Manuscript: #49405v2

Title: Characteristics of planktonic and sediment bacterial communities in a heavily polluted urban river

General comments:

This study aims to investigate the spatiotemporal dynamics of both planktonic and sediment bacterial populations in the urban river and the associated environmental factors. The authors addressed previous comments and suggestions, which strengthen the paper, however, there are some issues that need to be addressed in the revised manuscript: (1) in several figures (Figures 3-6, 8) different sample names (SFe1-5, SAu1-5) are provided without clarification in the Materials & Methods section, whereas in the latter section only sampling sites (L1 – L5) are mentioned. This might be confusing for some readers and needs to be clarified. (2) The authors provided some seasonal information for the sampling sites (although the temperatures for each season might have been switched), additional information such as rainfall can be extremely helpful. I have noticed that nutrient levels during winter was higher than in summer that might be linked to for e.g. rainfall events and river hydraulics. Also, when nutrients are high it might select for specific taxa that can influence diversity. (3) The authors need to use certain terms consistently throughout the manuscript. For example, water bacterial communities are referred to as both plankton and bacterioplankton communities. They should decide on one term and use it consistently. (4) I appreciate the additional statistical analysis that the authors performed. In the Discussion section the authors stated that the water bacterioplankton community was seeded from the sediment community. It will be very interesting to see the co-occurrences between these two communities and potential functionality for each community. This can elevate the paper than just explaining diversity in the river. (5) The authors have tried to improve the Discussion & Conclusion sections. However, I feel that they should also discuss the significance of the results in terms of: (i) What do the results imply about the health and status of the river and for use by the community? (ii) Is it possible to infer long-term consequences on bacterial communities (water column and sediment) as well as river health if pollution continues? (iii) Can the results be used for any monitoring purposes? (iv) Can the authors also mention the limitations of this study and plans for future improvements. I believe that if the authors discuss the significance of the results and how it can be used for future improvement that it will enhance the paper.

Specific comments: (based on pdf version)

Introduction

Line 69: considerably modifies

Line 73: sewage discharge was not mentioned in the previous sentences. Is this part of residential discharge? If so, it should be mentioned so that sewage, residential discharge and dam construction form one component that’s used for the aim in the next paragraph

Materials & Methods

Line 91: what is meant by “whole water quality”?

Line 94: …two adjacent sampling sites is approximately 8-12km.

Line 100: and phosphorus etc.

Line 102-103: Why is the air temp for winter warmer than for summer? Maybe the two temperatures were switched? Can the authors also add the mean annual rainfall for each season? It will be important to know if high rainfall over a specific season (prior to the sampling dates) causes a 'wash-out' effect of communities and nutrients

Line 108: methods described in literature (*China Environmental Protection Agency, 2002; Wang, 2012*) and are shown in Tables S1 and S2, respectively.

Line 124: protocol by *Caporaso et al. (2010).*

Line 127: was further obtained using the UPARSE pipeline (*Edgar, 2013*).

Line 133: One-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) test, was used to determine significant differences ($P < 0.05$) between alpha-diversity indices and the relative abundances of potential bacterial pathogens among sample groups.

- The authors calculated statistics between sample groups (SFe1-5, SAu1-5) but failed to mention the groups in the Materials & Methods section. This can cause confusion with readers as the authors only mentioned the sites (L1 – L5)

Line 136-142: Potential relationships between physicochemical properties and: (i) bacterial community richness; (ii) diversity; and (iii) proportion of the major bacterial groups were examined with Spearman rank correlation using the software SPSS 20.0. The similarity of bacterial communities (i.e. the shared OTUs among all sites) were analyzed with a Venn diagram. Beta-diversity using Bray-Curtis dissimilarity (*Bray and Curtis,1957*) was used to examine the difference in the overall community composition between samples and visualized on a PCoA plot.

Line 143: Permutational multivariate analysis of variance (PerMANOVA) was performed to test for significant

Line 145: (i) A sentence is required to state why multivariate analysis was used. E.g. 'Multivariate analysis was used to determine which physicochemical properties had the biggest impact on planktonic and sediment bacterial communities. '

(ii) Although the authors stated in their rebuttal letter that species data were log transformed and environmental data were z-score standardized prior to beta diversity calculations, it is important to mention it in the text as well.

(iii) Did the authors test for VIF and performed ANOVA of parameters before running the final RDA model?

Line 145: (DCA) was first applied

Results

Line 170: What about the winter samples? Where there any differences between HS and RI areas?

Line 172-173: The authors need to rephrase or elaborate this sentence; the exact meaning is unclear.

Line 180: There's an error in the Figure 3 legend. The coloring for 'Sediment (winter)' should be blue and not green

Line 180: PerMANOVA confirmed that the bacterial communities…

Line 182: Bacterioplankton communities for summer samples significantly differed from winter samples ($R^2 = 0.731$, $P= 0.011$)

Line 185: HS and RI sediment communities are all clustered together on the PCoA plot, not apparent how they are significantly different

Line 212-213: Delete this sentence, it's redundant

Line 215: Not sure what the authors mean by 'dissimilarity'. Do they maybe mean the 'presence' of potential pathogens?

Line 216: 12 major bacterial genera (relative abundance > 0.1% in each sample)

Line 227: and taxa (*Proteobacteria* and *Alphaproteobacteria*) displayed positive correlations with water temperature ($P < 0.05$ or $P < 0.01$)

Line 230: negative correlation with water NH_4^+–N and TOC concentrations ($P < 0.05$).

Line 232: In addition, water temperature and TP were identified as key drivers determining the abundance of *Actinobacteria* ($P < 0.05$). Water temperature also correlated to *Patescibacteria* ($P < 0.05$).

- Was the correlation with *Patescibacteria* positive or negative?

Line 237: Figure 6: Instead of using latitude and longitude in the RDA, the authors can use distance between sampling points as a variable.

- What about TP? It's situated close to the winter samples and would thus suggest influence on communities.
- It seems that the authors included all environmental variables in the RDA. Did they test for multicollinearity (VIF) and which variables were significant (anova) before constructing the final RDA model?

Line 240: The VPA indicated that season, nutrients,

Line 242: Season, nutrients, and

Line 246: exhibited a positive correlation with sediment NH_4^+–N, NO_3^-–N, and TN ($P < 0.05$) concentrations (Table 4).

Line 251: What do the authors mean by "in direct proportion"?

Line 255-257: *Bacteroidetes* showed a positive correlation with TP and TOC ($P < 0.05$ or $P < 0.01$). RDA analysis for sediment communities showed that sediment physicochemical factors explained 44.5% and 31.4% of the total variation (Figure 8), respectively.

- Again, it seems that the authors included all environmental variables in the RDA. Did they test for multicollinearity (VIF) and which variables were significant (anova) before constructing the final RDA model?

Line 261-265: The VPA showed that season, nutrients, and geographic distance contributed 60.6% of the variation in the sediment bacterial community (Figure 7b). Season and nutrients explained 50.9% of the variation, indicating a major contribution in shaping the sediment bacterial community.

Discussion

Line 268: *Bacterial community richness and diversity in Liangtan river*

Line 273: What do the author mean by “a certain urban river”? Is it for the Liangtan river?

Line 275-279: This is a repetition of the results and can be deleted.

Line 286-289: HS and RI areas in summer (P<0.05), suggesting spatial distribution of bacterial communities. The spatial change of bacterial community diversity was dependent on sampling date. *Dai et al. (2016)* indicated a remarkable spatial variation of bacterial Shannon diversity in summer compared to spring, which further support our results.

- Spatial differences will be dependent on distance, whereas temporal differences will be dependent on sampling date.

Line 291: Previous literature suggest that

Line 297: previously (*Mao et al. 2019; Zhang et al. 2019)*,

Line 304: *Bacterial community composition in Liangtan river*

Line 308: What do the author mean by “a certain urban river”? Is it for the Liangtan river?

Line 309: PerMANOVA revealed strong heterogeneity

Line 311: Did the authors compare their results to all rivers or just urban rivers?

Line 313: As proposed by *Staley et al. (2015)*, sediment can contribute up to 50% of the community composition in the water column explaining the big overlap.

Line 317-321: Noticeably, the genera *Pseudomonas*, *Arcobacter*, and *Acinetobacter* were detected in high abundance in water and/or sediment samples. Additionally, taxa displayed higher abundances in the RI area compared to the HS area (Table S2) that may be attributed to the high nutrient sources from industrial and residential sewage.

Line 332: Interestingly, the genera *Bacillus* and *Clostridium* (phylum *Firmicutes*) were found mainly in sediment samples from RI area associated with the presence of untreated fecal sewage.

Line 334: “*Cyanobacteria* was another predominant group…” Start a new paragraph from this sentence

Line 336: might be associated with the hydropower station. Similar observations were documented for the Guadiana river (*Domingues et al., 2014*) and Jiangling river (*Wang et al., 2018*).

Line 342: What do the authors mean by “direct proportion”? Does it mean that Cyanobacteria correlated with NH4, TN and TOC?

Line 343: These results suggest environmental dominators drive potential responses of bacterial communities to the domestic sewage-polluted conditions and construction of hydropower station.

- Which responses? Community structure?

Line 347-353: The VPA revealed a major contribution of season and nutrients in shaping planktonic and sediment bacterial communities, yet the effect of geographical distance on bacterial community structure was negligent. Environmental factors are more likely to contribute to variation in bacterial composition than geographical distance at small or intermediate spatial scales (less than 3000 km; *Martiny et al. 2006*). Hence, environmental factors (season and nutrients) were more important than geographical distance in shaping bacterial community structure in the Jiangling River.

Line 356: phosphorus and nitrogen are the major factors

Line 363: in Liangtan river