Manuscript: #49405

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. Although the results of this study are valid, there are several points that need to be addressed. (1) The aim was to investigate "spatiotemporal" dynamics, however, only temporal trends were investigated and discussed. Distances between sampling points were not reported, so it is not clear whether spatial trends in community dynamics were possible/observed. (2) A site map, as well as description anthropogenic activities that impact each sampling site, will be beneficial. Also, a short description of summer and winter season can be helpful. This will provide a more comprehensive picture of the river and allow better understanding of the data. (3) Additional statistical analyses and ordination analysis are required. Statistical analyses were not performed on alpha and beta diversity metrics and are necessary to extend the validity of the results. (4) Although the authors determined which environmental parameters influenced communities, it will be interesting and more meaningful to see which anthropogenic activities impacted their diversity and distribution. This can be achieved by variation partitioning analysis or a similar type of analysis. (5) It will also be good to infer relationships/interactions between OTUs within communities and/or between communities (e.g. water and sediment). This will provide a deeper insight into community dynamics but also strengthen the paper. (6) Raw data for environmental parameters measured were not supplied. It will be helpful to add a table with the values of environmental parameters so the reader can trace back any patterns/variations to the raw data. That being said, the English language, grammar style and usage throughout the manuscript was good.

Specific comments:

Introduction

p. 8 line 62-73: The transition between the first (surrounding terrestrial environment) and second (dams) part of the paragraph is not fluent. An extra sentence(s) is necessary to inform the reader how dams play a role in aquatic ecosystem.

p. 8 line 76: I recommend that the authors elaborate on the aim and objectives of the study. They mentioned how anthropogenic activities and dams can alter bacterial communities in the penultimate paragraph (paragraph 3), so this should form part of the aim and objectives.

Materials & Methods

Sites description and sample collection

- A site map of the sampling area and points will be helpful. The map can also show which sites are impacted by which anthropogenic activities. A description of the different seasons (summer vs winter) will also help the reader to understand the temporal trends in the data.

- Which of the samples served as control samples? It will be useful to have control samples to fully comprehend the impact of residential and industrial areas/activities on bacterioplankton and sediment communities.
- What is the type of pollution sources in the residential and industrial areas?

p. 9 line 91: are located downstream and upstream…
p. 9 line 92: …industrial areas. Additionally, Sites L3 and L4 are adjacent to Xiyong and Tuzhu sewage plants, respectively.
p. 9 line 96: were the plexiglass water and tube core samplers sterilized prior to sampling?
p. 9 line 102: are shown in Tables S1 and S2…

Molecular analyses

- Were data log/square root transformed prior to beta diversity calculations and RDA analysis?
- Were environmental data standardized (z-score) prior to RDA analysis?
- I recommend that the authors perform principal component analysis (PCA) to determine the most important gradients in environmental data.
- It will be useful if the authors can perform additional statistical analysis on the alpha and beta diversity metrics. For example, ANOVA, Kruskal-Wallis or Wilcoxon tests can be used on alpha diversity metrics to determine statistical differences between samples. For beta diversity, PERMANOVA (adonis/adonis2) can be used to determine if there is a significant difference in the centroid & dispersion of upstream and downstream communities. This can be supplemented with betadispersivity (betadisper) tests.
- Water and sediment samples were plotted on two separate RDA plots. It will be useful to make one RDA plot for all samples. It will be easier for the reader to see/determine which environmental parameters influence which samples.

p. 10 line 107: to retain microbial cells from **river** water
p. 10 line 109: according to manufacturer instructions.
p. 10 line 110: using the primer set
p. 10 line 117: further processed as previously described (*Caporaso et al., 2010*). Chimeric reads were discarded using UCHIME (*Edgar et al., 2011*).
p. 10 line 125-127: Did the authors perform weighted or unweighted Unifrac? Why did they use UPGMA clustering to visualize beta diversity? I believe visualization of beta diversity will be better with a PCoA or NMDS plot.
p. 11 line 131: Were Spearman rank correlations performed on absolute or relative abundances? It is better to use absolute abundance for this purpose because relative abundance data suffers from apparent correlations. That being said, the paper by Quinn et al. 2017 (https://www.nature.com/articles/s41598-017-16520-0) is very helpful to understand compositional data and correlations.

p. 11 line 131-136: I commend the authors for applying DCA to determine which ordination analysis fits their data, very few papers report on this. However, the description of the process can be shortened (e.g. Redundancy analysis (RDA) was performed (*Lepš and Šmilauer, 2003*) based on the DCA results.)

Results

- The aim was to investigate "spatiotemporal" dynamics; however, the paper only looks at temporal trends. Distances between sampling points were not reported and results on the aspect are lacking.
- Values for environmental parameters were not reported. The authors stated that some samples showed seasonal variations while others showed no variation. It will be helpful to add a table with the values of environmental parameters measured so the reader can trace back any patterns/variations to the raw data.
- Alpha diversity indices are presented in text. I recommend that indices are shown as box plots (with median values and interquartile ranges indicated on plots); statistical differences between groups can also be indicated on plots. Visualization of indices will make it easier to follow spatio-temporal trends.
- It will also be interesting to know if samples in industrial and residential areas (L3-L5) were richer and more diverse than L1 & L2
- Were pathogenic or opportunistic pathogens present in samples? Particularly for samples L3 and L4 that were sampled closed to sewage plants.
- Authors refer to "significance/significant" results in text (p. 12 line 155 & 167) but they fail to mention if statistical analyses were performed on alpha or beta diversity metrics in the Materials & Methods sections. If such analyses were performed it have to be clearly stated. Conversely, if analyses were not performed then authors cannot refer to results as "significant"; others words such as "noteworthy/important/substantial" might be of better use.

Bacterial community richness and diversity

p. 11 line 151: than the corresponding water sample.

Discussion

I recommend the authors organize the Discussion by importance of issues:

- First paragraph: State and interpret key findings. Provide the answer to the research question with supporting information. Although the first paragraph interprets some of the key findings, it does not answer the question about beta diversity.
- Middle paragraphs: Compare and contrast your findings with those of other published results. Organize the topics from most to least important. Explain any discrepancies, unexpected findings, and limitations. The paragraph discussing the possible impacts of anthropogenic impacts on bacterioplankton communities were interesting and enlightening. It will good to have a comparison with sediment communities.
- Last paragraph: Provide concluding summary with significance/implication of the study