Anonymous Referee #2

We thank the reviewer for their time and review of our paper. We have made the revisions as per their suggestions and feel the manuscript is improved as a result.

There are a few areas where tense usage is not consistent.
We have revised the text and corrected the tense usage.

Line 25 : "characterize" should be "characterized"
The text has been corrected.

Line 26 : " find" should be "found"
The text has been corrected.

Lines 37-38: I think it should be "Mangroves span tidal zones that are characterized by periodic tidal flooding, such that environmental conditions, such as salinity, vary greatly across small spatiotemporal scales.
We agree with the reviewer and have revised the sentence.

Line 39: This sentence is a bit awkward because it sounds like you are describing and impact. Instead, you could say something about what tidal zones mangroves span. This might be good as you don't really mention Sublittoral, Intertidal, or Supralittoral anywhere in your introduction.
We agree with the reviewer and have revised the text to better define the tidal zones studied. We have also included them in the abstract to help the reader.

Lines 54-56: Awkward wording, try to make a little clearer.
We have revised the sentence.

Line 69: "as" should be "is"
The text has been corrected.

Line 79: Remove "Importantly"
We have removed the word from the text.

Line 108: make sure you put "gene" after "16S rRNA"
We revised and corrected the text.

Line 129: Make sure you mention that vegan is an R package. You don't seem to mention R in this section or the citation for R. Additionally Jari Oksanen is the creator of the vegan package. I'm not sure where the Dixon 2003 citation comes from.
https://www.rdocumentation.org/packages/vegan/versions/2.4-2

We apologize for the oversight and have amended the text and included citations to R (and python). While the Dixon citation is the initial announcement of the vegan package, we agree that it is not uptodate and have also included a contemporary citation.

Line 135 & 136: "As we are using 16S rRNA amplicon sequencing a crucial limitation must be considered in evaluating our results". The tense is incorrect and it would be good to elaborate a little more on what the crucial limitation is and why it should be considered.
We have revised the sentence and elaborated the text in order to make it clearer for the readers.

Lines 196 – 200: It seems like you need to report more information on these results. What are the 8 families you found to be significantly different between/amongst zones? What 4 families were least abundant in the intertidal zone?
We have revised the text and added more details to the reported results in the section.

Lines 201-206: Needs clarification similar to comment above. Which 7 families contributed to carbon metabolism, etc. It is also important to cite the figure at some point in this paragraph.
We have added more details to the reported results and a citation to the corresponding figure.

Line 207: Spacing between words is off
We have corrected the formating.

Lines 299-300. The last sentence is a bit repetitive and unclear
We agree with the reviewer and have revised the text to make it clearer.

Figure 3. It is a good idea to include the number of dimensions used and the stress for the nMDS analysis
We agree with the reviewer and have updated the figure, adding the dimensions parameter and stress values.

Figure 4. When you say "To have been labelled with a metabolic pathway" are you referring to pathway enrichment? If so maybe point that out so that the reader can see the connection between this and the figure legend.
We agree with the reviewer that the text was unclear, we have revised the caption to read; "Pathway enrichment requires taxa to have at least 10% of three KOs in that pathway in any zone."

Anonymous Referee #3

Review: Biogeosciences "Tidal influences on the structure and function of prokaryotic communities in the sediments of a pristine Brazilian mangrove"

The authors present a study that is highlighting differences in community composition in the sediment of mangroves across different tidal zones. Apart from focusing on the community composition the authors also try to predict community functioning by predicting functions from 16S rRNA gene sequences via Picrust2. In the updated version of the manuscript the limitations of predicting functions based on taxonomic affiliation are better addressed than in the original manuscript. However, some critical points of the manuscript need to be revised and addressed.

We thank the reviewer for their time and insightful commentary. The paper has gained scope and breadth based on their feedback and we hope that these improvements show.

Comments

Abstract and Introduction

- The abstract would benefit from some sharpening and highlighting actual findings, such as abundant taxa discovered and how that differs from other studies in mangroves that discovered much greater diversity in mangrove sediments (see e.g. Zhou et al., 2017, Zhang et al. 2021. We agree with the reviewer and have revised the abstract to reflect more the main findings and how they compare with similar studies. A portion of this comparison is with the Zhou data and is included in our Supplemental as Figures 11, 12, and 13. However, we urge caution in the comparison, as no sublittoral measure was made and we can only infer that the mud flat described by Zhou can be treated as an intertidal equivalent. Further comparisons (although not re-analysis of data) with more or less similar datasets were also made for numerous other studies as is reflected in Supplemental File 2.

- The introduction seems to have improved significantly compare to the original version of the manuscript. However, it could still need some sharpening. Especially the explanation of study site, aim and hypotheses could be brought more "to the point" to become clearer to the reader. We appreciate the reviewers' kind words as to our improvement. We have revised the text in order to make the central points clearer to the readers.

Results
- Figure 2: I find this way of plotting the abundance data very hard to read compared to a normal stacked bar chart. E.g. finding the abundances of Bathyarchaeia is almost impossible. For the main text a simple stacked bar chart on phylum level might be easier to read and interpret. The figure has been changed and is now displaying the taxonomic levels of phylum and class. The figure with taxonomic families has been moved to the supplemental material.

- under 3.3. the enrichment of specific taxa and their potential function across zones is highlighted. This paragraph would benefit greatly if the connections between tidal zone,

dominant taxa and function would become more clear and easier to understand. At this point the text is hard to read with numerous pathway codes and repetitions.

We apologize for the difficulty. We have revised the figure to help make the context more clear and have edited the text to remove unnecessary pathway codes.

Figure 5 does support the reader in understanding the text, however also here highlighting the different predicted functions more clearly could be helpful.

We agree with the reviewer and have revised the text and figure to avoid repetitions and excessive citation of codes. The figure 5 has been modified to allow a better understanding of the results described in the text.

- What about archaeal taxa found in the sediment?

Unfortunately, we did not find any archaea that satisfied the statistical conditions to be included in this step of the analysis. This may in part be due to the incomplete representation of them in our 16S rRNA gene amplicon library, as the reviewer points out. To aid the reader in this, we have added clarifying text about the archaeal taxa to the results and discussion sections.

Discussion

- How does the community composition, especially in the sublittoral zone, compare to other environments such as shallow marine sediments in coastal areas?

We have added select papers from coastal marine studies to our literature review and have added to our discussion how these distinctly different environments compare to mangrove sediments, according to the literature.

- Studies that have performed metagenome sequencing on mangrove sediments are more suitable to predict functions. How to the findings of this study compare to those of other studies (e.g. Zhang et al., 2021). Trying for highlight differences and similarities would certainly benefit the manuscript.

We agree with the reviewer and have revised the text in order to add comparisons between our results and other work that sought to predict microbial functions in the sediments, using both metagenomic and 16S rRNA gene amplicons. We do, indeed, find a significant difference in our samples being predominantly *Firmicute* instead of *Proteobacteria,* an interesting difference.

- Recent studies using metagenomics on mangroves, but also other environments have shown, that especially yet uncultivated microorganisms are often being not detected by primer sets frequently used for amplicon sequencing (Eloe-Fadrosh et al., 2016). However, these microbes are often dominant parts of the microbial community. Could key players in mangrove sediments have been overlooked?

We agree with the reviewer and have emphasized the important limitations of the use of 16S rRNA gene amplicon sequencing for the identification of some relevant taxa in these microbiomes.

Minor comments:

- the correct term is "16S rRNA gene amplicon sequencing"
We have corrected the term throughout the manuscript.

- Supplemental table 1: change "metagenomics method" to just "Method". DGGE, T-RFLP and amplicon sequencing are not metagenomics! More recent metagenomic studies are completely missing from this table as well as being ignored in the manuscript.
We have changed the term and revised the table to add more recent microbiome studies.

- Figure 1: stay consistent between capital and lower-case letters in figure and figure legend (e.g. (a) vs (A))
We have revised the text and corrected inconsistencies.