Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2019-214-AC2, 2019

Biogeosciences Discussions
Open Access
EGU

Interactive comment on "Biogeographical distribution of Microbial Communities along the Rajang River-South China Sea Continuum" *by* Edwin Sien Aun Sia et al.

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Received and published: 2 September 2019

We would like to thank Ref #2 for the comments and suggestions which helped to improve the manuscript significantly. Our point-by-point responses are posted below, with the reviewer's comments being quoted first and our response (R) below each comment.

The manuscript of Sia et al. describes a study of bacterial communities' distribution in a section of the Rajang River. Overall, the quality and content of the paper is in line with similar publications on lotic bacterial communities, where the community composition is linked to environmental parameters. The strongest point of the study is that is covers

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multiple time points (different seasons) and several salinity zones. The authors also made an attempt to estimate potential functions of the bacterial communities. I would like to note a detailed and comprehensive Discussion section. However, some revision is necessary. Certain results need to be verified, methods described more in details (please see specific comments). English language could be improved; the manuscript is not free of mistakes and misprints.

Some specific questions and comments:

P 5 L 146 – it is not clear for me how is classification into freshwater and brackish water described in Fig. 1(B). Possibly that is due to the poor quality of the map.

R: Thank you for pointing this out. We removed this sentence "as described in Fig 1. (B)" as Fig. 1(B) is to show the areas with peat only.

P 5 L 150, 152 – Are you sure that those were polycarbonate filters? GF are usually glass fiber filters.

R: Thank you for pointing this out. The correct filter used was Nuclepore™ Track-Etched Polycarbonate Membrane Filter. We have removed the (GF/C) description.

P 5 L 156 – Incorrect reference. Caporaso et al. 2012 describe QIIME pipeline, not Illumina sequencing.

R: Agreed. Changed to Bentley et al. (2008) which describes the first paper that Illumina was based upon.

P 5 L 156 – Could you please add more information on DNA extraction and library preparation procedures, for example, which primers were used for amplification?

R: Thank you for pointing this out. We have included the relevant information in the methods section. It now reads: ....A total of 117 filters were recovered (1 x 3.0 μm was discarded due to contamination) and immediately stored at -20 °C and sent to the Australian Centre for Ecogenomics (ACE), Brisbane for DNA extraction, library prepa-

ration and processing utilizing the Illumina (Bentley et al., 2008) platform.

2.2 Illumina Sequencing and Bioinformatics Analyses Initial upstream processes were carried out by the Australian Centre for Ecogenomics utilizing the ACE mitag pipeline (ACE, 2016). The primers utilized were based on the V3 – V4 hypervariable regions of the 16S rRNA gene.

P 6 L 163 – Reference for Mothur pipeline missing.

R: Thank you for pointing this out. The relevant citation was added (Schloss et al., 2009)

P 6 L 175 –Reference for the GreenGenes database missing.

R: Thank you for pointing this out. The relevant citation was added (DeSantis et al., 2006)

P 7 L 215 – Can you explain why the sequencing depth was so low, especially for some samples? Was it on purpose?

R: Thank you for this question. The minimum sequencing depth was 10,000 reads per sample. After QC and removal of unknown sequences, some samples were left with a very low read count. Given the general lack of data from these systems and to 'lose' as little information /samples as possible, we chose a low read number for the subsampling.

P 7 L 215 – Were the sequences deposited to a public database?

R: As of now, the sequences have yet to be deposited in a public database. They will be submitted in the coming days.

P 7 L 232 – Are you sure it is "brackish peat" and not "freshwater peat", which seems to me from Fig.2?

R: Yes, thank you for pointing this out. "Brackish peat" was changed to "freshwater

peat”

P 8 L 247-249 – This observation is not obvious to me from Fig. 3.

R: Agreed, this portion was removed.

P 8 L 258-259 – was the difference between OTU counts statistically significant?

R: The results shown were plotted based on the calculations from the estimate_richness function in the phyloseq package, and hence the observation was more a qualitative observation.

P 10 L 324 – I didn’t find any description of the results separately for free-living and particle-attached bacteria, however you discuss them a bit in chapter 4.1 in relation to Supp. Fig. 3. Were the results pooled together for free-living and particle-attached bacteria in Fig. 2-7?

R: Thank you for pointing this out. Yes, for Figures 2 – 7 the results were pooled together for discussion as the difference between free-living and particle-attached bacteria did not exhibit clear distinction and hence was not further elaborated. The following sentence was added in Section 2.2: “Apart from the results and discussion shown for free-living and particle-attached bacteria, the remaining discussion is based on the pooled results of both components”

P 11 L 378-380 – How does the dominance of Proteobacteria indicate its role in nitrogen cycling? Please explain how it is complementary to Cyanobacteria bloom, the message is unclear.

R: The sentence was rephrased as “In a study by Yang et al. (2013), the dominance of Protebacteria influenced the nitrogen cycle via the processes of nitrification and denitrification, in which aeration would increase its abundance and result in higher mortality of cyanobacteria.

P 12 L 394- 397 – “In contrast, most extreme environments show” this sentence sounds

strange and needs to be rephrased.

R: Agreed, this sentence was changed to “In most of these studies, Deinococcus-Thermus was found in low abundance (e.g. 1% in Antarctic marine environments, 1.5% in hypersaline soils; Giudice and Azzaro, 2019; Vera-Gargallo et al., 2019) when compared to the Rajang River.”

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