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Interactive comment

Interactive comment on "Diversity, distribution and nitrogen use strategies of bacteria in the South China Sea basin" by Yuan-Yuan Li et al.

Yuan-Yuan Li et al.

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Reviewer 2 comments: This study focuses on the 16S and nifH community structure of the South China Sea. It is a description study with a few sampling points at various depths. The main problem of this paper, is the lack of a narrative. The reader will not be sure what is the main finding of this study against already acquired knowledge. The nifH amplicon approach could be mentioned, but as the nifH primers can only capture a small proportion of the community, the findings could be used in support of other results, rather than forming the main findings narrative. In general, the study is worth publishing as it describes the community snapshot of the SCS. However, this paper needs re-writing in order to better show the importance of the findings.

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Answer: Thanks for the reviewer's comments and suggestions. We made extensive changes to this manuscript and re-wrote it according to the reviewer's suggestions. Firstly, the revised manuscript was focused on the community dynamics of the SCS, both the contents related to nifH and nitrogen utilization were removed from our new manuscript. Moreover, redundancy analysis between environmental factors and community structure (Fig. 4) as well as the overall PICRUSt prediction (Fig. 5; Fig. 6) were conducted, and these results were added to the revised manuscript. Secondly, we reconstructed the storyline consisting mainly of "16S rRNA", "environmental influence" and "PICRUSt predictions", in the revised manuscript. Main findings were summarized and discussed with other published studies in the revised manuscript. The importance of our findings lied in the fact that the divergence of bacterial community function and distribution between the nSCS and the sSCS was first reported at the basin scale. Our results again emphasized that bacterial community structures were influenced by environmental factors and provided evidence that the SCS basin exhibited functional zonation among depths which enriched different metabolic potentials.

Introduction: Could you add a few a paragraph about how does the SCS microbiome structure, N-fixation and nutrient limitation etc. compares with other seas. As a reader I would like to know either SCS is an anomaly or does it represent a typical coastal microbiome.

Answer: Thanks for the reviewer's suggestion. We re-wrote the Introduction section in the revised manuscript. New information about the geographical feature, microbiome structure and nutrient limitation of the SCS were added. Comparison with other oceanic regions were also added to the Discussion section. Briefly, the composition of bacterial community was similar with other Pacific Ocean regions with typical oligotrophic characteristics, but exhibited seasonal changes due to the influences of monsoon and eddy.

Results: I find the use of percentage values almost irrelevant considering that you have a single biological replicate per sampling site/depth. I can agree that there is a pattern

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of depth vs surface vs location at the global community level (the whole community pattern as represented on PCoA), but the percentage differences would definetely change with more replications. Please, add that the single replicate does not allow for any statistical analysis to be conducted and instead of using numbers, please just state either some phyla/genera seem to be more/less abundant for a specific sampling point.

Answer: Thanks for the reviewer's comments and suggestions. We agreed that the percentage values used here were irrelevant. Most of the percentage values were removed from the manuscript. A few were retained to show the importance of the species in the survey area. Discussions about the limitation of statistical analysis in present study were added to the Conclusion and Recommend section.

Results: I would like to see a PCA or similar analysis linking nutrient, salinity, temperature with the community structure. Is the influence of the nutrients, salinity etc. smaller or greater than the location? Can you separate them? What I am asking, is the knowledge about salinity and nutrients status of the sampling location enough to predict the likely microbial community structure?

Answer: Thanks for the reviewer's suggestions. We conducted the redundancy analysis between environmental factors and the community structure in the revised manuscript (Fig. 4) according to the reviewer's suggestion. Detailed results and discussion were also added. The results showed that 99.7% of the variance in community structure could be explained by the environmental factors, including nitrate (62.3% of contribution), phosphorus (60.1%), temperature (68.4%), salinity (47.6%), depth (52.4%) in this study. Our results supported the viewpoint that both environment factors and sampling depth were strongly correlated to the bacterial community structure.

(a small remark) please use 100,000 instead of 100, 000 in your sequencing number reports Answer: Corrected.

For the nifH part, please clearly state that no nifH primers are able to provide a comprehensive nifH community profile. Different studies chose different primers. You are BGD

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unravelling a part of nifH community. While the comparisons between sites are valid, please remember that this is just a part of the community, and quite likely most of this community is still our of our reach.

Answer: Thanks for the reviewer's suggestions. We deleted it for this part was removed from the revised manuscript.

Figure 1, please add a legend, what depth does the colour signify Answer: Corrected. Please see the attached Fig. 1.

Figure 2a-d please increase the font size of the labels (graph bottoms) Answer: Corrected. Please see the attached Fig. 2.

Figure 3cd plase correct *Bacteira to Bacteria, please explain, which groups are included in this category in the figure legend. Answer: Corrected. Please see the attached Fig. 3. *Bacteria meant those unclassified bacterial groups.

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201706CSC sampling station 50 m 100 m 250 m 500 m 20°N 750 m 1000 m SEATS 1250 m 1500 m 2000 m 15°N 2500 m SS1 B1 3000 m 3500 m 4000 m • A2 C1° Ocean Data View 4500 m 5000 m 10°N 5500 m

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6000 m

Discussion paper



120°E

115°E

Fig. 1. Sampling sites of the South China Sea basin

110°E



Fig. 2. Differences in bacterial community richness, diversity and structure from horizontal and vertical bacterial samples in the Northern and Southern SCS: (a) The richness of the bacterial community; (b) T

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Deltaproteobacteria Alphaproteobacteria

Gammaproteobacteria

50%

25%

0% SEATS Surface

SEATS BAIN

SEATS 2001

SEATS JEON

ss1-surface

551-10511

(c)

5522000

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Fig. 3. Relative abundances of bacterial compositions of the SCS at phylum level: (a-b) Horizontal and vertical bacterial composition; (c-d) Taxonomic groups of Proteobacteria in bacterial community. *Unclas

A2 surface

B1 surface

C1-Surface

sst.750m





Alteromonadaceae

SEATs 68m Vibrionaceae

SEATs 200m

Acinetobacter

Salinity

Salinisphaeraceae

SAR202

SAR406

SS1_750m

SS1_200m

NO3 SiO3 Decta Decanospirillales Pseudoalteromondatace SAR332 Acidimicrobiales

1.5

SEATs_750m

NO2

SS1_105m

S. <u>___</u>

1.0

Ì.

-1.5

DO

Flavobacteriaceae SAR116 Prochlorococcus

Synechococcus 4

OMI clade 🚽 SAR86 -Rhodobacteraceae

*Cyanobacteria

Chl a

SEATs surface

SS1_surface

Rhodospirillaceae SAR11

A2 surface

C1 surface

B1_surface

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SEATS-750m SEATS-200m SEATS-68m SEATS-surface



Fig. 5. Abundances and distributions of KEGG pathways predicted from PICRUSt: (a) In all sampes; (b) Different depths from SEATS; (c) Different depths from SS1.



Fig. 6. Abundances and distributions of KEGG tier 3 KO categories predicted from PICRUSt: (a) In surface samples; (b) Vertical profiles in SEATS and SS1.



