

***Interactive comment on “Differential analysis of prokaryotic communities from pristine mangrove tidal zone sediments reveal distinct structures and functional profiles” by Carolina Oliveira de Santana et al.***

**Anonymous Referee #1**

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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 mi-

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crobes 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 PICRUS 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 PICRUS. 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.

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.

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.

Line 40: Explain the role of mangrove ecosystems in climate change mitigation

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?

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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?

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?)

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

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.

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.

Figure 1: Explain what Abundance (K) means in the figure caption

Figure 3: Why present results from both Jaccard and Bray-Curtis beta diversity metrics?

Figure 3: Include descriptions of (A) and (B) in figure caption.

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.

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

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just metabolism-associated KOs of KOs associated with all pathways?

Line 245: Don't need this last sentence.

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.

Line 261: Are site and zone used interchangeably here?

Line 273: Functional abundances for KOs weren't measured...they were extrapolated from the 16S data.

Line 378: These functional profiles are predicted from amplicon data, and were not actually measured in this study.

Technical corrections: Line 25, abstract: Sentence should be rewritten. It's grammatically awkward as is.

Line 37/38: remove the "the" before tropical, and remove "of Earth"

Line 47: Run-on sentence

Line 57: "latter" instead of "later"

Line 64: "relative to most mangrove forests" instead of "relative to most mangroves"

Line 117: missing "of" in "with 5 ul the forward..."

Line 150: Sentence is grammatically awkward. Rephrase.

Line 188: Avoid contractions

Line 193: Plural use of families and singular use of genus

Line 239: Sentence is grammatically awkward. Rephrase.

Figure 5 caption is written awkwardly and should be rephrased.

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KEGG should be capitalized throughout.

Figure 6: elemental pathways should not be capitalized.

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