN.R-project.org/package=vegan).

In this study, we used the "odds ratio" to assess microbial preference to the PA or FL lifestyles. As defined by Ganesh et al. (2014), the formula of the "odds ratio" is as:

odds ratio = log 10 (relative abundance in PA fraction / relative abundance in FL fraction)

a positive value indicates the PA preference, while a negative value signifies the FL preference (Suter et al., 2018).

2.5 Quantification of 16S rRNA gene and cell abundance estimation

The copy number of microbial 16S rRNA gene for PA and FL fractions were estimated with 7500 Real-Time PCR System (Applied Biosystems, ThermoFisher, UK). The primer sets used were 341f/518r for bacteria (Dilly *et al.*, 2004) and 344f/519r for archaea (Bano *et al.*, 2004) with about 200 bp amplified DNA fragments. The PCR products of bacterial and archaeal 16S rRNA gene were first cloned into a pUC18 plasmid vector (Takara Bio Inc, Japan), and then transformed into *E. coli* DH5 α. The recombinant plasmids were extracted and purified, and ~~was~~ subsequently diluted 10-folds as the standards for real-time PCR reactions. R^2 for the standard curves varied between 0.994 and 0.996, indicating a well linear relationship over the concentration ranges used in our study. PCR reaction was carried out in a 20 μL amplification volume. The reaction mixture contained 1 μL of DNA template, 0.15 μM forward and reverse primers, and 10 μL Power SYBR Green PCR Master Mix (Life technologies, UK). The PCR amplification conditions included: 95°C, 10 min to activate polymerase; 95°C, 15 sec, 60°C, 1 min, 40 cycles. A negative control was used to monitor potential contamination and agarose gel electrophoresis helped to confirm the absence of nonspecific amplification. Melt curves were generated using the Applied Biosystems real-time PCR system software with default thresholds. Each sample had triplicate amplifications and the average copy number of 16S rRNA gene were calculated. To make a direct comparison between bacterial and archaeal abundances, we converted copy number of 16S rRNA gene into cell abundance based on the assumption that on average, a bacterial cell ~~had~~ has 4.08 16S rRNA gene copies while archaea ~~contained~~ contains 1.71 copies per cell (Lee *et al.*, 2009). 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.

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3. Results

3.1 Environmental parameters of the water columns

Fundamental environmental parameters, including temperature, salinity, pH, DO and DOC/POC are listed in Table 1. In general, they showed similar vertical trends with the normal pelagic ocean. Salinity increased gradually from ~ 33.84 PSU at 50 m to ~ 34.52 PSU at 200 m and 1,000 m, then maintained at around 34.60 PSU at greater depths until 4,000 m. DO concentration was the highest (~ 204.5 μM) at surface water, and decreased gradually to the lowest (~ 83.9 μM) at 1,000 m depth, then increased gradually from ~ 102.0 μM at 2,000 m to ~ 113.5 μM at 4,000 m. Nitrite concentrations of the water columns at all depths were below the detection limit. Concentrations of nitrate, phosphate, and silicate were continuously increasing from the surface to 1,000 m depth, and then remained at relatively constant levels (Table 1).

As expected, age of the seawater determined from $\Delta^{14}C_{DIC}$ was youngest at the surface and increased with depth linearly, varying from about 106 to 1650 years. The upper water layers (50 m and 200 m) from the two stations had the youngest and nearly the same ages, around 106 years. Ages of 1,000 m and 2,000 m in G3 station were almost identical, around 1,180 years, and increased to 1,600 years at 3,000 m and 1,750 years at 4,000 m. By contrast, age of 1,000 m in J5 station was ~ 1,310 years, and remained relatively stable below 1,000 m with the age of about 1,650 years (Table 1). DOC concentrations ranged from 63.07 to 40.34 μmol/L, with the highest at the surface and the lowest at the deep. However, POC concentrations varied greatly between 0.5 and 2.1 μmol/L and showed great variations. The POC concentrations were highest at 3,000 m of the G3 station (1.8 μmol/L) and at 1,000 m of the J5 station (2.1 μmol/L) (Table 1).

3.2 Microbial cell abundances

The estimated abundances of bacteria and archaea were about 10^6 ~ 10^9 cells L^{-1} and ~~10^6~~-10^5 ~ 10^7 cells L^{-1}, respectively (Fig. 1). The FL bacterial fraction generally accommodated higher cell abundances (varying from 0.62 ×10^7 to 1.65 ×10^8 cells L^{-1}), several times higher than their corresponding PA fraction (1.85±0.02 ×10^6 ~ 1.70-90 ×~~10^9~~-10^8 cells L^{-1}). However, one remarkably lower abundance of FL bacterial fraction than PA fraction was detected in the surface water (50 m) of the G3 station where PA bacterial abundance was up to 1.23-70 ×10^9 cells L^{-1}, two orders of magnitude higher than that of the FL fraction (1.62 ×10^7 cells L^{-1}) (Fig. 1a). Similar to bacteria, the FL archaeal fractions usually showed higher cell abundances than their PA fractions (Fig. 1b). The only exception was also at the depth of 50 m of G3 station where the estimated PA archaeal cell abundance (6.50±0.01 ×10^7 cells L^{-1}) was much higher than that of FL archaeal fraction (1.01 ×10^6 cells L^{-1}). FL archaeal fraction had the cell abundances between 2.70 ×10^5 and 8.62±0.03 ×10^6 cells L^{-1}, while PA archaeal fractions fluctuated between 1.9028 ×10^5 and 6.50±0.01 ×10^7~~5.54 ×10^6~~ cells L^{-1} (Fig. 1). ~~The upper seawater layers (50 m and 200 m) were also inhabited with the highest abundance of archaea. FL archaeal fraction had the cell abundances between 1.01 ×10^6 and 8.62 ×10^6 cells L^{-1}, while that of PA archaeal fraction ranged from 1.28 ×10^5 to 6.50 ×10^7 cells L^{-1}. At other depths, cell densities of~~

~~archaeal FL fraction varied between 1.01 - 3.88 ×10^6 cells L^{-1} and 0.74 - 8.62 ×10^6 cells L^{-1} for G3 and J5 stations, respectively. PA archaeal fraction fluctuated between 1.90 ×10^5 and 5.54 ×10^6 cells L^{-1}. Similar to bacteria, the FL archaeal fractions usually showed higher cell abundances than their PA fractions (Fig. 1b).~~

3.3 Estimation of microbial diversity

Totally ~~92~~91,~~041~~692/81,~~761~~332 and ~~73~~72,~~094~~590/~~97~~93,~~611~~059 valid sequences of bacterial 16S rRNA gene were obtained for FL/PA fractions of G3 and J5 stations, respectively. ~~The average valid sequences, including both PA and FL bacteria were 14, 354 sequences per depth.~~ Based on the 97% similarity, these FL and PA bacterial sequences were defined into a total of 6,~~666~~320 operational taxonomic units (OTUs) in which 1,982 OTUs belonged to singletons and were finally removed from the valid OTU table (Table S1). ~~The number of OTUs in the FL and PA bacterial fractions at each depth ranged from 214 to 1,470 (Table S1).~~ Correspondingly, 50,~~736~~727/41,~~719~~511 and 44,~~456~~443/~~38~~37,~~333~~751 archaeal sequences were determined for FL/PA archaeal fractions of G3 and J5 stations. Attempt to determine PA archaeal sequence from 3,000 m depth of G3 station and 4,000 m depth of J5 station failed because of technical reasons. ~~The average number of archaeal sequences (including PA and FL archaea) were 7,966 sequences per depth.~~ A total of 1,~~071~~070 archaeal OTUs were defined and ~~the number of OTUs for the FL and PA archaeal fractions varied from 82 to 275~~329 OTUs were considered as singletons (Table S2). The sequence~~i~~ing depths of 16S rRNA gene were shown in their rarefaction curves (Fig. S2).

Shannon's diversity (H) and Chao1 were calculated to estimate microbial diversity of both PA and FL fractions at all depths (Fig. 2 and Fig. ~~S2~~S3). In most cases, the H indices of the bacterial FL fractions were ~~always~~ usually higher than their PA counterparts at each depth (Fig. 2). H index of FL and PA bacterial fractions gradually increased from 50 to 1,000 m, decreased in the intermediate water of~~from 1,000 to~~ around 2,000 m depth, and increased again ~~from~~at ~~2,000~~3,000 and~~to~~ 4,000 m (Fig. 2a). Archaeal H index varied along the vertical profiles with a ~~Similar~~ trend similar to bacteria, and ~~,~~FL archaea generally had higher H index values than the PA fraction (Fig. 2b). In addition, it was further observed that even at the same depth, the values of H index between two stations fluctuated a lot. ~~The H index was usually the lowest at the surface, increased to the highest value at 200 m or 1,000 m and decreased continuously into the deep (Fig. 2b).~~ Chao1 index showed nearly similar variation trends for both PA and FL microbial fractions (Fig. ~~S2~~S3).

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. Overall, three groups were distinguished, the surficial 50 m group, the FL group, and the PA group (Fig. 3). One ~~incompact~~ group, consisted exclusively of samples at 50 m depth, separated the microbes in the surface from those in the rest of the water column of both stations, irrespective of microbial lifestyles (FL or PA). However, the other two groups were separated mainly based on the FL and PA lifestyles. It is interesting to note that the FL bacterial samples clustered into one group where samples were further partitioned with respect to depth (Fig. 3a). Canonical correspondence analysis (CCA) showed that fundamental environmental parameters including depth, DO, salinity, seawater age, DOC and POC concentration, and silicate exerted potential impact on variations of FL and PA microbial

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communities along the water column (Fig. 4, Fig. ~~S3~~S4). Mantel test further indicated that all those factors, except POC concentration (P =0.164), were the statistically significant variables associated with variation of PA and FL fractions (P =0.001).

3.4 Taxonomic compositions of the PA and FL bacterial and archaeal fractions

Taxonomic compositions of FL and PA bacterial fractions and their relative abundances are presented in Fig. 5. At phylum level, bacterial sequences were mainly assigned into *Proteobacteria* (*α*-, *β*-, *γ*-, and *δ*-), *Actinobacteria*, *Cyanobacteria*, *Planctomycetes*, *Bacteroidetes*, *Marinimicrobia* (SAR406 clade), *Chloroflexi*, *Firmicutes*, *Acidobacteria*, *Gemmatimonadetes*, ~~*Gracilibacteria*~~ *Nitrospinae* and *Verrucomirobia*. The taxa at ~ family level with relatively high abundances (>3%) on average in either PA or FL fraction were further shown in Fig. 6.

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It is clear that *α*- and *γ*-*Proteobacteria* were the dominant lineages in both the FL and PA fractions at nearly all depths. In most cases, the sum of *α*- and *γ*-*Proteobacteria* accounted for ~ 40% to nearly 90% (Fig. 5). Moreover, their relative abundances in different PA and FL fractions and different stations also varied widely. Within the *α*-*Proteobacteria*, the dominant families included *Methylobacteriaceae*, *Phyllobacteriaceae*, *Rhodobacteraceae* and *Erythrobacteraceae* (Fig. 6). Members of the families *Methylobacteriaceae* and *Erythrobacteraceae* occurred commonly in both fractions at almost all depths but usually with higher proportions in PA fractions. The family *Rhodobacteraceae* occurred commonly in both fractions at every depth (1 % ~ 20%), while the *Phyllobacteriaceae* was dominantly distributed in the PA fraction of 2,000 m depth of J5 station with > 60% proportions. In addition, another important lineage within *α*-*Proteobacteria* is SAR11 clade (now named as *Pelagibacterales*) (Grote et al., 2012). It was clearly revealed that SAR11 clade showed relative higher abundances in FL fractions than PA fractions. Moreover, at depths above 1,000 m, SAR11 clade had a far higher proportion than the deep ocean and the maximum levels occurred at 200 m depth (20% ~ 24%) (Fig. 6, Table S1). *γ*-*Proteobacteria* ~~is~~ was another lineage with the highest abundance overall. Its relative abundances changed significantly with depths and in different fractions. The minimum abundances were only 1% ~ 5%, while the maximum were up to 73% ~ 80% (Fig. 5 and Table S1). Moreover, G3 station generally had higher *γ*-*proteobacteria* proportions than that of J5 station on average. As shown in Fig. 6, although sequences of *γ*-*Proteobacteria* were classified into multiple families, actually only two families *Alteromonadaceae* and *Pseudoalteromonaodaceae* exhibited absolutely dominant prevalence in the bacterial populations. The *Pseudoalteromodaceae* populated predominantly the PA fractions in 50 m and 200 m depths (66% ~ 75%), while the *Alteromonadaceae* mainly dominated the PA fractions in the deep water, particularly at 2,000 m and 3,000 m depths. *δ*-*Proteobacteria* also had a common distribution in both fractions of all depths, usually accounting for less than 10% proportions in most samples (Fig. 5), and SAR324 clade members contributed significantly to the dominance of the *δ*-*Proteobacteria* (Fig. 6). *Actinobacteria* and *Cyanobacteria* were abundantly distributed only in the surficial 50 m depth, and by sharp contrast, their proportions in other depths were less than 5%. Other bacterial lineages which had a wide distribution in all depths but only with minor abundances in both fractions included *Planctomycetes*, *Bacteroidetes*, *Marinimicrobia* (SAR406 clade), *Chloroflexi*, *β*-*Proteobacteria*, *Firmicutes*, *Gemmatimonadetes* and *Verrucomicrobia* (Fig. ~~S4~~S5).

Majority of archaeal amplicons were mainly fallen into the *Nitrosopumilales* and several uncultured taxonomic lineages. (Fig. 7 and Fig. S5S6). Both FL and PA archaeal fractions at all depths were principally populated by the order *Nitrosopumilales* Marine Group I (MGI) (formerly referring to MGI.1a, a subclade of MGI) (Qin et al., 2017) of the *Thaumarchaeota* and Marine Group II (MGII) of the *Euryarchaeata*. Members from MGI the *Nitrosopumilales* and MGII lineages generally contributed more than 80% relative abundances in their respective clone libraries. MGI The *Nitrosopumilales* was always one of the most abundant clades along the vertical profiles except in the topmost FL and PA fractions. Within the MGI group, only a small part of members were annotated into the cultured genus *Nitrosopumilus* and *Candidatus* Nitrosopelagicus, while the majority of them fell into those uncultured subclades (Table S2). MGII clade exhibited a wide distribution along the water columns, and it usually accounted for the large proportions in both archaeal size fractions. The photic layer (~ 50 m depth) contained the highest abundances of MGII clade, particularly in FL fractions with up to ~ 80% proportions. By sharp contrast, the lowest abundances of MGII occurred at 2,000 m (G3 station) and 3,000 m (J5 station) depths, making up <20% percentagesproportions. The third most abundant clade overall is Marine Group III (MGIII) of the *Euryarchaeata*. MGIII representatives were mainly dispersed in the FL fractions with 5% ~ 18% abundances, while they were absent from most of the PA fractions. However, the relative abundances of MGIII members in PA fractions of 1000 m depth could be as high as 30% ~ 45% (Fig. 7). The order *Methanosarcinales* of *Euryarchaeata* was detected commonly in most PA fractions, but it had the higher abundance only in the upmost 50 m depth (~ 29.7%) (Fig. 7). Another sample accommodating relatively much *Methanosarcinales* was the PA faction of 3,000 m in J5 station with 9.1% proportion. Within the *Euryarchaeata*, another clade of methanogens, *Methanobacteriales*, was also detected from both size fractions but with low relative abundances (<5%) (Fig. 7, Fig. S5S6, Table S2). In addition, other archaeal lineages included *Woesearchaeota* (formerly known as the DHVEG-6 group), Miscellancous Crenarchaeotic Group (MCG, now named as *Bathyarchaeota*), the *Halobacteriales* of the *Euryarchaeata* and Marine Benthic Group A (MBG-A) of the *Thaumarchaeota*. They just provided a limited contribution to archaeal populations (Fig. S5S6).

3.5 Bacterial preference to PA or FL lifestyles

Odds ratio was used to assess the preference of bacterial taxonomic lineages to the PA or FL lifestyle. A positive odds ratio indicates PA preference or higher abundance in the PA fraction, while a negative value suggests FL preference or higher abundance in the FL fraction. The bacterial lineages dominating the PA fractions come exclusively from *α*- and *γ*-*Proteobacteria* with some relatively abundant *δ*-*Proteobacteria* and *Planctomycetes* at specific depths (Fig. 65). By contrast, although the predominant lineages of FL fractions also mainly consisted of members of *α*- and *γ*-*Proteobacteria*, other abundant lineages were more diverse including *Actinobacteria*, *Cyanobacteria*, *Bacteroidetes*, *Marinimicrobia* and *δ*-*Proteobacteria*, as shown in Fig. 5. As shown in Fig. 8, we listed those lineages at ~ family level with high proportions (> 1%) with their odds ratios along the depth profiles. It was suggested that At family level, themost of the absolutely dominant families clades of PA fractions comprised of the *Phyllobacteriaceae*, and *Methylobacteriaceae*, *Erythrobacteraceae*, *Rhodobacteraceae* (*α*-*Proteobacteria*), and *Pseudoalteromonadaceae*, and *Alteromonadaceae* (*γ*-*Proteobacteria*).) (Fig. 6) and they showed a clear preference to PA lifestyle at different depths (Fig. 8). However, the α-proteobacterial *Rhodobacteraceae* and *Erythrobacteraceae* prevailing in PA

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fractions preferred to different lifestyles at different depths (Fig. 8). Compared with those PA-preferred lineages, there is a wider range of lineages showing preference to FL lifestyle. ~~,~~ These~~Except for these prevalent families, there is a wide range of lineages also showing preference to particle-attached lifestyle but with relatively low abundance (Fig. 6 and Fig. 8). These minor lineages are mainly populated by the families *Oceanospirillaceae* and *Alcanivoracaceae* (*γ-Proteobacteria*), *Sandaracinaceae* and *Bdellovibrionaceae* (*δ-Proteobacteria*), *Burkholderiaceae* (*β-Proteobacteria*), *Saprospiraceae* (*Bacteroidetes*), *Planctomycetaceae* and *Phycisphaeraceae* (*Planctomycetes*), SAR406 clade (*Marinimicrobia*), *Cryomorphaceae* and *Flavobacteriaceae* (*Bacteroidetes*), *Propionibacteriaceae*, *Nocardioidaceae* and *Corynebacteriaceae* (*Actinobacteria*).~~

~~The predominant lineages of FL fractions mainly consisted of members of *Actinobacteria*, *Cyanobacteria*, *Bacteroidetes*, *α-* and *δ-Proteobacteria*, as shown in Fig.5. At family level, the~~ phylogenetic lineages ~~with showing a FL preference~~ are mainly populated by the ~~families~~ OM1 clade and Sva0996 marine group (*Actinobacteria*), ~~SAR324 clade and *Nitrospinaceae* (*δ-Proteobacteria*),~~*Nitrospinaceae* (*Nitrospinae*), ~~*Cyanobacteria*,~~ *Planctomycetaceae* (*Planctomycetes*), SAR11 clade (*α-Proteobacteria*), SAR324 clade (*δ-Proteobacteria*), SAR86 clade and *Thioglobaceae* (*γ-Proteobacteria*). It is important to point out that a considerable number of bacterial lineages exhibited their preferences to both PA and FL lifestyles, though preferring differently at different depths or locations (Fig. 8). Actually, ~~*Comamonadaceae* (*β-Proteobacteria*), *Erythrobacteraceae*, SAR11 clade, *Methylobacteriaceae*, *Bradyrhizobiaceae*, *Rhodobacteraceae*, *Hyphomonadaceae* (*α-*Proteobacteria), *Phycisphaeraceae* and *Phycisphaeraceae* (*Planctomycetes*), SAR406 clade, *Saprospiraceae*, *Chitinophagaceae*, *Cryomorphaceae*, *Flavobacteriaceae*, *Flammeovirgaceae* (Bacteroidetes) (Fig. 8). However, compared with counterparts of PA fractions, their abundances in FL fractions are low without absolute dominance.~~Except for these prevalent families, there is a wide range of lineages also showing preference to particle-attached lifestyle but with relatively low abundance (Fig. 6 and Fig. 8). These minor lineages are mainly populated by the families *Oceanospirillaceae* and *Alcanivoracaceae* (*γ-Proteobacteria*), *Sandaracinaceae* and *Bdellovibrionaceae* (*δ-Proteobacteria*), *Burkholderiaceae* (*β-Proteobacteria*), *Saprospiraceae* (*Bacteroidetes*), *Planctomycetaceae* and *Phycisphaeraceae* (*Planctomycetes*), SAR406 clade (*Marinimicrobia*), *Cryomorphaceae* and *Flavobacteriaceae* (*Bacteroidetes*), *Propionibacteriaceae*, *Nocardioidaceae* and *Corynebacteriaceae* (*Actinobacteria*).

~~A~~at OTU level, ~~near~~less than 1/2 of the total OTU ~~numbers~~ (2005 out of 4338 OTUs) ~~are~~were shared by PA and FL fractions (Fig. S7). Phylogenetically, these PA/FL-shared OTUs ~~are~~were ~~mainly~~mostly fallen into *α-*, *γ-*, *δ-Proteobacteria*, *Planctomycetes*, *Chloroflexi*, *Bacteroidetes*, *Marinimicrobia* and *Actinobacteria*. ~~Moreover,~~ The taxonomic components of the PA/FL-shared OTUs at different levels are approximately ~~primarily~~ similar to ~~those of~~ OTUs retrieved exclusively from either the PA fractions or the FL fractions (Table S1, Fig. S7).

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4. Discussion

4.1 Comparison of microbial abundance and diversity between PA and FL fractions

PA bacterial and archaeal fractions show generally lower abundance and taxonomic richness than their FL counterparts and constitute a small fraction of the total abundances. Our results are consistent in principle with previous reports on various pelagic environments, in either the euphotic zone, twilight or the dark deep ocean (Turley and Stutt, 2000; Simon et al., 2002; Ghiglione et al., 2007; Rieck et al., 2015). However, in some eutrophic and notably particle-rich marine ecosystems, for example, marine snow or estuaries, PA bacterial fractions were present in higher local concentrations and greater diversity than FL bacteria (Caron et al., 1982; Karner and Herndl, 1992; Turley and Mackie, 1994; Garneau et al., 2009). In upper photic zone, PA bacterial abundance and their contribution to total bacterial biomass are highly variable, and depend largely on the quantity and quality of suspended organic particles (Cammen and Walker, 1982; Simon et al., 2002; Doxaran et al., 2012). This is indeed the case in the South China Sea. As shown in Fig. 1, at 50 m and 200 m depths of G3 station, PA bacterial abundances outnumbered FL bacteria by nearly 2 ~ 100 times, whereas J5 station has an opposite trend. However, as shown in Table 1, these two stations have almost the same environmental parameters, particularly in POC concentrations. One possibility may be that G3 and J5 have different POC compositions, attributable to different origins of organic matter (Chen et al., 2015; He et al., 2016; Liang et al., 2018). Although bacteria attaching to particles are of relatively lower abundance compared to free-living cells in the pelagic ocean, they are consistently metabolically more active with higher extracellular enzymatic activities (Karner and Herndl, 1992) and cell-specific thymidine incorporation rates (Turley and Mackie, 1994; Turly and Stutt, 2000). Therefore, PA bacteria often play a comparable role to free-living bacteria in hydrolysis or decomposition of marine organic matter, biomass production and carbon cycling (Griffith et al., 1994; Turly and Stutt, 2000; Liu et al., 2015). The decline of bacterial abundance and richness along the depth profile is largely owing to the gradual decreasing availability of usable organic carbon (Smith, 1992; Turly and Stutt, 2000; Jiao et al., 2014). It is interesting that the mid-water around 2000 m depth showed the lowest bacterial diversity (Fig. 2, Fig. S3). One possibility is that 1,500-2,000 m is roughly a ~~rough~~ boundary for different water masses in the deep, central basin of the South China Sea. The deep water masses (>2600 m) of the central basin coming from the western Pacific Ocean through the Bashi Channel are relatively rich in nutrients than the mid-water masses of the SCS. Therefore, it may cause a relative increase in microbial diversity in deep water masses such as those at 3,000 m and 4,000 m. In addition, some "old, deep" water from the bottom of the central basin will also rise to around 2,000 m depth because of the basin-scale circulation. These old waters are relatively enriched in refractory DOC (RDOC), remained after microbial utilization of labile DOC during their circulation, potentially reducing microbial diversity. This hypothesis is partly supported by the seawater age at J5 station. It is shown that the age of seawater at 2,000 m depth of J5 station is 1,670 years, roughly equal to those of deep waters at 3,000 m and 4,000 m (1,680 years and 1,610 year). In contrast, archaea are commonly much lower in cell abundance and community diversity compared with their bacterial counterparts at the same depths (Fig. 1, Fig. 2 and Fig. ~~S2~~S3). The relative abundance of archaeal populations in total prokaryotes increases gradually with depth, indicative of a potential rising impact on biogeochemical cycle in marine environments. In addition, pronounced distinction in microbial community structures

of PA and FL assemblages were observed along the depth profile, which were well supported by results of statistical analyses (Fig. 3). It is expectable that PA microbial fraction differs ~~taxonomically~~ from FL fraction, considering their discrepant activity patterns for survival. Related discussions are shown below.

4.2 Environmental factors potentially shaping microbial community structure

Several environmental parameters ~~were supposed to~~ played a pivotal role in structuring microbial communities of seawater. Hydrological condition (e.g. depth)~~Depth~~, together with age and salinity of water mass, are a key subset of environmental drivers (Fig. 4). Recent studies have shown that microbial populations in the meso-/ bathypelagic ocean are largely dissimilar to those of the epipelagic zone (Salazar et al., 2015; Milici et al., 2017; Liu et al., 2018a), indicative of a crucial environmental selection process exerted by depth. In our study, PCoA analysis revealed that PA and FL fractions from the surficial zone (50 m) were clustered into a separate but relatively loose group distant from other depths (Fig. 3), indicative of the influence imposed from depth in shaping microbial community structures. Several bacterial lineages, including *Cyanobacteria*, *Actinobacteria*, *δ-Proteobacteria*, *Marinimicrobia* (SAR406 clade) and *Firmicutes* with distinct distributing stratification contribute to this dissimilarity (Fig. 5). *Cyanobacteria* and *Actinobacteria* belong to typical phototrophs (Mizuno et al., 2015) and they are prevalently distributed in euphotic zones. By contrast, δ-proteobacterial SAR324 clade, as shown in our results, are primarily found in mesopelagic waters (200 ~ 1,000 m) (Fuhrman and Davis, 1997; Wright et al., 1997). SAR406 clade has a ubiquitous distribution across diverse marine niches, however, its high abundance always occurs within the mesopelagic zones, ~ five times or higher than in surface ocean (Yilmaz et al., 2016). Archaeal population components also reflect the impact~~ion~~ of depth. Euphotic zones hold less abundant thaumarchaeotal ~~MGI~~ *Nitrosopumilales* and more euryarchaeotal *Methanosarcinales* and *Woesearchaeota* (Fig. 7), while marine thaumarchaeotal groups are more abundant in meso- and bathypelagic waters (Karner et al., 2001; Mincer et al., 2007; Varela et al., 2008). In addition, Salazar et al. (2016) found that sampling depth appears to have a more direct impact on free-living bacterial communities. Our results are highly consistent with this observation in that FL bacterial fractions from the same depth grouped together irrespective of their sampling locations (G3 or J5 station) (Fig. 3a).

DO concentration is observed to strongly affect particle flux and particle transfer efficiency from euphotic zone to the deep sea since remineralization of organic particles appears to be oxygen-dependent (Laufkotter et al., 2017; Cram et al., 2018). ~~It~~ DO is considered as one of the ~~best subsets of~~most crucial ~~–~~environmental variables for shaping the compositions of particle-attached bacterial assemblages (Salazar et al., 2016). Some taxonomic lineages are directly affected by oxygen. For example, a ~~most~~ recent study found that oxygen is one of the key factors driving the distribution and evolutionary diversity of *Woesearchaeota* (Liu et al., 2018b). POC and DOC can be substrates for both PA and FL communities, respectively (Azam and Malfatti, 2007; Zhang et al., 2016; Liu et al., 2019). 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). We hypothesize that the quality rather than the quantity of POC imposes a decisive influence on microbial populations, especially in the deep, dark ocean. During ~~the~~ POC sinking from surface through the water column, and also as seawater ages, the labile organic matter becomes increasingly decomposed, while the more

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refractory material remains and resists further degradation (Simon et al., 2002). In such cases, utilization of the ~~refractory~~ POC in the deep sea by microorganisms depends on the quality and quantity of the remaining POC. Meanwhile, in older seawater, DOC also become more refractory because free-living microorganisms preferentially utilize labile DOC and the remained refarcotory DOC gradually accumulates, which potentially affect microbial community structures. Among common nutrients, silicate exhibited statistically significant correlation with microbial distributions (Fig. ~~S3~~S4), and this is ~~out of our expectation~~unexpected because the SCS generally ~~shows~~ exhibits N- or P-limited ~~in~~ phytoplankton production (Wu et al., 2003; Chen et al., 2004). However, recent research found that near the sampling site of this study, there is a clear silicon deficiency in the euphotic zones shallower than 75 m (Huang et al., 2015), which directly influences the diversity and biomass of phytoplankton (for example, diatom), and consequently, the quantity and quality of POM transported to the deep along the vertical water columns, and finally exerts a potential impact on microbial communities. Some bacterial lineages such as the *Rhodobacteraceae*, *Flavobacteriaceae*, *Oceanospirillaceae* and SAR11 clade, commonly retrieved in our present study, have been confirmed to be closely related to marine diatom blooms (Zhang et al., 2018; Monnich et al., 2020). ~~–~~Actually, microbial community structure and their distribution along the water column profile are a comprehensive combination impacted by multiple environmental variables.

4.3 Specialist or generalist for PA and FL lifestyle: clues from bacterial community compositions

It was ~~suggested~~ indicated that PA and FL bacterial fractions generally accommodated different ~~phylogenetic~~ community compositions along the depth profiles (Fig. 3), consistent with previous reports in various marine ~~niches~~ habitats (Acinas et al., 1997; Moeseneder et al., 2001; Ghiglione et al., 2009; Salazar et al., 2015). However, in most cases, taxonomic compositional disparity between the two filtration fractions does not seem much apparent at least at phylum level (Fig. 5). Actually, a few studies also confirmed that at high taxonomic ranks, bacteria show conserved lifestyles either in association with particles or as free-living microorganism (Eloe et al., 2011; Salazar et al., 2015; Liu et al., 2018a). The pronounced contrast in population compositions of the two filtration fractions was unveiled only at greater taxonomic level and a considerable number of phylogenetic taxa exhibited different preferences to PA or FL lifestyles. ~~As~~ It was shown in Fig. 5 and Fig. 6~~,~~ that as the most abundant members, *α*- and *γ*-*Proteobacteria* occurred prevalently in both filtration fractions, but at the family level, most of predominant bacterial lineages of PA and FL fractions were significantly ~~significantly~~ divergent, indicating their preference to different microhabitats shaped by organic particles and environmental parameters. The dominant lineages in PA fractions were mainly associated with the families *Pseudoalteromonadaceae* and *Alteromonadaceae* within *γ*-*Proteobacteria*, and the *Methylobacteriaceae* and *Phyllobacteraceae* within *α*-*Proteobacteria*. These γ-proteobacterial members are usually retrieved from diverse marine habitats as the typical PA clades, and they are believed to have the abilities to degrade/utilize HMW organic compounds with higher nutrient requirements (DeLong et al., 1993; Crespo et al., 2013). The adhesion to particles could make them increase nutrients acquisition and avoid the nutrient-depleted conditions (Crespo et al., 2013). By contrast, members of *α*-*Proteobacteria* are rarely reported as the dominant lineages of PA fraction or particle-attached preference (Crespo et al., 2013; Rieck et al., 2015; Suzuki et al., 2017), which is inconsistent with our results revealing α-proteobacterial lineages frequently prevailed as PA members. Further phylogenetic ~~assignment~~ analysis revealed that the majority of α-proteobacterial PA members

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~~exclusively~~ belonged to the genus *Methylobacterium* which are strictly aerobic, facultatively methylotrophic bacteria, and can grow on a wide range of carbon compounds (Green, 2006). They probably benefit from the particle-attached lifestyle, making their high requirements for organic matters easily to achieve. Compared with bacterial PA counterparts, FL bacterial communities are more diverse, and dominant populations are scattered in more phylogenetic taxa with relatively homogeneous proportions (Fig. 8). Among the predominant lineages, the actinobacterial OM1 cade and cyanobacteria ~~dominantly~~ dominate ~~govern~~ the upper surficial waters (Fig. 6), likely attributed to their phototrophic behaviors. Although actinobacteria are recognized as ubiquitous members of marine bacterioplankton (Giovannoni and Stingl, 2005), they are scarcely reported with predominance (Milici et al., 2016a). ~~Recently,~~ Ghai et al. (2013) revealed the OM1 clade members possess the smallest cell sizes with streamlined genome, representing a typical adaption to oligotrophic condition (Giovannoni et al., 2014) which well agrees with the oligotrophic environments in the SCS (~~liLi~~Gong et al., 20142). Other predominant FL lineages include α-proteobacterial SAR11 clade, δ-proteobacterial SAR324 clade, and *Marinimicrobia* (SAR406 clade), all usually being the most ubiquitous free-living bacterial lineages and dominantly distributed in epi- and mesopelagic zones (Grote et al., 2012; Tarn et al., 2016; Yilmaz et al., 2016; Milici et al., 2017; Liu et al., 2018a). Genomic information ~~underlines~~ suggests that although these clades have a flexible metabolism utilizing multiple hydrocarbon compounds, they generally lack of carbohydrate-active enzyme genes for the attachment to and the degradation of particulate organic matter (Peoples et al., 2018), consistent with their preference to free-living lifestyle rather than particle-attachment (Eloe et al., 2011; Salazar et al., 2015; Tarn et al., 2016). In addition, the percentages of SAR11 clade revealed here seem to be relatively lower~~er~~ compared with those ~~of~~reported in previous studies ~~in which~~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 ~~the low detection~~underestimation of SAR11 clade and bias the interpretation of their relative abundances (Aprill et al., 2015).~~–~~

In addition to those predominant lineages mentioned above, there are a couple of bacterial taxa showing evident PA or FL preferences. At ~ family level, these PA- or FL-preferred taxa are well hinted by their odds ratio between PA and FL fractions. These bacterial lineages are characterized by low abundances or occasional occurrences in water columns (Fig. 6, Table S1) but high odds ratio (absolute value) (Fig. 8), indicating their strong preferential divergence in the two size fractions. ~~As shown in Fig. 8, such families with PA preference were mainly derived from the phyla/classes *Actinobacteria* and *γ-Proteobacteria*, together with several families from *Bacteroidetes*, *Planctomycetes* and *δ-Proteobacteria*, while FL-preferred lineages are mostly distributed within *α* , *γ-Proteobacteria*, *Actinobacteria* and *Bacteroidetes*, along with certain groups of *β* , *δ-Proteobacteria*, *Planctomycetes* and *Firmicutes*.~~ The majority of these lineages are recorded consistently about their PA- or FL preferences in previous studies, and commonly possess the ability to hydrolyze and utilize complex carbon sources. Although their abundance is low, these relatively minor populations can still effectively influence local microhabitats because of their high specificity for organics. In contrast, there are still some populations which are scarcely reported. For example, Sva0996 marine group, an actinobacterial group, is retrieved occasionally from marine sediments and upper ocean (Bano and Hollibaugh, 2002; Wang et al., 2018). Orsi et al. (2016) first found this group prefers to free-living lifestyle in upper seawater and have the ability to assimilate phytoplankton-derived dissolved protein. Our present results suggest that Sva0996 group are flexible to adapt PA or FL lifestyles at the surface seawater because two lifestyles occur concurrently. Moreover, the distribution of Sva0996 group is not

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restricted only in upper photic ocean, and they can survive in meso- and bathypelagic seawaters with the significant preference for free-living lifestyle (~~odds ratio for FL preference is up to 3.93~~Fig. 8). However, due to lack of pure culture or their genome information, it is not yet ~~nothing is available~~possible ~~–~~to elaborate their preferences for ~~selection between~~ PA and FL lifestyles ~~due to lack of pure culture or their genome information~~.~~–~~

A high proportion of bacterial lineages are revealed to co-occur in both PA and FL fractions (Fig. 8 and Fig. S7)~~. At OTU level, more than 1/3 of total OTU numbers (2402 out of 6964 OTUs) are shared by PA and FL fractions (Fig. 9). Phylogenetically, these PA/FL shared OTUs are mainly fallen into α, γ, δ *Proteobacteria*, *Planctomycetes*, *Bacteroidetes* and *Actinobacteria*. Moreover, taxonomic components of PA/FL shared OTUs at different levels are primarily similar to those of OTUs retrieved exclusively from PA fractions or FL fractions (Table S1, Fig. 9)~~, indicating that a considerable amount of bacterial lineages potentially have PA and FL dual lifestyle strategies ~~(Bauer et al., 2006; Gonzalez et al., 2008)~~. On the one hand, as shown in Fig. 6, a few bacterial ~~lineages~~ lineages ~~such as *Flavobacteriaceae*, *Planctomycetaceae*, *Rhodobacteraceae*, *Erythrobacteraceae*, *Burkholderiaceae*, *Nitrospinaceae*, SAR324 clade, *Alteromonadaceae*, *Pseudomonadaceae* and *Salinisphaeraceae* co-~~occur in PA and FL fractions at least at one of the same depths with approximately equivalent abundances. In such cases, their odds ratios are close to zero or minor range (Fig. 8), indicating that these bacteria are able to employ two different survival strategies at the same time. On the other hand, ~~some~~lots of taxa ~~including the families Sva0996 marine group, *Flavobacteriaceae*, *Phycisphaeraceae*, *Rhodobacteraceae Methylobacteriaceae*, *Erythrobacteraceae*, *Pseudoalteromonadaceae*, *Halomonadaceae* and *Moraxellaceae*,~~ show divergent preferences to PA or FL lifestyles at different depths or different locations. This is clearly evident by the shift or conversion of their odds ratios at different depths along the vertical profiles of water column (Fig. ~~9~~8), indicative of their different adaption tactics to different environments. One possible explanation is that most of the marine bacteria are generalists with dual life strategies (Bauer et al., 2006; Gonzalez et al., 2008), and able to grow in suspension as well as on particles (Lee et al., 2004; Grossart et al., 2006, 2010). For instance, PA bacteria must be capable of surviving freely in the water column to migrate and colonize new organic particles (Ghiglione et al., 2007; Crespo et al., 2013). Bacterial populations may switch their lifestyles between free-living and particle-attachment, depending on substrate availability and the surrounding chemical triggers (Grossart, 2010; D'Ambrosio et al., 2014). To date, one exception, the genus *Scalindua* in the *Planctomycetes* phylum, which is a known marine chemoautotroph involved in anammox, is exclusively observed in FL fractions in previous studies (Fuchsman et al., 2012; Ganesh et al., 2014; Suter et al., 2018). However, it is absent from our water columns.

4.4 Archaeal community preferences to PA and FL lifestyles

Samples of PA and FL archaeal fractions were also separated into different groups by statistical analysis (Fig. 3b~~)~~~~(~~, Table S3), indicating their ~~phylogenetically~~ different community structures. However, because most of OTUs belonged to uncultured archaeon, it is impossible to assign them into taxonomic lineages at finer level. Thus, the distinction of archaeal population compositions between PA and FL fractions was unnoticeable (Fig. 7). The ~~MGI~~ *Nitrosopumilales* under MGI and MGII are the most abundant taxa in both PA and FL archaeal fractions. The thaumarchaeal ~~*MGI Nitrosopumilales* thaumarchaea~~ are one of the most abundant and cosmopolitan chemolithoautotrophs

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in the dark ocean (~~Karner~~ Konneke et al., 200~~1~~5) and responsible for much of the ammonia oxidation in this environment for their common metabolism of aerobic ammonia oxidation. Corresponding to their autotrophic metabolism, MGI (including *Nitrosopumilales*) generally exhibit free-living preference and are the prevalent archaeal taxa in free-living fractions below euphotic zone (Smith et al., 2013; Salazar et al., 2015; Tarn et al., 2016). However, different from our results, a few studies showed that MGI dominated both the PA and FL archaeal populations and no obvious distinction was observed in abundance and ecotype of MGI (Eloe et al., 2011; Jin et al., 2018). To date, only a few pure cultures of marine MGI, small rods with a diameter of 0.15~0.26 μm and a length of 0.5 ~ 1.59 μm and no flagella were observed (Könneke et al., 2005; Qin et al., 2014), suggesting that their occurrence in PA fraction is not caused by pore size of filter to fractionate different assemblages. One possibility is that decomposition of organic particles continuously releases ammonia and MGI can easily acquire high concentrations of ammonia by attaching to particles, especially in oligotrophic area. Recent studies provide another explanation to particle-attached MGI that some MGI cultures are obligate mixotrophy that rely on uptake and assimilation of organic compounds (Alonso-Sáez et al., 2012; Qin et al., 2014). In such case, PA lifestyle is in favor of their nutrient requirements. MGII have a wide distribution in the open ocean and as shown in our results, they are the dominant archaeal community generally within the upper euphotic zone (Massana et al., 2000; Martin-Cuadrado et al., 2015). Recently, they have been found, however, to be also abundant in deep-sea waters (Baker et al., 2013; Tarn et al., 2016; Liu et al., 2018a), showing a wider adaption to diverse marine habitats in addition to the photic zone. MGII are thought to be heterotrophs, and have the ability of degrading proteins and lipids (Iverson et al., 2012; Orsi et al., 2015). Metagenomes revealed a number of genes encoding cell adhesion, degradation of high molecular weight organic matter and photoheterotrophy (Rinke et al., 2019; Tully et al., 2019), evidencing their potentiality to utilize organic particles as important growth substrates. All these findings imply MGII's preference to particle-attached lifestyle, and they are frequently detected from PA fractions in size-fractionated studies (Iverson et al., 2012; Orsi et al., 2015; Tran et al., 2016). However, in a few studies including our present study, MGII are also identified as the dominant archaeal components from FL fractions, with equal or even more abundance than PA fractions (Fig. 7). Further studies confirm that genome contents and populations of free-living MGII are distinct from those of particle-attached MGII (Orsi et al., 2015; Rinke et al., 2019), suggesting their metabolic evolution and adjustment to niche partitioning. In addition, MGIII also occurred commonly in both fractions (Fig. 7). MGIII are usually retrieved as minor components of deep mesopelagic and bathypelagic communities (Galand et al., 2009; Tarn et al., 2016). Like MGII, to date no cultured representative of MGIII leads to little is known about their ecological and physiological characteristics. Function prediction from metagenomes suggest that MGIII are aerobic (or facultative anaerobic), motile, and heterotrophic, and potentially can utilize lipid, proteins and polysaccharides as major energy source (Martin-Cuadrado et al., 2008; Haro-Moreno et al., 2017). Recently, a novel lineage of MGIII genomes preferring to live in the photic zone was recovered, consistent with previous few studies and our present results in which MGIII populations are obtained from the euphotic zone with a considerable abundance (Galand et al., 2009, 2010). Moreover, recent findings also indicate that MGIII are inclined to be attached to other microorganisms (particle-attached preference) and only sporadically be released to the surrounding environments (free-living lifestyle) (Haro-Moreno et al., 2017).

In addition, there are several other archaeal lineages with remarkable differences in abundance between PA and FL fractions. The order *Methanosarcinales* and *Methanobacteriales*, affiliated to the

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phylum *Euryarchaeota* and retrieved exclusively from PA fractions (Fig. 7), belong to strictly anaerobic methanogens. Their preference to particle-attached lifestyle in the water column ~~environments~~ is intelligibl~~y convinced~~e. Within normal water column, seawater is usually oxic in spite of low oxygen concentration ~~and~~. ~~o~~Only on or inside the organic particles where heterotrophic microbes attach and digest organic matter using oxygen as electron acceptor, local anoxic niches are developed with the gradual exhaustion of ambient ~~oxygen~~ oxygen, and become suitable for the survival of anaerobic methanogens. Members of the *Woesearchaeota* were abundantly derived from the PA fraction of the upper seawater. In marine environments, *Woesearchaeota* are distributed restrictively in marine sediments (Lipsewers et al., 2018) or deep-sea hydrothermal vents (Takai et al., 1999), and are scarcely detected from pelagic seawater masses. Recent studies suggest that woesearchaeotal lineages are mostly retrieved from anoxic environments (Castelle et al., 2015; Liu et al., 2018b). Moreover, genomic metabolic analysis indicates *Woesearchaeota* have an anaerobic heterotrophic lifestyle with conspicuous metabolic deficiencies (Probst et al., 2017; Liu et al., 2018b), implying a potential syntrophic or mutualistic partnership with other organisms (Castelle et al., 2015; Liu et al., 2018b). It is further demonstrated that *Woesearchaeota* tend to co-occur with typical anaerobic methanogens from the *Methanomicrobia* and *Methanobacteria* constituting a potential consortia (Liu et al., 2018b). In our present results, at several depths, the *Methanosarcinales* of the *Methanomicrobia* and the *Methanobacteriales* of the *Methanobacteria*, together with *Woesearchaeota*, were detected concurrently, implying to a large extent their potential syntrophic partnership.

4.5 Potential vertical connectivity of microbial populations along the depth profile

Microbial distribution at different depths to a certain extent implicates their potential vertical connectivity along the water column profile. It has been suggested that the sinking of organic particles formed in upper euphotic zone is a main vector in transferring prokaryotes from the surficial ocean to deep waters (Mestre et al., 2018). Those surficial lineages, usually belonging to putative photosynthetic/photoheterotrophic, bacteriochlorophyll~~Bchl~~ a-containing microorganism or strict epipelagic/euphotic inhabitants, are reliable indicators to hint their downward transportation if they are detected from meso- or bathypelagic waters. For example, cyanobacteria are typical photosynthetic bacteria and their distribution is thought to be confined to the euphotic zone, with commonly observed maximum depths of about 150 ~ 200 m. In the present study, however, cyanobacterial lineages were retrieved throughout the whole water column (Fig. 5 and Fig. 6), especially at 4,000 m depth where cyanobacteria account for nearly 12% of the PA communities. Although a recent study revealed that cyanobacteria can dominate the deep continental subsurface microbial communities with the potential for a hydrogen-based lithoautotrophic metabolism instead of photosynthesis (Puente-Sanchez et al., 2018), these indigenous deep cyanobacteria were classified into the genera *Calothrix*, *Microcoleus* and *Chroococcidiopsis*, phylogenetically different from those prevailing in our study (*Prochlorococcus*, *Synechococcus*). Jiao et al. (2014) observed substantial *Prochlorococcus* populations at 1,500 m depth in the South China Sea, and suggested that multiple physical processes, including internal solitary waves and mesoscale eddies were responsible for the occurrence of these “deep *Prochlorococcus*”. However, in our study area, ages of seawater increase gradually from the surface to the deep along the water column profile in a normal time sequence (Table 1), refuting this possibility. Thus, a reasonable postulation here is that the sinking particles function as vectors and convey cyanobacteria attaching on particle surfaces from epipelagic zone into deep-sea waters. Likewise, members of the family

Erythrobacteraceae, which are largely represented by OTUs within the genus *Erythrobacter*, are also present abundantly in both PA and FL fractions at 4,000 m depth (Fig. 6). *Erythrobacter* spp. belong to putative bacteriochlorophyllBchl a-containing, aerobic anoxygenic photoheterotrophic bacteria and are thought to be distributed only in the euphotic upper ocean (Kolber et al., 2000; Koblížek et al., 2003). SAR11 clade, are potentially photoheterotrophic (Gomez-Pereira et al., 2013; Evans et al., 2015) and ubiquitous in global photic zones as one of the most abundant bacteria (Morris et al., 2002). We observed that members of SAR11 clade are distributed across the whole water columns, especially in mesopelagic aphotic depths with relatively high proportions. Other lineages specializing in inhabiting surface seawater but was also retrieved from the deep ocean include γ-proteobacterial SAR86 clade, SAR116 clade of marine Roseobacter and SAR202 clade within *Chloroflexi*. The majority of the OTUs within these "surface lineages" have been retrieved from the meso-/bathypelagic ocean and can be traced back simultaneously to those present in surface waters, suggesting their potential origin from the upper epipelagic zones.

5. Conclusions

In this study, we systematically compared bacterial and archaeal community structures within two different filtration fractions representing particle-attached and free-living lifestyles at different depths in the South China Sea. As revealed in previous studies, whatever for eother bacteria or archaea, the FL fractions usually show higher cell abundance and diversity than their PA counterparts at most depths. A set of environmental factors including depth, salinity, seawater age, DOC, POC, DO and silicate are considered playing important roles in structuring PA and FL microbial communities along the depth profile. On the one hand, as the result of adapting to different organic substrates available, PA and FL fractions generally accommodate significantly significantly divergent microbial compositions at each depth. At fine taxonomic levels, a considerable number of microbial lineages exhibited pronounced preferences to PA or FL lifestyles, also with distinct distributing stratificationstratified distribution along the depth profile. A few microbial taxa show potentially PA and FL dual lifestyle strategies, able to switch according to substrate availability and environmental variations, and implying versatile metabolic flexibility. In addition, according to some special microbial lineages supposed to be restricted in upper euphotic zones, we found that the sinking organic particles likely function as vectors into transfer prokaryotes transfer from surfaceicial ocean to deep waters, indicatingve of the potential vertical connectivity of prokaryotes along the water column profile.

Data availability

The pyrosequencing data obtained from the 454 sequencing of 16S rRNA genes were deposited in the Sequence Read Archive (SRA) database under accession ID PRJNA546072 for bacterial sequences and PRJNA546071 for archaeal sequences.

Author contribution

JL and JF designed the experiments, and JL, LG, JW and BW carried them out. JL, SB, LZ and LS treated and analyzed the sequence data. JL and JF wrote the manuscript with contributions from all co-authors.

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Competing interests

The authors declare that they have no conflict of interest.

References

Acinas, S.G., Rodríguez-Valera, F., Pedrós-Alió, C.: Spatial and temporal variation in marine bacterioplankton diversity as shown by RFLP fingerprinting of PCR amplified 16S rDNA, FEMS Microbiol. Ecol., 24, 27-40, 1997.

Alldredge, A.L., Cole, J.J. and Caron, D.A.: Production of heterotrophic bacteria inhabiting macroscopic organic aggregates (marine snow) from surface waters, Limnol. Oceanogr., 31, 68-78, 1986.

Alonso-Sáez, L., Waller, A.S., Mende, D.R., Bakker, K., Farnelid, H., Yager, P.L., Lovejoy, C., Tremblay, J.-É., Potvin, M., Heinrich, F., Estrada, M., Riemann, L., Bork, P., Pedrós-Alió, C. and Bertilsson, S.: Role for urea in nitrification by polar marine Archaea, Proc. Natl. Acad. Sci. USA., 109, 17989-17994, 10.1073/pnas.1201914109, 2012.

Arístegui, J., Gasol, J.M., Duarte, C.M., Herndl, G.J.: Microbial oceanography of the dark ocean's pelagic realm, Limnology and Oceanography, 54, 1501-529, 2009.

Azam, F., and Malfatti, F.: Microbial structuring of marine ecosystems, Nature Rev. Microbiol., 5, 782-791, 2009.

Baker, B.J., Sheik, C.S., Taylor, C.A., Jain, S., Bhasi, A., Cavalcoli, J.D., Dick, G.J.: Community transcriptomic assembly reveals microbes that contribute to deep-sea carbon and nitrogen cycling, ISME J., 7, 1962-973, 10.1038/ismej.2013.85, 2013.

Bano, N., and Hollibaugh, J.T.: Phylogenetic composition of bacterioplankton assemblages from the Arctic Ocean, Appl. Environ. Microbiol., 68, 505-518, 2002.

Bano, N., Ruffin, S., Ransom, B., and Hollibaugh, J.T.: Phylogenetic composition of Arctic Ocean archaeal assemblages and comparison with antarctic assemblages, Appl. Environ. Microbiol., 70, 781-789, 2004.

Bauer, M., Kube, M., Teeling, H., Richter, M., Lombardot, T., Allers, E., Wurdemann, C.A., Quast, C., Kuhl, H., Knaust, F., Woebken, D., Bischof, K., Mussmann, M., Choudhuri, J.V., Meyer, F., Reinhardt, R., Amann, R.I., and Glockner, F.O.: Whole genome analysis of the marine Bacteroidetes '*Gramella forsetii*' reveals adaptations to degradation of polymeric organic matter, Environ. Microbiol., 8, 2201-2213, 2006.

Cammen, L.M., and Walker, J.A.: Distribution and activity of attached and free-living suspended bacteria in the bay of fundy, Canadian Journal of Fisheries and Aquatic Sciences, 39(12), 1655-1663, 1982.

Caron, D.A., Davis, P.G., Madon, L.P., and Sieburth, J.M.: Heterotrophic bacteria and bacteriovorous protozoa in oceanic macroaggregates, Science, 218, 795-797, 1982.

Castelle, C., Wrighton, K., Thomas, B., Hug, L., Brown, C., Wilkins, M., Frischkorn, K.R., Tringe, S.G., Singh, A., Markillie, L.M., Taylor, R.C., Williams, K.H. and Banfield, J.F.: Genomic expansion of domain archaea highlights roles for organisms from new phyla in anaerobic carbon cycling, Current Biology, 25(6), 690-701, 2015.

Chen, W., Cai, P., Dai, M., Wei, J.: ^{234}Th/ ^{238}U disequilibrium and particulate organic carbon export in the northern South China Sea, Journal of Oceanography, 64, 417-428, 2008.

Chen, Y.L., Chen, H.Y., Karl, D.M., Takahashi, M.: Nitrogen modulates phytoplankton growth in spring in the South China Sea, Cont. Shelf Res., 24, 527-541, 2004.

Chen, M., Liu, H., Song, S. and Sun, J.: Size-fractionated mesozooplankton biomass and grazing impact on phytoplankton in northern South China Sea during four seasons, Deep-Sea Res. Part. II. Top. Stud. Oceanogr., 117, 108-118, 2015.

Cram, J.A., Weber, T., Leung, S.W., McDonnell, A.M.P., Liang, J.H., Deutsch, C.: The role of particle size, ballast, temperature, and oxygen in the sinking flux to the deep sea, Global Biogeochemical Cycles, 32(5), 858-876, 2018.

Crespo, B.G., Pommier, T., Fernadez-Gomez, B., and Pedros-Alio, C.: Taxonomic composition of the particle-

attached and free-living bacterial assemblages in the Northwest Mediterranean Sea analyzed by pyrosequencing of the 16S rRNA, MicrobiologyOpen, 2, 541-552, 2013.
Crump, B.C., Baross, J.A., and Simenstad, C.A.: Dominance of particle-attached bacteria in the Columbia River estuary, USA., Aquat. Microbial. Ecol., 14, 7-18, 1998.
D'Ambrosio, L., Ziervogel, K., MacGregor, B., Teske, A., and Arnosti, C.: Composition and enzymatic function of particle-associated and free-living bacteria: a coastal/offshore comparison, ISME J., 8, 2167-2179, doi:10.1038/ismej.2014.67, 2014.
Dang, H.Y., Chen, R.P., Wang, L., Shao, S.D., Dai, L.Q., Ye, Y., Guo, L., Huang, G., and Klotz, M.G.: Molecular characterization of putative biocorroding microbiota with a novel niche detection of epsilon- and zetaproteobacteria in Pacific Ocean coastal seawaters, Environ. Microbiol., 13(11), 3059-3074, 2011.
DeLong, E.F., Franks, D.G., and Alldredge, A.L.: Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages, Limnol. Oceanogr., 38, 924-934, 1993.
Dilly, O., Bloem, J., Vos, A., Munch, J.C.: Bacterial diversity in agricultural soils during litter decomposition, Appl. Environ. Microbiol., 70, 468-474, 2004.
Doxaran, D., Ehn, J., Bélanger, S., Matsuoka, A., Hooker, S., Babin M.: Optical characterisation of suspended particles in the Mackenzie River plume (Canadian Arctic Ocean) and implications for ocean colour remote sensing, Biogeosciences, 9, 3213-3229, 10.5194/bg-9-3213-2012, 2012.
Eloe, E.A., Shulse, C.N., Fadrosh, D.W., Williamson, S.J., Allen, E.E., and Bartlett, D.H.: Compositional differences in particle-associated and free-living microbial assemblages from an extreme deep-ocean environment, Environ. Microbiol. Rep., 3, 449-458, 2011.
Evans, P.N., Parks, D.H., Chadwick, G.L., Robbins, S.J., Orphan, V.J., and Golding, S.D., Tyson, G.W.: Methane metabolism in the archaeal phylum bathyarchaeota revealed by genome-centric metagenomics, Science, 350, 434-438, 2015.
Fuchsman, C.A., Staley, J.T., Oakley, B.B., Kirkpatrick, J.B., and Murray, J.W.: Free-living and aggregate-associated Planctomycetes in the Black Sea, FEMS Microbiol. Ecol., 80, 402-416, 2012.
Fuhrman, J.A., and Davis, A.A.: Widespread Archaea and novel Bacteria from the deep sea as shown by 16S rRNA gene sequences, Marine Ecol. Prog. Series, 150, 275-285, 1997.
Galand, P.E., Casamayor, E.O., Kirchman, D.L., Potvin, M., Lovejoy, C.: Unique archaeal assemblages in the Arctic Ocean unveiled by massively parallel tag sequencing, ISME J., 3, 860-869, 2009.
Galand, P.E., Gutiérrez-Provecho, C., Massana, R., Gasol, J.M., & Casamayor, E.O.: Inter-annual recurrence of archaeal assemblages in the coastal NW Mediterranean Sea (Blanes Bay microbial observatory), Limnology & Oceanography, 55(5), 2117-2125, 2010.
Galand, P.E., Lovejoy, C., Pouliot, J., Vincent, W.F.: Heterogeneous archaeal communities in the particle rich environment of an arctic shelf ecosystem, J. Mar. Syst., 74, 774-782, 2008.
Garneau, M.È., Vincent, W.F., Terrado, R., Lovejoy, C.: Importance of particle-associated bacterial heterotrophy in a coastal Arctic ecosystem, J. Mar. Syst., 75, 185-197, 2009.
Ganesh, S., Parris, D.J., DeLong, E.F., Stewart, F.J.: Metagenomic analysis of size-fractionated picoplankton in a marine oxygen minimum zone, ISME J., 8, 187-211, 10.1038/ismej.2013.144, 2014.
Ghai, R., Mizuno, C.M., Picazo, A., Camacho, A., Rodriguez-Valera, F.: Metagenomics uncovers a new group of low GC and ultra-small marine Actinobacteria, Sci. Rep., 3, 2471, doi:10.1038/srep02471, 2013.
Ghiglione, J.F., Conan, P., and Pujo-Pay, M.: Diversity of total and active free-living vs. particle-attached bacteria in the euphotic zone of the NW Mediterranean Sea, FEMS Microbiol. Lett., 299, 9-21, 2009.
Ghiglione, J.F., Mevel, G., Pujo-Pay, M., Mousseau, L., Lebaron, P., and Goutx, M.: Diel and seasonal variations in abundance, activity, and community structure of particle-attached and free-living bacteria in NW Mediterranean Sea, Microbial Ecology, 54, 217-231, 2007.

867 Giovannoni, S.J., Cameron, Thrash J., Temperton, B.: Implications of streamlining theory for microbial
868 ecology, ISME J., 8, 1553-1565, doi:10.1038/ismej.2014.60, 2014.
869 Giovannoni, S.J. and Stingl, U.: Molecular diversity and ecology of microbial plankton, Nature, 437, 343-348,
870 10.1038/nature04158, 2005.
871 Gómez-Pereira, P.R., Kennaway, G., Fuchs, B.M., Tarran, G.A., and Zubkov, M.V.: Flow cytometric
872 identification of Mamiellales clade II in the Southern Atlantic Ocean, FEMS Microbiol. Ecol., 83, 664-671,
873 2013.
874 Gong, G.C., Liu, K.K., Liu, C.T., Pai, S.C.: The chemical hydrography of the South China Sea west of Luzon
875 and a comparison with the West Philippine Sea, Terr. Atmos. Ocean Sci., 3, 587-602, 1992.
876 Gonzalez, J.M., Fernandez-Gomez, B., Fernandez-Guerra, A., Gomez-Consarnau, L., Sanchez, O., Coll-Llado,
877 M.: Genome analysis of the proteorhodopsin-containing marine bacterium *Polaribacter* sp. MED152
878 (Flavobacteria), Proc. Natl. Acad. Sci. USA, 105, 8724-8729, 2008.
879 Green, P.N.: *Methylobacterium*, In: The Prokaryotes: A Handbook on the Biology of Bacteria, edited by:
880 Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.H., and Stackebrandt, E., Springer, New York, 5,
881 257-265, 2006.
882 Griffith, P., Shiah, F., Gloersen, K., Ducklow, H.W., Fletcher, M.: Activity and distribution of attached bacteria
883 in Chesapeake Bay, Mar. Ecol. Prog. Ser., 108, 1-10, 1994.
884 Grossart, H.P.: Ecological consequences of bacterioplankton lifestyles: changes in concepts are needed,
885 Environ. Microb. Rep., 2, 706-714, 2010.
886 Grossart, H.P., Kiorboe, T., Tang, K., Allgaier, M., Yam, E.M., Ploug, H.: Interactions between marine snow
887 and heterotrophic bacteria: aggregate formation and microbial dynamics, Aqua. Microb. Ecol., 42, 19-26,
888 2006.
889 Grossart, H.-P., Tang, K.W., Kiørboe, T., and Ploug, H. Comparison of cell-specific activity between free-
890 living and attached bacteria using isolates and natural assemblages, FEMS Microbiol. Lett., 266, 194-200,
891 2007.
892 Grote, J., Thrash, J.C., Huggett, M.J., Landry, Z.C., Carini, P., Giovannoni, S.J., and Rappé, M.S.: Streamlining
893 and core genome conservation among highly divergent members of the SAR11 clade, mBio, 3, e00252-12,
894 doi:10.1128/mBio.00252-12, 2012.
895 Haro-Moreno, J.M., Rodriguez-Valera, F., López-García, Purificación, Moreira, D., and Martin-Cuadrado,
896 A.B.: New insights into marine group III Euryarchaeota, from dark to light. The ISME Journal, 11, 1102-
897 1117, 2017.
898 He, X., Xu, D., Bai, Y., Pan, D., Chen, T.A., Chen, X., Gong, F.: Eddy-entrained Pearl River plume into the
899 oligotrophic basin of the South China Sea, Cont. Shelf Res., 124, 117-124, 2016.
900 Hollibaugh, J.T., Wong, P.S., and Murrell, M.C.: Similarity of particle-associated and free-living bacterial
901 communities in northern San Francisco Bay, California, Aquat. Microb. Ecol., 21, 103-114, 2000.
902 Huang, Y.N., Chen, F.J., Zhao, H., Zeng, Z., and Chen, J.F. Concentration distribution and structural features
903 of nutrients in the northwest of South China Sea in winter 2012, Journal of Applied Oceanography, 34, 310-
904 316, 2015.
905 Iverson, V., Morris, R.M., Frazar, C.D., Berthiaume, C.T., Morales, R.L., Armbrust, E.V.: Untangling genomes
906 from metagenomes: revealing an uncultured class of marine Euryarchaeota, Science, 335, 587-590, 2012.
907 Jiao, N., Luo, T., Zhang, R., Yan, W., Lin, Y., Johnson, Z.I., Tian, J., Yuan, D., Yang, Q., Zheng, Q., Sun, J.,
908 Hu, D., and Wang, P.: Presence of *Prochlorococcus* in the aphotic waters of the western Pacific Ocean,
909 Biogeosciences, 11, 2391-2400, 2014.
910 Karner, M.B., DeLong, E.F., Karl, D.M.: Archaeal dominance in the mesopelagic zone of the Pacific Ocean,
911 Nature, 409, 507-510, 2001.

912 Karner, M. and Herndl, G.J.: Extracellular enzymatic activity and secondary production in free-living and
913 marine-snow-associated bacteria, Marine Biology, 113, 341-347, 1992.
914 Kiorboe, T., and Jackson, G.A.: Marine snow, organic solute plumes, and optimal chemosensory behavior of
915 bacteria, Limnol. Oceanogr., 46,1309-1318, 2001.
916 Koblížek, M., Béjà, O., Bidigare, R.R., Christensen, S., Benitez-Nelson, B., Vetriani, C., Kolber, M.K.,
917 Falkowski, P.G. and Kolber, Z.S.: Isolation and characterization of *Erythrobacter* sp. strains from the upper
918 ocean, Arch. Microbiol., 180, 327-338, https://doi.org/10.1007/s00203-003-0596-6, 2003.
919 Kolber, Z.S., Van Dover, C.L., Niederman, R.A., Falkowski, P.G.: Bacterial photosynthesis in surface waters
920 of the open ocean, Nature, 407, 177-179, 2000.
921 Könneke M, Bernhard, A.E., de la Torre, J.R., Walker, C.B., Waterbury, J.B. and Stahl, D.A.: Isolation of an
922 autotrophic ammonia-oxidizing marine archaeon, Nature, 437, 543-546, 2005.
923 Laufkötter, C., John, J. G., Stock, C.A., and Dunne, J.P.: Temperature and oxygen dependence of the
924 remineralization of organic matter, Global Biogeochemical Cycles, 31, 1038-1050, 2017.
925 Lee, C., Wakeham, S., Arnosti, C.: Particulate organic matter in the sea: the composition conundrum, Ambio.,
926 33, 566-575, 2004.
927 Lee, Z.M.-P., Bussema, C., and Schmidt, T.M.: rrnDB: documenting the number of rRNA and tRNA genes in
928 bacteria and archaea, Nucleic. Acids Res., 37, 489-493, 2009.
929 Li, J., Wei, B., Wang, J., Liu, Y., Dasgupta, S., Zhang, L.: Variation in abundance and community structure of
930 particle-attached and free-living bacteria in the South China Sea, Deep Sea Res. Part II Top. Stud. Oceanogr.,
931 122, 64-73, 10.1016/j.dsr2.2015.07.006, 2015.
932 Liang, W., Tang, D., Luo, X.: Phytoplankton size structure in the western South China Sea under the influence
933 of a 'jet-eddy system', J. Mar. Syst., 187, 82-95, 2018.
934 Lipsewers, Y.A., Hopmans, E.C., Sinninghe Damsté, J.S., and Villanueva, L.: Potential recycling of
935 thaumarchaeotal lipids by DPANN Archaea in seasonally hypoxic surface marine sediments, Organic
936 Geochemistry, 119, 101- 109, 2018.
937 Liu, R.L., Wang, L., Liu, Q.F., Wang, Z.X., Li, Z.Z., and Fang, J.S.: Depth-resolved distribution of particle-
938 attached and free-living bacterial communities in the water column of the New Britain Trench. Frontiers in
939 Microbiology, 9, 625, 2018a.
940 Liu, X., Li, M., Castelle, C. J., Probst, A. J., Zhou, Z., & Pan, J.: Insights into the ecology, evolution, and
941 metabolism of the widespread woesearchaeotal lineages, Microbiome, 6(1), 102, 2018b.
942 Liu, S., Riesen, A., and Liu, Z.: Differentiating the role of different-sized microorganisms in peptide
943 decomposition during incubations using size-fractioned coastal seawater, Journal of Experimental Marine
944 Biology and Ecology, 472, 97-106, 2015.
945 Long, R.A., and Azam, F.: Microscale patchiness of bacterioplankton assemblage richness in seawater, Aquatic
946 Microbial Ecology, 26, 103-113, doi: 10.3354/ame026103, 2001.
947 Martin-Cuadrado, A.-B., Garcia-Heredia, I., Moltó, A.G., López-Úbeda, R., Kimes, N., López-García, P.,
948 Moreira, D., Rodriguez-Valera, F.: A new class of marine euryarchaeota group II from the Mediterranean
949 deep chlorophyll maximum, ISME J., 9, 1619-1634, doi:10.1038/ismej.2014.249, 2015.
950 Martin-Cuadrado, A.B., Rodriguez-Valera, F., Moreira, D., Alba, J.C., Ivars-Martinez, E., Henn, M.R.:
951 Hindsight in the relative abundance, metabolic potential and genome dynamics of uncultivated marine
952 archaea from comparative metagenomic analyses of bathypelagic plankton of different oceanic regions,
953 ISME J., 2, 865-886, 2008.
954 Massana, R., Delong, E.F., and Pedros-Alio, C.: A few cosmopolitan phylotypes dominate planktonic archaeal
955 assemblages in widely different oceanic provinces, Applied and Environmental Microbiology, 66(5), 1777-
956 1787, 2000.

957 Meng, F., Dai, M., Cao, Z., Wu, K., Zhao, X., Li, X., Chen, J., and Gan, J.: Seasonal dynamics of dissolved
958 organic carbon under complex circulation schemes on a large continental shelf: the northern South China
959 Sea, Journal of Geophysical Research: Oceans, 122, 9415-9428, 2017.
960 Mestre, M., Ruiz-Gonzalez, C., Logares, R., Duarte, C.M., Gasol, J.M., and Sala, M.M.: Sinking particles
961 promote vertical connectivity in the ocean microbiome, Proc. Natl. Acad. Sci. USA, 115, 6799-6807, 2018.
962 Mevel, G., Vernet, M., Goutx, M., and Ghiglione, J.F. Seasonal to hour variation scales in abundance and
963 production of total and particle-attached bacteria in the open NW Mediterranean Sea (0-1000 m),
964 Biogeosciences, 5, 1573-1586, doi:10.5194/bg-5-1573-2008, 2008.
965 Milici, M., Deng, Z.L., Tomasch, J., Decelle, J., Wos-Oxley, M.L., Wang H., Jáuregui, R., Plumeier, I., Giebel,
966 H.A., Badewien, T.H., Wurst, M., Pieper, D.H., Simon, M., Wagner-Döbler, I.: Co-occurrence analysis of
967 microbial taxa in the Atlantic Ocean reveals high connectivity in the free-living bacterioplankton, Front.
968 Microbiol., 7, 649, 10.3389/fmicb.2016.00649, 2016.
969 Milici, M., Vital, M., Tomasch, J., Badewien, T.H., Giebel, H.A., Plumeier, I.: Diversity and community
970 composition of particle-associated and free-living bacteria in mesopelagic and bathypelagic Southern Ocean
971 water masses: evidence of dispersal limitation in the Bransfield Strait, Limnol. Oceanogr., 62, 1080-1095,
972 10.1002/lno.10487, 2017.
973 Mincer, T.J., Church, M.J., Taylor, L.T., Preston, C., Karl, D.M., DeLong, E.F.: Quantitative distribution of
974 presumptive archaeal and bacterial nitrifiers in Monterey Bay and the North Pacific Subtropical Gyre,
975 Environ. Microbiol., 9, 1162-1175, 2007.
976 Mizuno, C.M., Rodriguezvalera, F., and Ghai, R.: Genomes of planktonic acidimicrobiales: widening horizons
977 for marine actinobacteria by metagenomics, mBio, 6(1), e02083-14, 10.1128/mBio.02083-14, 2015.
978 Moeseneder, M.M., Winter, C., and Herndl, G.J.: Horizontal and vertical complexity of attached and free-
979 living bacteria of the eastern Mediterranean Sea, determined by 16S rDNA and 16S rRNA fingerprints,
980 Limnol. Oceanagr., 46, 95-107, 2001.
981 Morris, S.A., Radajewski, S., Willison, T.W., Murrell, J.C.: Identification of the functionally active
982 methanotroph population in a peat soil microcosm by stable-isotope probing, Appl. Environ. Microbiol., 68,
983 1446-1453, 2002.
984 Orsi, W.D., Smith, J.M., Wilcox, H.M., Swalwell, J.E., Carini, P., Worden, A.Z.: Ecophysiology of uncultivated
985 marine euryarchaea is linked to particulate organic matter, ISME J., 9, 1747-1763, 2015.
986 Ortega-Retuerta, E., Joux, F., Jeffrey, W.H., and Ghiglione, J.F.: Spatial variability of particle-attached and
987 free-living bacterial diversity in surface waters from the Machenzie River to the Beaufort Sea (Canadian
988 Arctic), Biogeoscience, 10, 2747-2759, 2013.
989 Peoples, L.M., Sierra, D., Oladayo, O., Qing, X., Alex, N., and Jessica, B.: Vertically distinct microbial
990 communities in the Mariana and Kermadec Trenches, PLOS ONE, 13, e0195102, 2018.
991 Poretsky, R.S., Sun, S., Mou, X., and Moran, M.A.: Transporter genes expressed by coastal bacterioplankton
992 in response to dissolved organic carbon, Environ. Microbiol., 12, 616-627, 2010.
993 Probst, A.J., Castelle, C.J., Singh, A., Brown, C.T., Anantharaman, K., Sharon, I., Hug, L.A., Burstein, D.,
994 Emerson, J.B., Thomas, B.C., Banfield, B.F.: Genomic resolution of a cold subsurface aquifer community
995 provides metabolic insights for novel microbes adapted to high CO2 concentrations, Environ Microbiol., 19,
996 459-74, 2017.
997 Puente-Sánchez, F., Arce-Rodríguez, A., Oggerin, M., García-Villadangos, M., Moreno-Paz, M., Blanco, Y.,
998 Parro, V.: Viable cyanobacteria in the deep continental subsurface, Proc. Natl. Acad. Sci. USA., 115(42),
999 10702-10707, doi:10.1073/pnas.1808176115, 2018.
1000 Qin, W., Amin, S.A., Martens-Habbena, W., Walker, C.B., Urakawa, H., Devol, A.H.: Marine ammonia-
1001 oxidizing archaeal isolates display obligate mixotrophy and wide ecotypic variation, Proc. Natl. Acad. Sci.

USA., 111, 12504-12509, 2014.
Qin, W., Heal, K.R., Ramdasi, R., Kobelt, J.N., Martens-Habbena, W., Bertagnolli, A.D., Amin, S.A., Walker, C.B., Urakawa, H., Könneke, M., Devol, A.H., Moffett, J.W., Armbrust, E.V., Jensen, G.J., Ingalls, A.E., Stahl, D.A.: *Nitrosopumilus maritimus* gen. nov., sp. nov., *Nitrosopumilus cobalaminigenes* sp. nov., *Nitrosopumilus oxyclinae* sp. nov., and *Nitrosopumilus ureiphilus* sp. nov., four marine ammonia-oxidizing archaea of the phylum Thaumarchaeota, Int. J. Syst. Evol. Microbiol., 67, 5067-5079, 2017.
Rieck, A., Herlemann, D.P.R., Jürgens, K., and Grossart, H.-P.: Particle-associated differ from free-living bacteria in surface waters of the Baltic Sea, Front. Microbiol., 6, 469, 2015.
Rinke, C., Rubino, F., Messer, L.F., Youssef, N., Parks, D.H., and Chuvochina, M., Brown, M., Jeffries, T., Tyson, G.W., Seymour, J.R., Hugenholtz, P.: A phylogenomic and ecological analysis of the globally abundant Marine Group II archaea (*Ca.* Poseidoniales ord. nov.). The ISME Journal, 13, 663-675, 2019.
Rinta-Kanto, J.M., Sun, S., Sharma, S., Kiene, R.P., and Moran, M.A.: Bacterial community transcription patterns during a marine phytoplankton bloom, Environ. Microbiol., 14, 228-239, 2012.
Salazar, G., Cornejo-Castillo, F.M., Benítez-Barrios, V., Fraile-Nuez, E., Álvarez-Salgado, X.A., Duarte, C.M., Gasol, J.M., Acinas, S.G.: Global diversity and biogeography of deep-sea pelagic prokaryotes, ISME J., 10, 596-608, 2016.
Salazar, G., Cornejo-Castillo, F.M., Borrull, E., Díez-Vives, C., Lara, E., Vaqué, D., Arrieta, J.M., Duarte, C.M., Gasol, J.M., Acinas, S.G.: Particle-association lifestyle is a phylogenetically conserved trait in bathypelagic prokaryotes, Mol. Ecol., 24, 5692-5706, 2015.
Simon, M., Grossart, H.P., Schweitzer, B., and Ploug, H.: Microbial ecology of organic aggregates in aquatic ecosystems, Aquat. Microb. Ecol., 28, 175-211, 2002.
Smith, D.C., Simon, M., Alldredge, A.L., and Azam, F.: Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution, Nature, 359, 139-142, 1992.
Smith, M.W., Allen, L.Z., Allen, A.E., Herfort, L., and Simon, H.M.: Contrasting genomic properties of free-living and particle-attached microbial assemblages within a coastal ecosystem, Frontiers in Microbiology, 4, doi: 10.3389/fmicb.2013.00120, 2013.
Suter, E.A., Pachiadaki, M., Taylor, G.T., Astor, Y., and Edgcomb, V.P.: Free-living chemoautotrophic and particle-attached heterotrophic prokaryotes dominate microbial assemblages along a pelagic redox gradient, Environ. Microbiol., 20, 693-712, 2018.
Suzuki, S., Kaneko, R., Kodama, T., Hashihama, F., Suwa, S., and Tanita, I., Furuya, K., Hamasaki, K.: Comparison of community structures between particle-associated and free-living prokaryotes in tropical and subtropical Pacific Ocean surface waters, Journal of Oceanography, 73(3), 383-395, 2017.
Takai, K., and Horikoshi, K.: Genetic diversity of archaea in deep-sea hydrothermal vent environments, Genetics, 152, 1285-1297, 1999.
Tarn, J., Peoples, L.M., Hardy, K., Cameron, J., Bartlett, D.H.: Identification of free-living and particle-associated microbial communities present in hadal regions of the Mariana Trench, Front. Microbiol., 7, 665, doi:10.3389/fmicb.2016.00665, 2016.
Teeling, H., Fuchs, B.M., Becher, D., Klockow, C., Gardebrecht, A., et al.: Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom, Science, 336, 608-611, 2012.
Tully, B.J.: Metabolic diversity within the globally abundant Marine Group II Euryarchaea offers insight into ecological patterns, Nat. Commun., 10, 271, https://doi.org/10.1038/s41467-018-07840-4, 2019.
Turley, C.M., and Mackie, P.J.: Biogeochemical significance of attached and free-living bacteria and the flux of particles in the NE Atlantic Ocean, Mar. Ecol. Prog. Ser., 115, 191-203, doi:10.3354/meps115191, 1994.
Turley, C.M. and Stutt, E.D.: Depth-related cell-specific bacterial leucine incorporation rates on particles and its biogeochemical significance in the Northwest Mediterranean, Limnol. Oceanogr., 45, 419-425,

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doi:10.4319/lo.2000.45.2.0419, 2000.
Varela, M.M., Vanaken, H.M., Sintes, E., Herndl, G.: Latitudinal trends of Crenarchaeota, and bacteria, in the meso- and bathypelagic water masses of the eastern north Atlantic, Environ. Microbiol., 10, 110-124, 2008.
Wang, Y., Wang, B., Dann, L.M., Mitchell, J.G., Hu, X., and Tang, H., Zhang, H., Shen, Y.: Bacterial community structure in the Bohai Strait provides insights into organic matter niche partitioning, Continental Shelf Research, 169, 46-54, 2018.
Wright, T.D., Vergin, K.L., Boyd, P.W. and Giovannoni, S.J.: A novel delta-subdivision proteobacterial lineage from the lower ocean surface layer, Appl. Environ. Microbiol., 63, 1441-1448, 1997.
Wu, J., Chung, S.W., Wen, L.S., Liu, K K., Chen, Y.L.L., and Chen, H.Y., Karl, D.M.: Dissolved inorganic phosphorus, dissolved iron, and trichodesmium in the oligotrophic South China Sea, Global Biogeochemical Cycles, 17(1), 8-1-8-10, 2003.
Yawata, Y., Cordero, O.X., Menolascina, F., Hehemann, J.-H., Polz, M.F., Stocker, R.: Competition-dispersal tradeoff ecologically differentiates recently speciated marine bacterioplankton populations, Proc. Natl. Acad. Sci. USA., 111, 5622-5627, doi:10.1073/pnas.1318943111, 2014.
Yilmaz, P., Yarza, P., Rapp, J.Z. and Glöckner, F.O.: Expanding the world of marine bacterial and archaeal clades, Front. Microbiol., 6, 1524, doi: 10.3389/fmicb.2015.01524, 2016.
Yung, C.-M., Ward, C.S., Davis, K.M., Johnson, Z.I., Hunt, D.E.: Insensitivity of diverse and temporally variable particle-associated microbial communities to bulk seawater environmental parameters, Appl. Environ. Microbiol., 82, 3431-3437, 2016.
Zhang, R., Liu, B., Lau, S.C.K., Ki, J.S., and Qian, P.: Particle-attached and free-living bacterial communities in a contrasting marine environment: Victoria Harbor, Hong Kong, FEMS Microbiol. Ecol., 61, 496-508, 2007.
Zhang, Y., Xiao, W., and Jiao, N.: Linking biochemical properties of particles to particle-attached and free-living bacterial community structure along the particle density gradient from freshwater to open ocean, J. Geophys. Res.: Biogeosci., 121, 2261-2274, doi:10.1002/2016JG003390, 2016.

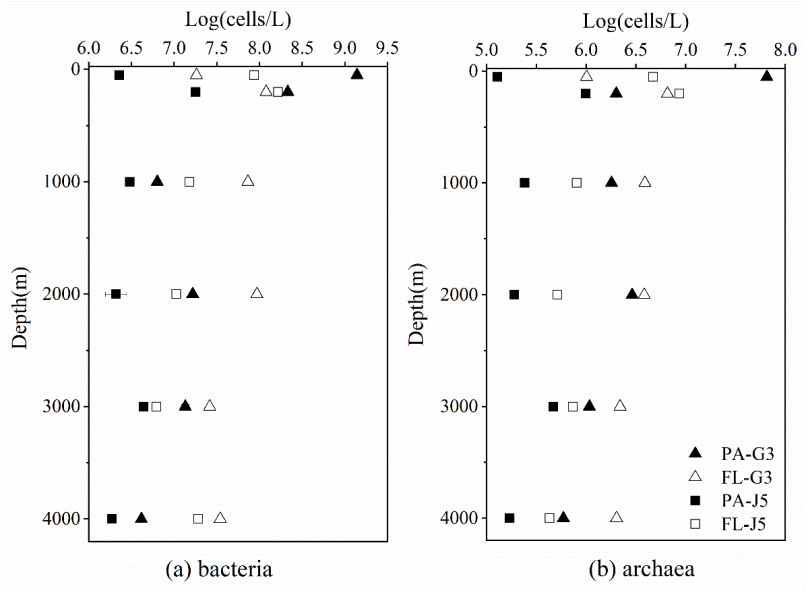


Figure 1. Bacterial and archaeal cell abundances in seawaters at different depths from G3 station and J5 station in the South China Sea, estimated from 16S rRNA gene copy abundances.

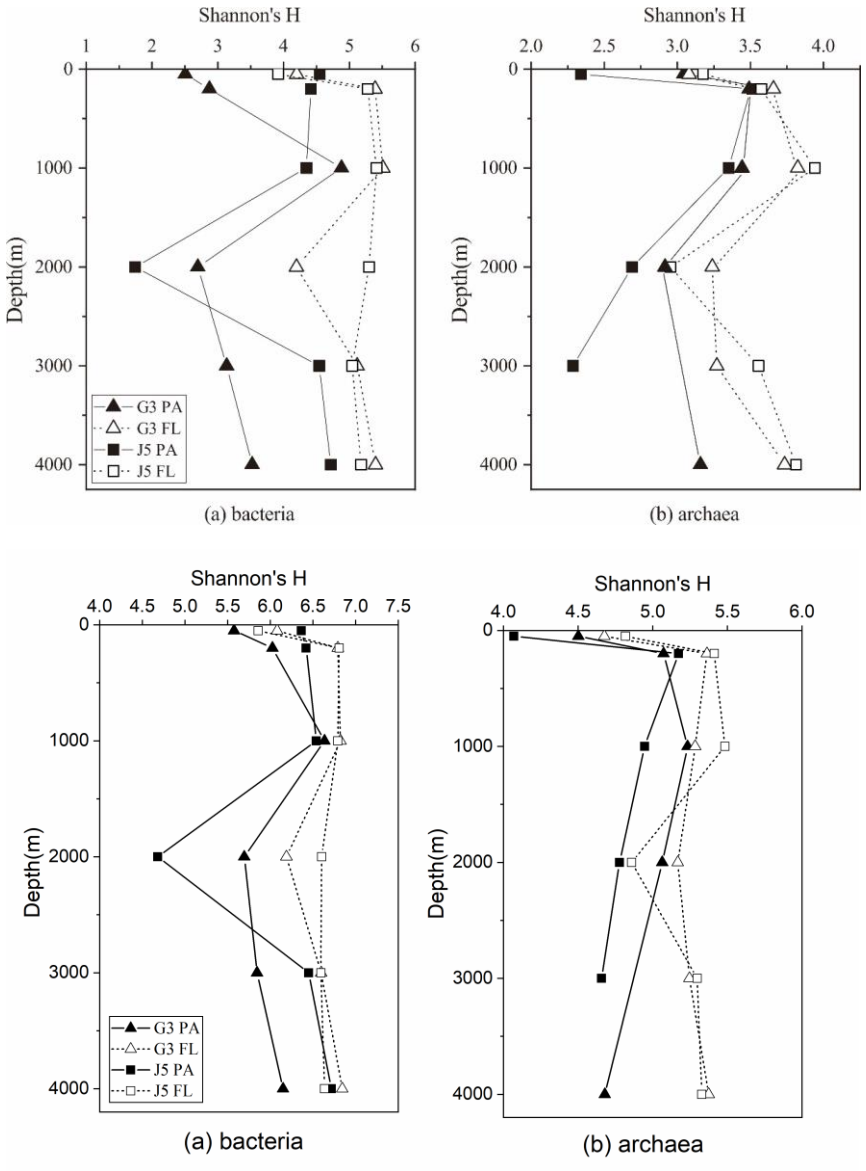


Figure 2. Shannon's diversity index calculated for all bacterial and archaeal communities of seawaters collected from G3 station and J5 station in the South China Sea.

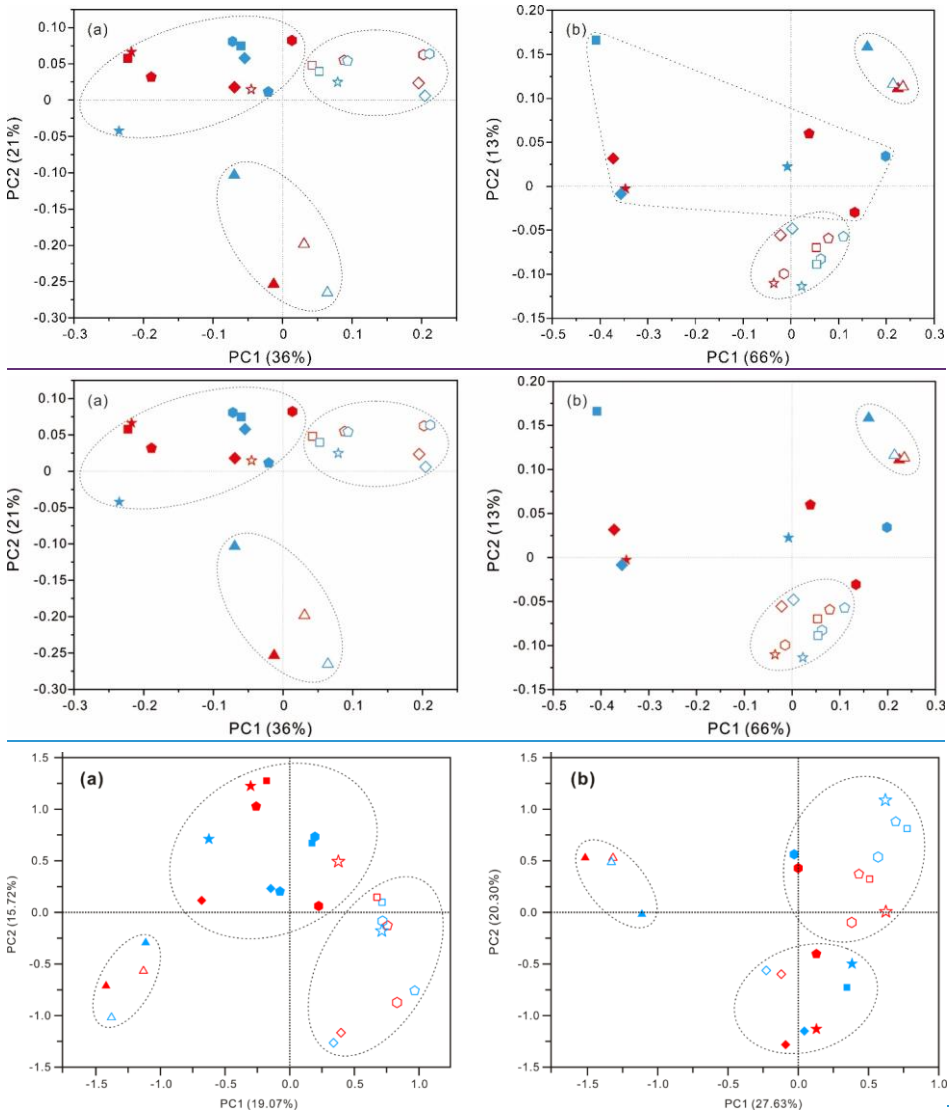


Figure 3. Results of PCoA analysis for particle-attached and free-living microbial fractions collected from seawater columns of the South China Sea. (a) PA and FL bacteria; (b) PA and FL archaea. Statistical analyses supported the groups with statistical significances (Table S3). Triangle: 50 m; rhombus: 200 m; hexagon: 1000 m; star: 2000 m; square: 3000 m; pentagon: 4000 m. Blue color: J5 station; red color: G3 station. Filled: particle-attached fraction; open: free-living fraction.

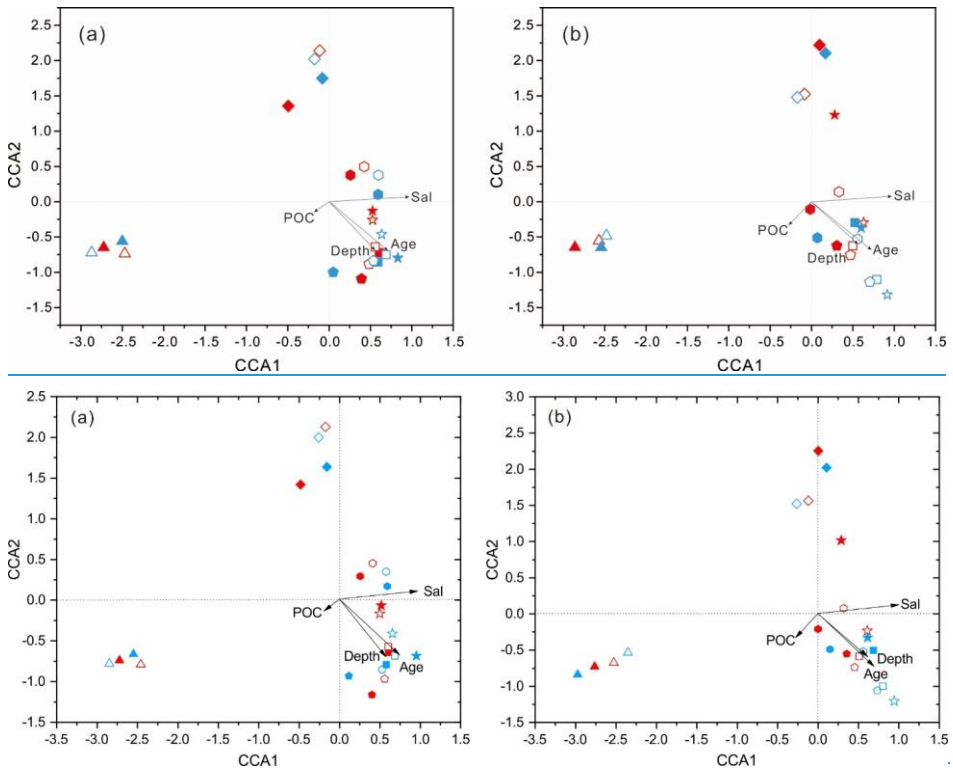


Figure 4. Results of CCA analysis to correlate several environmental factors including POC, seawater age, salinity and depth to PA and FL microbial communities collected from seawater columns of the South China Sea. (a) PA and FL bacteria; (b) PA and FL archaea. Triangle: 50 m; rhombus: 200 m; hexagon: 1000 m; star: 2000 m; square: 3000 m; pentagon: 4000 m. Blue color: J5 station; red color: G3 station. Filled: particle-attached fraction; open: free-living fraction.

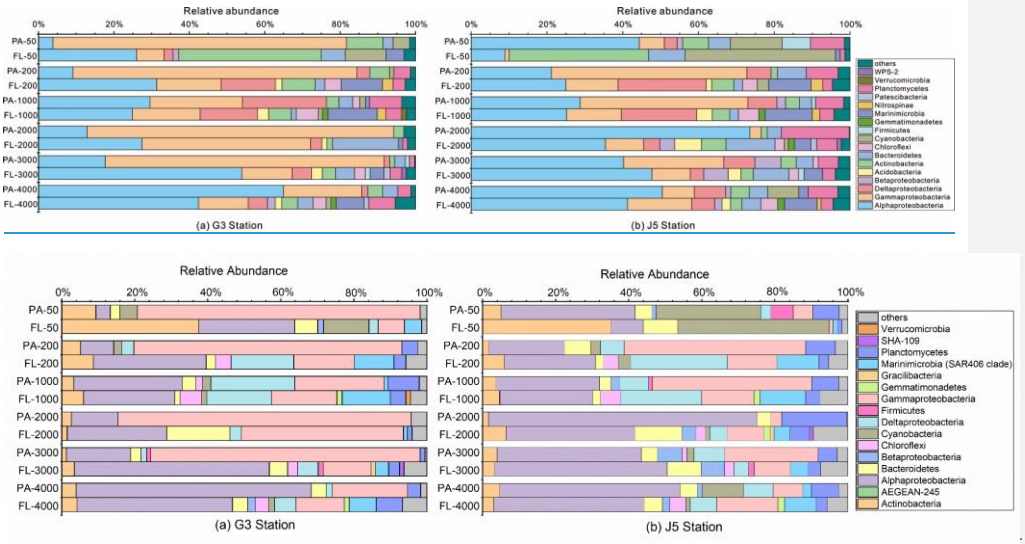


Figure 5. Taxonomic compositions of particle-attached and free-living bacterial communities of seawaters at different depths along two different water columns in the South China Sea. (a) G3 station; (b) J5 station. The phylum or class which has less than 1% proportions is classified into "others" (Fig. ~~S4~~S5).


G3 Station
J5 Station
Acidobacteria
uncultured Subgroup 6
Actinobacteria
Actinomarinaceae/OM1 clade
Sva0996 marine group
Bacteroidetes
Flavobacteriaceae
Cyanobacteria
Cyanobiaceae
Marinimicrobia
SAR406 marine metagenome
SAR406 uncultured bacterium
Nitrospinae
Nitrospinaceae
Planctomycetes
Planctomycetaceae
Alphaproteobacteria
Methylobacteraceae
Phyllobacteraceae
Rhodobacteraceae
SAR11 Clade I
SAR11 Clade II
Unclassified SAR11
Erythrobacteraceae
Betaproteobacteria
Burkholderiaceae
Deltaproteobacteria
uncultured NB1-j
SAR324 marine metagenome
uncultured SAR324 cluster
Gammaproteobacteria
Alteromonadaceae
Pseudoalteromonadaceae
Unclassified Alteromonadales
uncultured HF0770_27E13
Alcanivoracaceae
Halomonadaceae
Oceanospirillaceae
Salinisphaeraceae
Thioglobaceae
50 PA
50 FL
200 PA
200 FL
1000 PA
1000FL
2000 PA
2000 FL
3000 PA
3000 FL
4000 PA
4000FL
0.1
0.3
0.5
0.7

1102

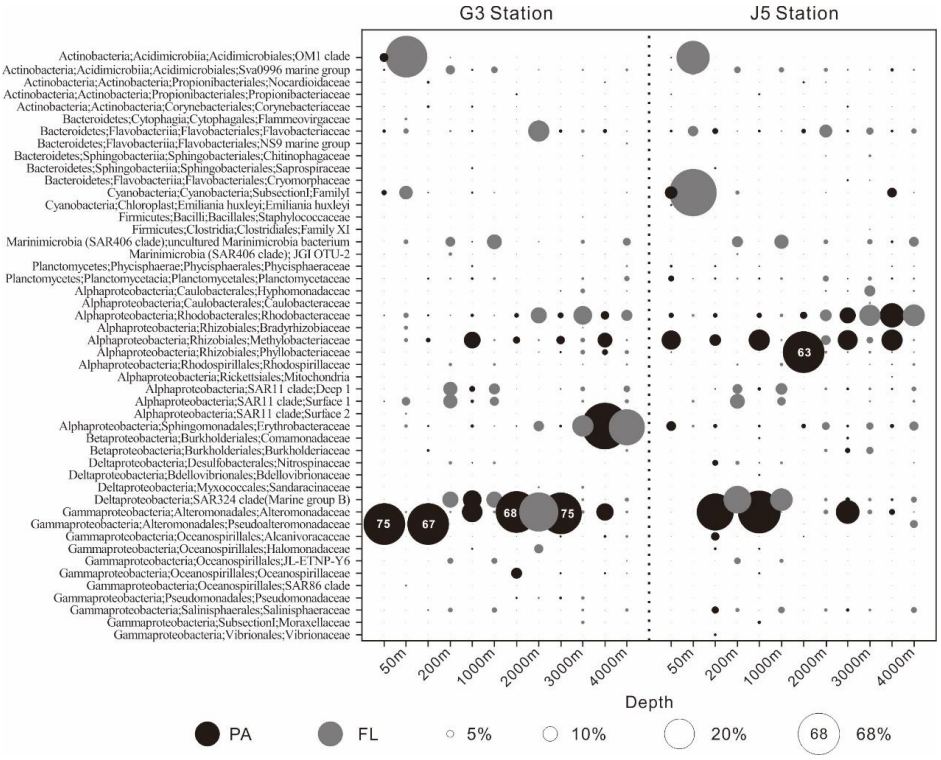
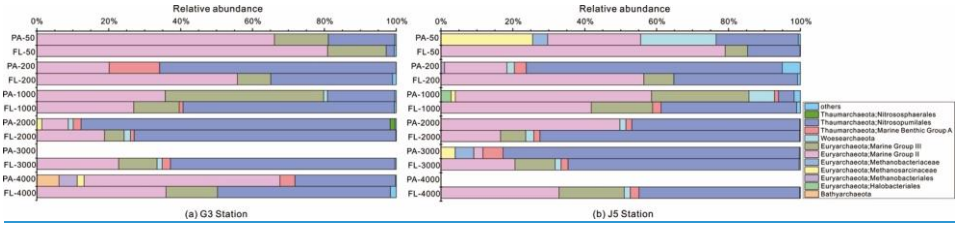


Figure 6. The relative abundances of families in particle-attached and free-living bacterial communities. Dark grey bubbles are the average relative abundances in the PA fraction, while light grey bubbles are the average relative abundances in the FL fractions. Scale is shown in the bottom, and the cycle with a number inside indicates actual relative abundance.



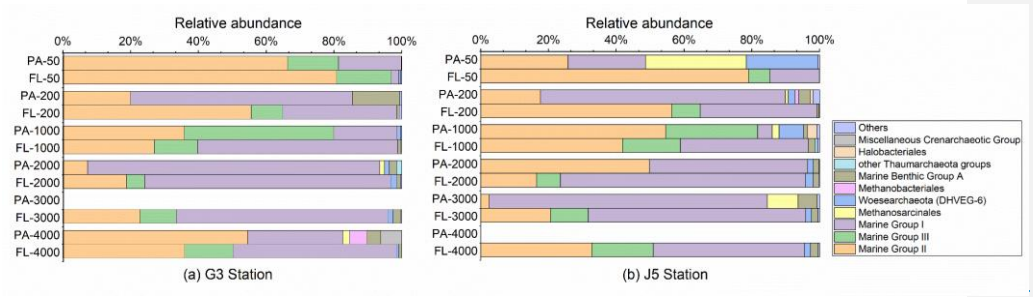
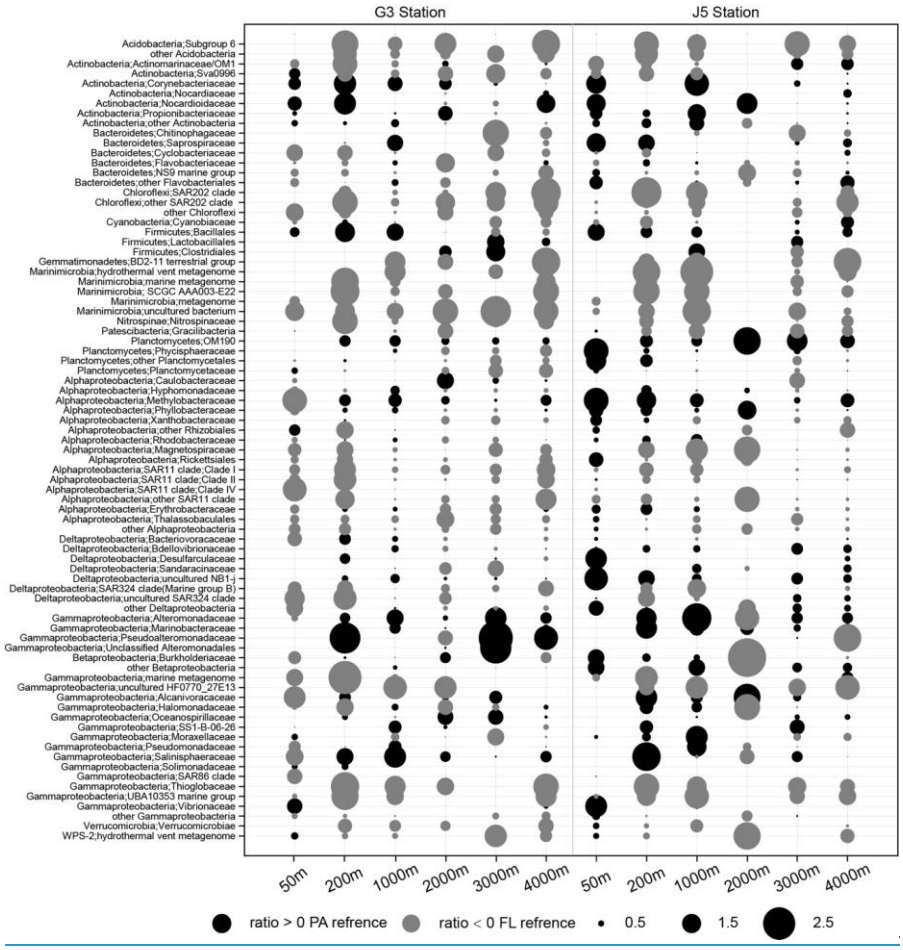


Figure 7. Taxonomic compositions of particle-attached and free-living archaeal communities of seawaters at different depths along two different water columns in the South China Sea. (a) G3 station; (b) J5 station. PA-3000 at G3 station and PA-4000 at J5 station indicate the samples failing in the sequencing of archaeal 16S rRNA gene. The archaeal lineages, at ~ phylum or class level, with less than 1% proportions is classified into "others" (Fig. ~~S5~~S6).


G3 Station
J5 Station
Acidobacteria;Subgroup 6
Actinobacteria;Actinomarinaceae/OM1
Actinobacteria;Sav0996
Actinobacteria;Corynebacteriaceae
Actinobacteria;Nocardiaceae
Actinobacteria;Nocardioidaceae
Actinobacteria;Propionibacteriaceae
Bacteroidetes;Chitinophagaceae
Bacteroidetes;Saprospiraceae
Bacteroidetes;Cyclobacteriaceae
Bacteroidetes;Flavobacteriaceae
Bacteroidetes;NS9 marine group
Chloroflexi;SAR202 clade
Cyanobacteria;Cyanobiaceae
Firmicutes;Bacillales
Firmicutes;Lactobacillales
Firmicutes;Clostridiales
Gemmatimonadetes;BD2-11 terrestrial group
Marinimicrobia;hydrothermal vent metagenome
Marinimicrobia;marine metagenome
Marinimicrobia;SCGC AAA003-E22
Marinimicrobia;metagenome
Marinimicrobia;uncultured bacterium
Nitrospinae;Nitrospinaceae
Planctomycetes;OM190
Planctomycetes;Phycisphaeraceae
Planctomycetes;Planctomycetaceae
Alphaproteobacteria;Caulobacteraceae
Alphaproteobacteria;Hyphomonadaceae
Alphaproteobacteria;Methylobacteraceae
Alphaproteobacteria;Phyllobacteraceae
Alphaproteobacteria;Xanthobacteraceae
Alphaproteobacteria;Rhodobacteraceae
Alphaproteobacteria;Magnetospiraceae
Alphaproteobacteria;SAR11 clade;Clade I
Alphaproteobacteria;SAR11 clade;Clade II
Alphaproteobacteria;SAR11 clade;Clade IV
Alphaproteobacteria;other SAR11 clade
Alphaproteobacteria;Erythrobacteraceae
Betaproteobacteria;Burkholderiaceae
Deltaproteobacteria;Bacteriovoracaceae
Deltaproteobacteria;Bdellovibrionaceae
Deltaproteobacteria;Desulfarculaceae
Deltaproteobacteria;Sandaracinaceae
Deltaproteobacteria;uncultured NB1-j
Deltaproteobacteria;SAR324 clade(Marine group B)
Gammaproteobacteria;Alteromonadaceae
Gammaproteobacteria;Marinobacteraceae
Gammaproteobacteria;Pseudoalteromonadaceae
Gammaproteobacteria;marine metagenome
Gammaproteobacteria;uncultured HF0770_27E13
Gammaproteobacteria;Alcanivoracaceae
Gammaproteobacteria;Halomonadaceae
Gammaproteobacteria;Oceanospirillaceae
Gammaproteobacteria;SS1-B-06-26
Gammaproteobacteria;Moraxellaceae
Gammaproteobacteria;Pseudomonadaceae
Gammaproteobacteria;Salinisphaeraceae
Gammaproteobacteria;Solimonadaceae
Gammaproteobacteria;SAR86 clade
Gammaproteobacteria;Thioglobaceae
Gammaproteobacteria;UBA10353 marine group
Gammaproteobacteria;Vibrionaceae
Verrucomicrobia;Verrucomicrobiae
WPS-2;hydrothermal vent metagenome
50m
200m
1000m
2000m
3000m
4000m
50m
200m
1000m
2000m
3000m
4000m
ratio > 0 PA refrence
ratio < 0 FL refrence
0.5
1.5
2.5

1115


G3 Station
J5 Station
Acidobacteria;Subgroup 6
other Acidobacteria
Actinobacteria;Actinomarinaceae/OM1
Actinobacteria;Sva0996
Actinobacteria;Corynebacteriaceae
Actinobacteria;Nocardiaceae
Actinobacteria;Nocardioidaceae
Actinobacteria;Propionibacteriaceae
Actinobacteria;other Actinobacteria
Bacteroidetes;Chitinophagaceae
Bacteroidetes;Saprospiraceae
Bacteroidetes;Cyclobacteriaceae
Bacteroidetes;Flavobacteriaceae
Bacteroidetes;NS9 marine group
Bacteroidetes;other Flavobacteriales
Chloroflexi;SAR202 clade
Chloroflexi;other SAR202 clade
other Chloroflexi
Cyanobacteria;Cyanobiaceae
Firmicutes;Bacillales
Firmicutes;Lactobacillales
Firmicutes;Clostridiales
Gemmatimonadetes;BD2-11 terrestrial group
Marinimicrobia;hydrothermal vent metagenome
Marinimicrobia;marine metagenome
Marinimicrobia; SCGC AAA003-E22
Marinimicrobia;metagenome
Marinimicrobia;uncultured bacterium
Nitrospinae;Nitrospinaceae
Patescibacteria;Gracilibacteria
Planctomycetes;OM190
Planctomycetes;Phycisphaeraceae
Planctomycetes;other Planctomycetales
Planctomycetes;Planctomycetaceae
Alphaproteobacteria;Caulobacteraceae
Alphaproteobacteria;Hyphomonadaceae
Alphaproteobacteria;Methylobacteraceae
Alphaproteobacteria;Phyllobacteraceae
Alphaproteobacteria;Xanthobacteraceae
Alphaproteobacteria;other Rhizobiales
Alphaproteobacteria;Rhodobacteraceae
Alphaproteobacteria;Magnetospiraceae
Alphaproteobacteria;Rickettsiales
Alphaproteobacteria;SAR11 clade;Clade I
Alphaproteobacteria;SAR11 clade;Clade II
Alphaproteobacteria;SAR11 clade;Clade IV
Alphaproteobacteria;other SAR11 clade
Alphaproteobacteria;Erythrobacteraceae
Alphaproteobacteria;Thalassobaculales
other Alphaproteobacteria
Deltaproteobacteria;Bacteriovoracaceae
Deltaproteobacteria;Bdellovibrionaceae
Deltaproteobacteria;Desulfarculaceae
Deltaproteobacteria;Sandaracinaceae
Deltaproteobacteria;uncultured NB1-j
Deltaproteobacteria;SAR324 clade(Marine group B)
Deltaproteobacteria;uncultured SAR324 clade
other Deltaproteobacteria
Gammaproteobacteria;Alteromonadaceae
Gammaproteobacteria;Marinobacteraceae
Gammaproteobacteria;Pseudoalteromonadaceae
Gammaproteobacteria;Unclassified Alteromonadales
Betaproteobacteria;Burkholderiaceae
other Betaproteobacteria
Gammaproteobacteria;marine metagenome
Gammaproteobacteria;uncultured HF0770_27E13
Gammaproteobacteria;Alcanivoracaceae
Gammaproteobacteria;Halomonadaceae
Gammaproteobacteria;Oceanospirillaceae
Gammaproteobacteria;SS1-B-06-26
Gammaproteobacteria;Moraxellaceae
Gammaproteobacteria;Pseudomonadaceae
Gammaproteobacteria;Salinisphaeraceae
Gammaproteobacteria;Solimonadaceae
Gammaproteobacteria;SAR86 clade
Gammaproteobacteria;Thioglobaceae
Gammaproteobacteria;UBA10353 marine group
Gammaproteobacteria;Vibrionaceae
other Gammaproteobacteria
Verrucomicrobia;Verrucomicrobiae
WPS-2;hydrothermal vent metagenome
50m
200m
1000m
2000m
3000m
4000m
50m
200m
1000m
2000m
3000m
4000m
ratio > 0 PA refrence
ratio < 0 FL refrence
0.5
1.5
2.5

1116

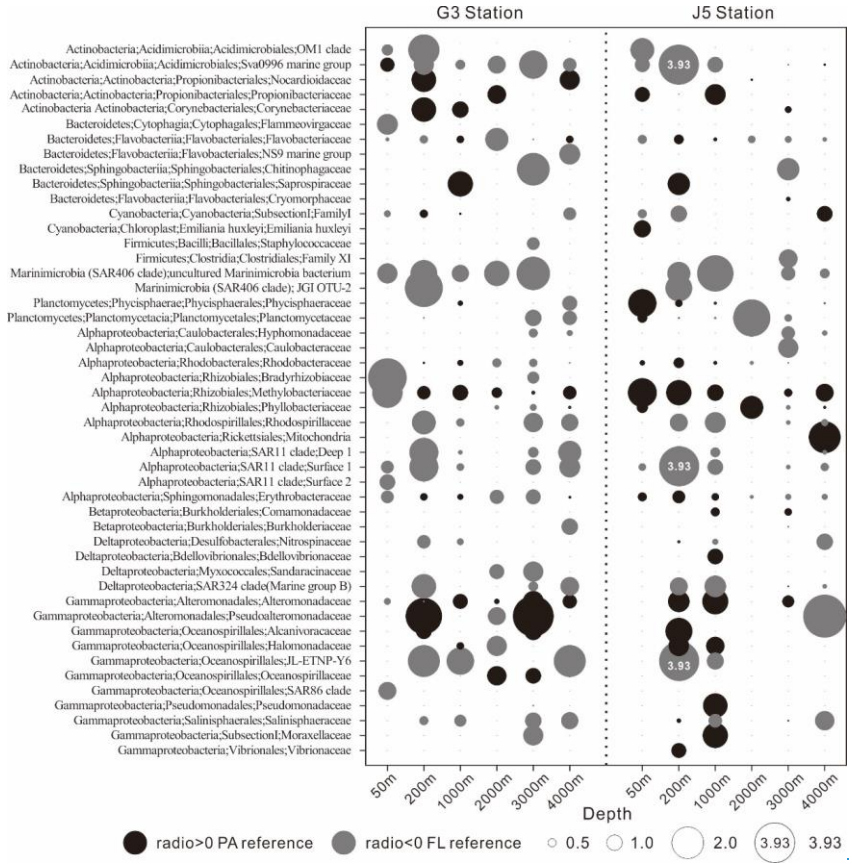


Figure 8. Odds ratio for each of the families with relatively abundant proportions in each sample. Dark grey bubbles represent the clades with a positive odds ratio, meaning the preference of PA lifestyle. Light grey bubbles represent the clades with a negative odds ratio, indicative of the FL preference. Scale is shown in the bottom, and the circle with a number inside indicates actual ratio (not proportional).

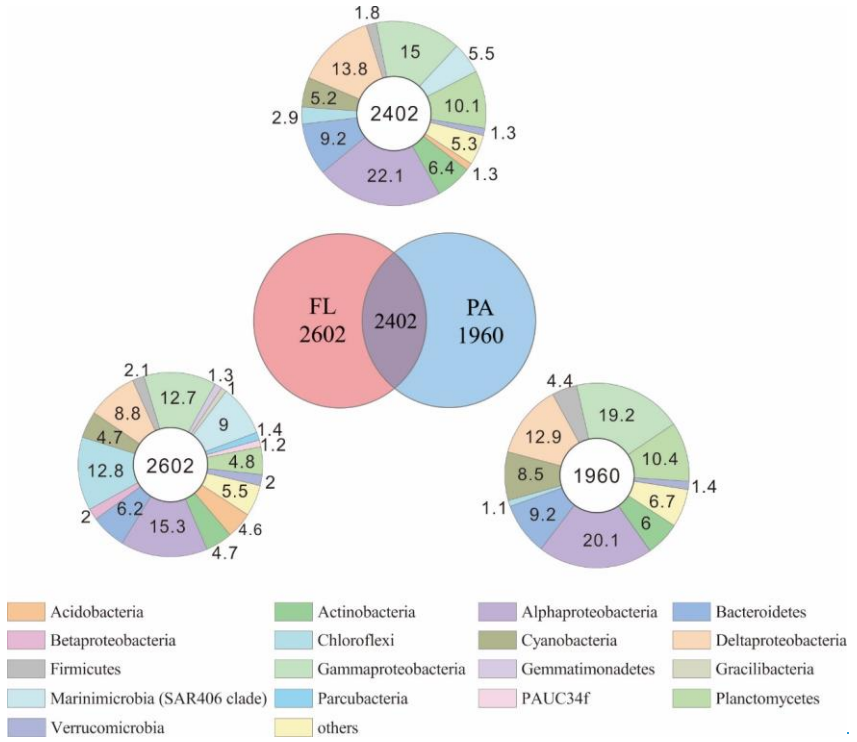


Figure 9. Numbers of each OTU sets including those exclusively found in PA fraction, FL fraction, and those shared by PA and FL fractions. Pie charts represent relative proportions of each bacterial lineages at phylum/class level.

Table 1. Environmental parameters of the water columns at different depths of G3 and J5 stations in the South China Sea

Depth (m)	G3 station										J5 station									
	T (°C)	Sal. (‰)	pH	DO (uM)	DOC (μM)	POC (μM)	Ages * (yr)	NO_3^- (μM)	PO_4^{2-} (μM)	Silicates (μM)	T (°C)	Sal. (‰)	pH	DO (uM)	DOC (μM)	POC (μM)	Ages * (yr)	NO_3^- (μM)	PO_4^{2-} (μM)	Silicates (μM)
50	25.80	33.81	8.02	204.3	63.07	1.5	109	BD	BD	2.27	23.60	33.88	8.02	204.8	67.77	1.6	108	0.12	BD	2.36
200	15.46	34.54	7.75	115.1	53.02	0.8	106	17.98	1.20	21.06	14.27	34.52	7.72	116	49.99	0.9	106	19.13	1.30	26.56
1000	4.68	34.51	7.51	85.5	49.34	1.2	1170	37.16	2.72	114.40	4.46	34.53	7.51	82.3	45.62	2.1	1310	37.04	2.73	121.93
2000	2.52	34.61	-	-	-	1.1	1190	-	-	-	2.49	34.61	7.52	102	41.67	0.9	1670	38.41	2.81	151.46
3000	2.36	34.62	-	-	42.94	1.8	1600	-	-	-	2.36	34.62	7.52	109.7	40.34	0.7	1680	38.16	2.79	145.03
4000	2.39	34.63	7.52	115.1	42.44	0.7	1750	38.48	2.82	141.81	2.43	34.62	7.53	111.8	46.52	1.2	1610	38.58	2.78	145.06

*$\triangle^{14}$C ages; BD: Below detection; -: no measurement.