

Reviewer comments are in black, our responses are in red.

## Reviewer #1

### General comments:

This study collected samples from 3 tidal zones of a pristine mangrove habitat for 16S amplicon sequencing and analysis. There needs to be a massive overhaul of the writing and synthesis of the results in order for this to be a publishable piece of work.

Generally, there are a lot of writing issues with the current manuscript, with grammar/spelling errors, typos, and run-on sentences throughout. The introduction needs the most work. There are abrupt transitions between paragraphs, and many concepts are not adequately introduced.

Specifically, the introduction needs to explain why it is important to study the different tidal zones in a mangrove habitat, why microbes are important to mangroves specifically, and what the broader implications are for this work.

The results section is too long and can be shortened. Much of the results describe methods which are already present in the methods section. The results also have discussion and implications that should be left in the discussion section. The PICRUSt results section reads like a combined results/discussion section, when it should be just the results. The discussion section primarily covers how current findings corroborate or conflict with previous findings. There is little synthesis of results, though the last two paragraphs of the discussion touch upon the beginnings of what could be synthesized from these results.

There also needs to be discussion of the limitations of using PICRUSt.

The wording throughout the manuscript needs to be changed to reflect the nature of the data (that this was done using 16S data, not metagenomes).

What is the novelty of this study, what are its contributions, and why is it important?

These are especially vital questions to answer because there are already studies characterizing pristine mangrove microbiomes.

The discussion needs to highlight how the findings fit into large ecological processes happening in the mangrove tidal zones, and not just a rehash of existing literature.

We appreciate the reviewers comments and thank them for their time and careful reading of our manuscript. We have revised the manuscript to address each of the reviewer's comments. We believe that the revised document more clearly articulates 1) why the study of tidal zones in pristine mangrove areas are important and how this research differs from previous work, 2) the methods that we have used and their potential limitations, and 3) how this work contributes to a deeper understanding of microbial ecology in mangrove areas. Furthermore, we have added a supplemental table with a short review of contemporary research on prokaryote populations in pristine and impacted mangrove tidal zones to aid the reader and provide additional context for our study. Throughout the manuscript the text has been revised and shortened, with references to methodology removed from the results section and a revised discussion section that

emphasizes the results and how our findings contribute to our understanding of this important ecology.

## Specific comments:

Line 21 mentions past metagenomic studies, but line 27 states this study used 16S rRNA amplicon sequencing. Currently it seems like the authors are using the two terms interchangeably. **We have changed the terms throughout the text to make clear that our approach is 16S rRNA amplicon sequencing and to distinguish this approach from metagenomics.**

Line 29: I think there should be some elaboration of how findings from this study contrast results from anthropogenically impacted mangroves in the abstract. It's not informative to the reader to just state that there is a difference. **We have rewritten the abstract and introduction to greater contrast the relation between this work and previous work on mangroves, and specifically mangrove tidal zone microhabitats.**

Line 40: Explain the role of mangrove ecosystems in climate change mitigation. **We have removed text referring to global climate change for concision.**

Line 45: This paragraph concludes with acknowledging the dependency of mangrove forests on the sediment microbiomes, but the first paragraph in the introduction wasn't written in a way that convinces me of this dependency. How, specifically, do sediment microbes benefit mangroves? **We have re-written the text to clarify how the sediment microbiome is important for the greater ecosystem functioning.**

Line 51: How does increased microbial diversity lead to an enrichment of microbes that play essential roles in ecosystem functioning? Which specific taxa are enriched by these dynamic conditions that would go on to maintain ecosystem functioning? **We have re-written the text to clarify that the statements about microbial roles in ecosystem functioning refer to general features of sediment microbiomes, as has been reported in the literature.**

Line 54: This is the first time that the sensitivity of the mangrove microbiome is introduced, which I found to be really surprising. I think there should be more of a lead up to this statement (what is the microbiome sensitive to? How is that sensitivity manifested?) **We have added a section, and supporting literature, that details the sensitivity of mangrove microbiomes to pollution, sea level, salinity change, and environmental degradation.**

Line 68: I have a problem with the term "16S rRNA amplicon metagenomics". 16S amplicon sequencing and metagenomics are two very different techniques. **The text has been corrected**

and now we refer to our method as 16S rRNA amplicon sequencing here and throughout the manuscript.

Line 76: Why did the authors hypothesize that the intertidal zone would have the highest microbial diversity? And also what would this mean ecologically? These types of information needs to be included in the introduction, particularly since mangrove zonation is not introduced until the last lines of the introduction. We have edited the text to introduce the concept of zonation at the beginning of the introduction and make clear that the hypotheses are based on previous ecological studies reported in the literature. We have also added an explanation of one ecological interpretation of this hypothesis and the consequences of our rejection of the hypothesis.

Line 80: But aren't there other studies that have looked at mangrove microbiomes under pristine conditions? I.e. Nogueira et al 2015. I don't have a problem with that, but this introduction is written in a way that implies this is the first study to look at pristine mangrove sediment microbiomes. We apologize for the confusion, we have amended the text and added a supplemental table to more clearly communicate the novelty and context of this work. The reviewer is correct that there are numerous studies of microbes in pristine mangroves. However the majority of these have not focused on microhabitats created by tidal variations, but only accessed the differences in communities between the impacted and pristine mangroves. To the best of our knowledge only two papers have assessed prokaryote population differences between mangrove tidal zones and both of these were performed in anthropogenically impacted mangroves. We have changed the text to clarify this difference between our study and these previous studies.

Figure 1: Explain what Abundance (K) means in the figure caption We have added this information to the figure caption.

Figure 3: Why present results from both Jaccard and Bray-Curtis beta diversity metrics? We now report only one distance metric (Jaccard) for the figure in the main text and the figures for additional tests were moved to the supplemental section.

Figure 3: Include descriptions of (A) and (B) in figure caption. The caption now refers to the A) and B) parts of the figure.

Results throughout: Do not need to include methods or even say "see methods" in the results section. See lines 216 and lines 228 as examples. We have excluded the mentions to methods in the text throughout the results section.

Line 234: This sentence is written confusingly and needs further elaboration. Does the word "Families" refer to protein families or microbial families? Additionally, are these just metabolism-associated KOs or KOs associated with all pathways? We apologize for the confusion. We have corrected the text and clarified that these are only metabolism-associated KOs and that we refer to taxonomic families.

Line 245: Don't need this last sentence. The sentence has been removed.

Line 261: This last sentence is a far overreach and makes no sense. The data does not support or even show there being a reduced abundance of KOs between the sites/zones. **We have removed the sentence from the text.**

Line 261: Are site and zone used interchangeably here? **We have edited the text so that only the term 'zone' is used to refer to microhabitats.**

Line 273: Functional abundances for KOs weren't measured...they were extrapolated from the 16S data. **This text has been amended to clarify this distinction.**

Line 378: These functional profiles are predicted from amplicon data, and were not actually measured in this study. **We have corrected the text to clarify our approach and its inherent limitations.**

## **Technical corrections:**

Line 25, abstract: Sentence should be rewritten. It's grammatically awkward as is. **We have rewritten the sentence.**

Line 37/38: remove the "the" before tropical, and remove "of Earth" **We have corrected the text.**

Line 47: Run-on sentence **We have corrected the text.**

Line 57: "latter" instead of "later" **We have corrected the text.**

Line 64: "relative to most mangrove forests" instead of "relative to most mangroves" **We have corrected the text.**

Line 117: missing "of" in "with 5 ul the forward..." **We have corrected the text.**

Line 150: Sentence is grammatically awkward. Rephrase. **We have clarified the sentence.**

Line 188: Avoid contractions **The text has been corrected.**

Line 193: Plural use of families and singular use of genus **The text has been corrected.**

Line 239: Sentence is grammatically awkward. Rephrase. **We have corrected the sentence.**

Figure 5 caption is written awkwardly and should be rephrased. **We have corrected the text.**

KEGG should be capitalized throughout. **We have corrected the text.**

Figure 6: elemental pathways should not be capitalized. **We changed the text on the figure.**