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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 #2

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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 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. P 5 L 150, 152 – Are you sure that those were polycarbonate filters? GF are usually glass fiber filters. P 5 L 156 – Incorrect reference. Caporaso et al. 2012 describe QIIME pipeline, not Illumina sequencing. 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? P 6 L 163 – Reference for Mothur pipeline missing. P 6 L 175 – Reference for the GreenGenes database missing. P 7 L 215 – Can you explain why the sequencing depth was so low, especially for some samples? Was it on purpose? P 7 L 215 – Were the sequences deposited to a public database? P 7 L 232 – Are you sure it is "brackish peat" and not "freshwater peat", which seems to me from Fig.2? P 8 L 247-249 – This observation is not obvious to me from Fig. 3. P 8 L 258-259 – was the difference between OTU counts statistically significant? 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? 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. P 12 L 394- 397 – "In contrast, most extreme environments show..." this sentence sounds strange and needs to be rephrased.

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