

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

Anonymous Referee #1

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Manuscript: bg-2019-214 Title: Biogeographical distribution of Microbial Communities along the Rajang River-South China Sea Continuum

A. General comments This study addresses the underlying factors that may influence the spatial and seasonal distribution of the prokaryotic communities and nutrient dynamics along the Rajang River, South China. Although the results of this study are valid and interesting, there are several points that need to be addressed. 1) Dividing sampling cruises into “wet season” and “dry season” may be more beneficial than referring to them individually. Authors mentioned both wet and dry seasons in the Methodology section (section 2.1), however, sampling cruises associated with each are lacking.

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2) The site map (Figure 1A) currently shows sampling points throughout the river with source types (Figure 1B), but illustration of anthropogenic activities along the river is missing. It will be helpful to add these as it's not clear which sections of the river are impacted by which activities. 3) Additional statistical analysis, such as PERMANOVA, may be used to infer the impact of anthropogenic activities (e.g. human settlements, effluents, transportation and sand dredging) and source types on beta diversity. Much of the Results and Discussion sections revolve around alpha diversity indices but very little is mentioned about beta diversity. 4) Potential functionality inferred from PICRUST showed clear distinction between samples when comparing source type. It would be interesting to see if potential functionality differed to such an extent when samples are compared by anthropogenic perturbations. 5) The general flow of the Discussion section needs improvement. The significance and contribution of the study will have a bigger impact when the Discussion is presented clear and logically. Also, the authors should double check the tense (present/past/passive) for each section. 6) Recheck format of in-text references. Not all citations are written in the same format.

B. Specific comments 1.0 Introduction p. 4 paragraph 6: The authors aimed to study microbial diversity and potential function in the Rajang River. Although this study is the first to investigate microbial diversity along a freshwater-marine gradient, with a tropical peatland component, the importance of the river in Malaysia and clear objectives need to be stated.

2.0 Methodology 2.1 Study area and sampling strategy p. 5 line 136-139: "According to Wetlands International (2015), the land surrounding the study sites is characterized by a range of anthropogenic activities, ranging from oil palm and sago plantations to human settlements as well as transportation and sand dredging activities (Fig. 1(B))." This is not clear from Fig 1B. Colours are associated with forested or non-forested lands, however, the map does not depict the different anthropogenic activities along the river.

2.2 Pyrosequencing and Bioinformatics Analyses → Change "Pyrosequencing"

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to “Illumina sequencing” in the subheading. The authors did not perform 454-pyrosequencing but Illumina sequencing. Did ACE also extract DNA from samples? If that’s the case, the authors should mention this in the beginning of this section.

2.4 Statistical Analyses and distLM model The authors used db-RDA to determine the impact of environmental variables on microbial diversity. The same method can be used to determine which parameters have an influence on specific bacterial taxa. Likewise, Spearman/Pearson correlations can be drawn between environmental parameters and taxa. The information inferred from these additional analyses can help the authors to link certain taxa to specific source types or pollution sources. I also suggest that anthropogenic inputs should be divided into the following categories: human settlements, effluents (from both palm oil and sago plantations), transportation and sand dredging. Variation partitioning, if possible, may then be used to determine which anthropogenic input, or source type, had the biggest impact on bacterial diversity along the river.

3.0 Results 3.3 Bacterial Distribution according to source type and cruise This section may be improved by organizing it into the following paragraphs: Mention the dominant taxa and their relative abundances. The author mentioned this in the Discussion section (section 4.1 line 333-335) but not in the Results section. Which taxa (dominant or specialized) were more predominant at specific source types and/or seasons? In this section, the authors acknowledge a higher Cyanobacterial abundance for the September 2017 marine and brackish peat samples. In the Discussion section they refer to the higher Cyanobacterial counts as “blooms” without prior testing (e.g. chlorophyll-a) as a proxy measure to confirm algal blooms. Since Cyanobacteria are more pronounced during warmer months, and anthropogenic activities close to the sampling areas can cause nutrient input and thus proliferation of Cyanobacteria, how valid is the assumption/statement made in the manuscript without additional measurements?

3.4 Alpha Diversity Indices How did the authors calculate the effect of land use

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and source type on alpha diversity indices? This was not mentioned in the Methodology section. Instead of comparing indices between cruises, the authors can make comparisons between seasons (e.g. compare the entire wet season with the dry season).

4.0 Discussion – I recommend that the authors re-write certain paragraphs of the Discussion section so that it may have a bigger impact on readers. Instead of naming all the different types of taxa in the river, focus on the important ones and what their roles are. How does the environment and different inputs (source types and anthropogenic activities) impact these taxa?

4.4 Possible pathogenic bacteria and/or anthropogenic influence and land-use change – Was *Flavobacterium* the only potential pathogen identified? I would suggest to start the paragraph with anthropogenic influence and land-use change. A second, shorter paragraph can discuss the potential pathogens

C. Technical comments

1.0 Introduction p. 3: Combine paragraphs 2 and 5. Both are discussing lotic environments and nutrient cycling; it will thus make more sense to combine these two. p. 4 line 93-93: Due to their high diversity and fast generation time, microbial communities are the first responders to environmental changes. . . . p. 4 line 96: Liao et al. (2019) showed that p. 4 line 97: delete “further” p. 4 line 97: Bruland et al. (2008) demonstrated that the p. 4 line 99-102: “Thus, as the Rajang River experiences two monsoonal seasons (Sa’adi et al., 2017) and is subjected to anthropogenic disturbances (Gaveau et al., 2016; Miettinen et al., 2016), it is thus fundamental to take into consideration both seasonal and anthropogenic influences on the microbial communities of the Rajang River.” This forms part of the aim and objective and should rather move to last paragraph p. 4 line 115: delete “hypothesized” p. 4 line 120-121: as well as anthropogenic disturbances (e.g. human settlements and plantations) on microbial succession. p. 4 line 121-122: Delete “Linear models are used to examine the relationship between the microbial community structure and their environment.”

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2.0 Methodology 2.1 Study area and sampling strategy p. 5 line 130: The region. . . p. 5 line 134: small tributaries p. 5 line 142: Which months were associated with the wet and dry season, respectively? p. 5 line 149: Approximately 250 – 500 mL of water. . . p. 5 line 153-156: A total of 117 filters were recovered (1 x 3.0 μm filter was discarded due to contamination) and immediately stored at - 20°C.

2.2 Pyrosequencing and Bioinformatics Analyses p. 5 line 160: Briefly, fastq files generated. . . p. 6 line 161: quality trimmed with fastqc, primer sequences. . . p. 6 line 162-163: High quality sequences were subsequently processed using the Mothur pipeline. p. 6 line 164: SILVA database p. 6 line 171: potential functional genes

2.3 Physico-chemical Data and Geochemical Analyses p. 6 line 179: in-between the cruises p. 6 line 189-191: Belawai samples (2°13'47.16"N, 111°12'19.04"E) were used in an incubation experiment to study the net primary productivity and respiration rate of the Rajang River. Technical triplicates were incubated in both light and dark set-ups (Refer to Supp. Table 1 for details).

2.4 Statistical Analyses and distLM model p. 6 line 195-197: to determine if the various terrestrial source types or different land use impacted bacterial community composition. p. 7 line 199: what type of normalization method was used? p. 7 line 202-204: "The authors would like to note that the distLM models are based on only the August 2016 and March 2017 cruise as there was a lack of physico-chemical data from the September 2017 cruise due to malfunctioning equipment." Delete this sentence, no need to mention this twice, at the end of the paragraph (lines 205-208) is sufficient

3.0 Results 3.1 Clustering of Samples according to ANOSIM Global Test Scores p. 7 line 215: A total of 74,690 high quality bacterial sequences. . .

3.2 Shifts in bacterial community structure p. 7 line 223-224: Delete this sentence, it's redundant p. 7 line 230: August 2016 (dry season) samples p. 7 line 231: September 2017 (wet season) samples p. 7 line 231: There were clear overlaps between samples from. . . p. 7 line 232-233: We also observed a gradual shift in bacterial composition

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from mineral soils and freshwater peat towards brackish and marine samples.

3.3 Bacterial Distribution according to source type and cruise p. 8 line 240: Delete “Fig 3 show that”

3.4 Alpha Diversity Indices p. 8 line 263-264: Rewrite the sentence p. 8 line 265: microbial communities varied significantly along the different source types. . . p. 8 line 266: . . . to be higher than that of March 2017. . . p. 9 line 276: Authors are referring to “upstream” samples in this sentence, which samples are these? They did not clearly differentiate between upstream and downstream samples in the Methodology section which is causing confusion in subsequent text.

3.5 Functional Profile of Bacterial Communities p. 9 line 289-290: Potential KEGG pathways between (i) marine and brackish peat, and (ii) freshwater peat and mineral soil were similar. There were differences between source types and seasons p. 9 line 290-292: Delete this sentence. It’s part of Discussion

3.6 Distance-based Linear Model of bacterial communities and environmental parameters p. 9 line 301: Dissolved Inorganic Phosphate (10.57%). p. 9 line 304: Delete “lastly” p. 9 line 305: Dissolved Inorganic Nitrogen (4.29%) respectively made up the. . . p. 10 line 308-309: Move this sentence to p. 9 line 300: “Marginal DistLM was performed in order to gauge the extent of physicochemical parameters or environmental variables accounting for a compelling proportion of variation in the bacterial communities. Significant vectors of environmental variables ($R^2 > 0.3892$, $P < 0.001$) were 308 calculated based on a linear model (DistLM) and plotted against the bacterial community composition as shown in Fig 7. Salinity was the single best predictor variable. . . “ p. 10 line 311-320: The distLM model clustered samples from the August 2016 cruise separately from the March 2017 samples. Brackish peat, as well as marine samples from August 2016, correlated more strongly with salinity, irrespective of land use. On the contrary, the March 2017 samples were found to cluster separately with DO. In addition, the August 2016 mineral soil samples correlated with silicate.

4.0 Discussion p. 10 line 332: Delete this subheading and move subheading 4.2 to 4.1 p. 10 line 335-342: in varying abundances, indicating high variation within the system. The majority of bacterial taxa were restricted to a relatively small number of assemblages. However, due to the heterogeneity of the Rajang River, substantial shifts in OTU diversity were shown, while exhibiting successional changes in community composition downstream. We observed abrupt shifts in terms of richness and diversity as well as bacterial distribution, which was structured according to macro-scale source types. Staley et al. (2015) proposed that variability in microbial communities were less due to the presence/absence but likely due to shifts in relative abundance of OTUs. p. 10 line 342: community composition, overlap between the core microbiome (i.e. free-living and particle-attached portions) of samples were not evident. p. 11 line 346: Change “further supported” with “demonstrated” p. 11 line 351: The short residence time in the Rajang River likely reflected a similar scenario to San Francisco Bay (Reference). p. 11 line 372-378: Delete these sentences. Beta-proteobacteria was already mentioned in the previous paragraph. p. 11 line 380: Were there really Cyanobacterial blooms? p. 12 line 385: Sphingomonas, a purple-sulfur bacteria, p. 12 line 391: indicating its preference for this environment. It’s interesting to note that most studies on... p. 12 line 394: 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. p. 12 line 397: Start new paragraph with: “There was a fundamental shift in bacterial community composition when taking the major taxa into consideration. There was a clear distinction between dry and wet seasons with an overall higher species richness and diversity for the dry season” For the wet season, focus on both the March and September cruises to make a conclusion

4.3 Factors determining bacterial community composition p. 13 line 421-427: Delete these sentences, was already mentioned in Methodology p. 13 line 427: There was a continual shift... p. 13 line 432: similar to findings by... p. 13 line 434: likely explaining the reduced relative abundances of some taxa. For example, Chloroflexi has a

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higher relative abundance upstream while *Deinococcus-Thermus* shows lower relative abundance downstream. p. 13 line 438: Delete “salinity gradients” p. 13 line 451: Salinity, DIP () and dissolved oxygen are major environmental drivers of species distribution (References). In this study, marine and brackish peat samples correlated well with salinity. p. 14 line 459-469: Not sure what the authors want to say here. Do they assume there was high or low bacterial productivity? p. 14 line 478-480: While the development of unique community structures was strongly influenced by spatial factors, seasonality also played a role. Seasonal variability was also observed between the. . . p. 14 line 485-490: Again, can the term Cyanobacterial bloom be accurately used? p. 15 line 494: “were similar in terms of climate. . .”

4.4 Possible pathogenic bacteria and/or anthropogenic influence and land-use change p. 15: Start the paragraph with line 515: “The results obtained from this study suggest that the run-off from anthropogenic activities alters the microbial community composition. Anthropogenic disturbances, in particular settlements and logging (secondary forest), led to higher diversity indices (Fig .6). On the contrary, sites surrounded by oil. . . .

5.0 Conclusion p. 16 line 543: The authors refer to “pristine and less pristine environments”. Which sites were classified as pristine, and which were less pristine? p. 16 line 545: The PICRUST results showed differences between source types p. 16 line 550: mixing experiments. This approach will contribute towards a better understanding of the response of microbial communities to anthropogenic perturbations, as well as their role in degrading peat-related run-off from. . .

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