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

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-, gamma-proteobacteria, 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). 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.

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).

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.

Provide the data for sequence depth (e.g. rarefaction curves) of 16S rRNA gene used in this study.

Specific comments L96: Marine Group III (MGII)→Marine Group (MGIII) L150: what amount of template DNA (ng) did author used? And provide the information about DNA

concentration (or amount) after DNA extraction. L152-155: provide the references of these primers. L161: provide the reference or URL for QIIME 1.9.1 software L177: provide the reference or URL 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. L379 "taxonomically" : add information about the taxonomic levels after this word (e.g. taxonomically (at least family or order?? level)). L386 "depth": I think it is better to correct "hydrological condition (e.g. depth)". L413: provide the R or Rho value before P value (R or Rho=????, P>0.05). 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"? L442-443: γ-proteobacterial (change italic to regular) 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. L462: What is (li)? Reference? L522-523 "statistical analysis": provide the R or Rho, and P values.

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). Figure 6: x-axis is confused. It is better to delete minor scale marks (e.g. those between 50m and 200m). Figure 7, Figure S5: provide the information about failed samples in the legend.

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