Dear Editor and reviewers,

We appreciate the thoughtful comments and valuable suggestions for our manuscript “Peanut-based intercropping systems altered soil bacterial communities, potential functions, and crop yield”. We have carefully reviewed the comments and have revised the manuscript accordingly. Please see detail in the revision, and our response to comments as follow:

Responses to the comments of Editor:

1. Were sequencing libraries mixed with PhiX? If so, how were these reads removed?

Response: Sequencing libraries were mixed with PhiX. Reads were removed during data demultiplexing. (Multiplexing refers to splitting multiplexed reads from different or the same lane based on the index, generates the sample's corresponding fastq file.)

Could mention this in the methods then.

2. How were sequencing adapter sequences removed from reads? This is usually done before analyzing with QIIME2.

Response: Sequencing adapter sequences were removed during the data demultiplexing.

So you have added this to the revised manuscript, but not said how adapters were removed.

Lines 185 & 186: What do you mean by ‘tail mass value’ and ‘average mass value’? Are you referring to Phred quality scores here? Again, what program was used to perform quality control? What version?

Line 192: ‘analyzed’ rather than ‘performed'?

2b. Was sequencing read quality observed before analyzing with QIIME2 (e.g. with fastqc or multiqc?)?

Response: Raw sequence reads were subject to quality control to obtain high-quality filtered reads for further analysis. Quality clipping of adapter sequences at 3′ and 5′ ends of reads was performed using the software fastp (https://github.com/OpenGene/fastp, v0.20.0), and reads were removed with length <50 bp, average base mass <20, and N bases.

No mention of fastp in the manuscript (well there was, but you’ve deleted it).

3. Was a positive control such as a mock microbial community used? To check if primers are more likely to amplify certain genera/don't amplify others.

Response: No positive control was set.

4. Was a negative control used, so starting with the DNA extraction kit, to remove any potential contaminant reads?

Response: Negative controls were used when performing amplification and were not used during sequencing.

Need to state this in the methods.

4b. Can you be more specific about the Nextflex kit used? There's a Rapid XP DNA-seq kit, a DNA-seq 2.0 kit, a cell-free DNA-seq kit, etc.

Response: The library construction kit used was the NEXTFLEX Rapid DNA-Seq Kit (Line

202).

4c. Barcodes used with this kit; do you re-use barcodes in combinations, or is each barcode only used for a single sample?

Response: In our study, each barcode was only used for a single sample (Line 209).

5. Consider adding ellipses to your NMDS plot, such as here: https://www.researchgate.net/figure/NMDS-showing-the-beta-diversity-of-A-Aedes-vexans-and-B-Culex-pipiens-microbiomes_fig3_339981588 (I'm not extolling virtues of the rest of the paper - I've not read it; I just googled for an example image).

Response: Thanks for your good suggestion. The ellipses have been added to the NMDS plot.

6. Line 202: you report the total number of ASVs. Could you report the number of sequencing reads per sample and a standard deviation across the replicates for each treatment?

Response: We have added the number of sequencing reads for each sample as well as the standard deviation of the replicates for each treatment, and the results were shown in “Supplemental Figure S3”.

No, figure S3 shows the number of ASVs per sample. How many sequencing reads were there per sample (either raw sequencing reads, or after denoising and removing chimeras).

Responses to the comments of Reviewer 1:

This paper examines the impacts of peanut-based intercropping on soil bacterial communities and crop yields, comparing it to monoculture. While the topic shows promise, the paper does have notable limitations and weaknesses that hinder its reliability and significance.

1) The field experiment conducted for only one year decreases the overall reliability of the research findings. Expanding the study duration could help enhance the robustness of the results.

Response: Thank you for your advice. In the future, we will extend the study duration for further research.

2) Although the paper investigates the effects of peanut-based intercropping on soil properties and crop yields, it fails to establish a clear link between bacterial communities and crop productivity. Establishing this relationship would significantly strengthen the paper's findings.

Response: Thank you for your advice. We developed the partial least squares path modeling analysis (Fig. 6) to further assess the link between bacterial communities and crop productivity in the revised manuscript.

3) The observed differences in bacterial communities between peanut-sorghum and peanut-millet intercropping are noteworthy, but further investigation is required to understand the underlying mechanisms and potential functions. This additional analysis

would provide valuable insights into the microbial dynamics related to intercropping systems.

Response: Thank you for your advice. We have added a more detailed analysis of differential bacterial communities in the revised manuscript.

Responses to the comments of Reviewer 2:

Basic reporting

The manuscript was written in professional English. There were a few grammatical errors and other major issues that need to be addressed to improve the readability of the manuscript.

Abstract:

Line 59: What is the broader impact of your study.

Response: It has been improved in the revision (details in Lines 51-53).

Introduction:

First paragraph: The introduction will read better if you start with the demerits of monocropping, then move to the merits of intercropping and why it is important. Thereafter, you can introduce why peanuts are considered in intercropping systems. Probably, because of its nitrogen fixing ability and other benefits listed in lines 70 – 73.

Response: We think this is an excellent suggestion. We have rewritten the first paragraph according to the suggestion. Thanks.

Line 89: Xue et al., 2023 did not work on maize and peanut. This citation is incorrect. They cited Chen et al. for this sentence. It is advisable that you read the direct authors that worked on maize/peanut system and cite them appropriately.

Response: Thank you for your note on this. We are very sorry for our negligence. The citation has been changed in the revised version (details in Line 84).

Last paragraph 99 -101: Please expand upon the knowledge gap being filled. Rewrite your objective and make it clear. What do inner mechanisms mean? Also, be specific on what you intend to use the Illumina Miseq platform to achieve. Did you carry out amplicon or metagenomic sequencing?

Response: Following your suggestion, we have rewritten our objective (details in Line 115-118). Amplicon sequencing was performed in this study.

Discussion:

The discussion should not be a repetition of results. This is an avenue to discuss your findings, how your findings fill the knowledge gaps mentioned in the introduction, the limitations of your study and discuss the broader impacts of your study.

Response: Many thanks for your helpful comments. We modified the discussion part and discussed our results comprehensively, which avoided repeating the results in the discussion part.

Experimental design

The experimental design (CRD or RCBD) was not mentioned in the manuscript. The data would have been more robust if replications were more than three, especially for a study set up in 2019. Every other aspect of the experimental design is okay.

Response: The field experiment was carried out using a randomized complete block design (RCBD) (details in Line 131).

Line 175: Please provide more information for sequence analysis. Did you use single or paired sequences for your analysis?

Response: Thank you for your advice. Our analysis was performed using paired sequences (details in Line 208).

Validity of the findings

The statistical tests applied are appropriate. Conclusion lacks depth. It failed to state clearly the broader impacts of the study.

Response: We have revised the conclusions to make them more detailed.

Additional comments

Other minor comments in the different sections

Minor issues

Abstract:

Line 43: change influences to influence

Response: Thank you for your advice. We rewrote the sentence. The “influcences” was still used in the revised sentence (details in Line 23).

Line 44: There are many studies out there showing the effect of intercropping on soil bacterial communities. So, “rarely been reported” is untrue.

Response: Thank you for your advice. We redescribed it in the revised manuscript (details in Line 24).

Line 51: This sentence, “The Actinobacteria, Proteobacteria, Acidobacteria, and Chloroflexi bacterial phyla were dominant across all cropping systems” can read better. I suggest writing it as: Bacterial phyla, including Actinobacteria, Proteobacteria ……, were dominant across all cropping systems.

Response: Thank you for your advice. We have revised according to your suggestion (details in Line 31-32).

Line 53: Spell enchanced correctly to enhanced. “Sorghum/peanut intercropping enchanced”

Response: We are very sorry for our negligence. It has been corrected in the revision, thanks (details in Line 33).

Line 76 -77: see my comments for line 44.

Response: Already revised according to the suggestion, thanks (details in Lines 71-72).

Line 84: Please specify the type of materials (organic?)

Response: Thank you for your advice. We replaced “materials” with “straw” in the revision (details in Line 78).

87: Soil or plant microbial communities?

Response: It is soil microbial communities. It has been improved in the revision, thanks (details in Line 82).

88: delete both “the”

Response: We have deleted "the" from the sentence. Thanks (details in Line 82-83).

89: Does amount of soil bacteria mean relative or absolute abundance or diversity? If it is relative abundance, it is important to state it because relative abundance is different from absolute abundance.

Response: Thank you for your comments. We reread the reference and changed the “amount” to “biomass” (details in Line 85).

Line 97: Clarify what you mean by high dwarf crops. Do you mean tall and dwarf crops?

Response: Thank you for this comment. We have rewritten the sentence carefully (details in Line 110).

Line 99: Do you mean that “more studies needed to understand the influence of intercropping on soil microbial communities need to be carried out in Shandong Province? If yes, please state it clearly.

Response: Thank you for this comment. We have rewritten the sentence carefully (details in Lines 110-111).

Line 104: State the different types of intercropping systems investigated.

Response: Thank you for this comment. We added the type of intercropping in the revision (details in Lines 113-114).

Materials and method:

Line 111-114. Cite the source of climate data.

Response: The climate data was collected from a small self-built weather station (details in Lines 128-129).

Line 115: Please provide the soil pH.

Response: According to your suggestion, we added the soil pH (details in Line 128).

Line 125-126: Change “are” to “were”. Millet and sorghum were harvested in early Sept.

Response: We have revised it according to your suggestion (details in Line 139).

Line 187: Please provide a reference for LDA > 3.5

Response: We have added a reference according to your suggestion (details in Line 226).

Line 188: Did you carry out a correlation analysis among soil physicochemical properties before selecting them for redundancy analysis? Strong and significant correlations among soil physicochemical properties can affect the RDA.

Response: We performed a correlation analysis of soil physicochemical properties and placed it in the Supplementary Information (Table S2).

Results

Line 198: Quantify the increase. It could be quantified in percent

Response: We have revised it according to your suggestion (details in Line 243).

Line 204: Delete the phrase, regarding unique ASVs.

Response: We deleted the phrase according to your suggestion (details in Line 263).

Line 210: delete “divided between” and replace with “among”

Response: We have revised it according to your suggestion (details in Line 268).

Line 221: “varies between treatments” should be “varied among treatments”

Response: We have revised it according to your suggestion (details in Line 278).

Line 250: move “further demonstrating that SOC could structure the bacterial community composition” to discussion.

Response: We have moved this sentence to the discussion (details in Lines 436-437).

Line 268: It would be nice to see the relationship between soil bacterial properties and total grain yield.

Response: We have supplemented the correlation analysis between soil bacterial properties and grain yield in Fig. 6.

Figures:

Overall comment: The figure can be rearranged, starting with total grain yield and LER, soil chemical properties before proceeding with the soil bacterial components.

Response: We have rearranged the figures according to your suggestion.

There are too many figures for the manuscript. I suggest that figures 1, 2, 3, and 8 to supplementary information.

Response: We have moved figures 1, 2, 3, and 8 to supplementary information according to your suggestion.

Figure 3: remove the lowercase letters on the figure since there was no statistical difference.

Response: We have revised it according to your suggestion.

Discussion:

Line 276: change “enhance” to “enhancing”

Response: We have revised it according to your suggestion (details in Line 341).

Line 278: Since your preceding sentence on line 276 talks about cereal/legume intercropping, it would be better to give an example of cereal/legume intercropping rather than using mulberry.

Response: We have revised it according to your suggestion (details in Line 342).

Line 279: Be specific with the two intercropping systems in your study. Name them

Response: We have revised it according to your suggestion (details in Line 363).

Line 282: Correct the grammar here. It should read: These results might be attributed to

Response: We have corrected it according to your suggestion (details in Line 365-366).

Line 291-292: This sentence is not clear, how does competitiveness of resident microorganisms affect differences in microbial diversity.

Response: We have added a detailed description in the revised manuscript (details in Line 376-380).

Line 296: Based on your beta diversity, cropping systems affected the bacterial community composition. Or do you have a different definition for bacterial community composition? If so, please state it.

Response: Thank you for this comment. We have redescribed it in the revised manuscript (details in Line 384-385).

Line 298-299: This sentence doesn't read clearly, please rephrase. An example: The dominant phyla include Actinobacateria, Proteobacteria ……., were consistent across all cropping systems, indicating that intercropping did not alter the dominant bacterial phyla. This is just an example; you can rewrite yours to read better.

Response: We think your suggestion is excellent. We have revised it according to your suggestion. Thanks (details in Line 386).

Line 301- 302: What is the implication of the result. You should discuss your result.

Response: Thank you for your advice. We have added the implication of result in the revised manuscript (details in Lines 390-392).

Line 315: How does increase in Streptomyces boost peanut growth and yield? Is it through the release of antimicrobial compounds or the mineralization of organic compounds to plant nutrients? Instead of repeating results in this section, it is important to discuss your results and the implications of your findings.

Response: Thank you for your advice. We have explained this further in the revised manuscript (details in Lines 419-424).

Line 315-322: You can rewrite this paragraph and discuss your result. For example: 'Intercropping in our study promoted bacterial genera that are potentially involved in N fixation and cycling activities. For example, there was an increase in the relative abundance of Microvirga and Acidibacter, known for their roles in n fixation and xxxx, under Sorg/Pean soils. Similarly, we found an increase in Kribbella and Reyranella under Mill/Pean soils."

Response: Thank you for your advice. We have rewritten this paragraph according to your suggestion (details in Line 412-417).

Line 323-325: I like these sentences, they read nice.

Response: We sincerely thankful for your positive response regarding our manuscript.

Line 327: Edaphic factors affect microbes indirectly. pH can affect nutrient availability and indirectly affect soil microbes.

Response: We have modified these two sentences according to your comment. Thanks (details in Line 426).

Line 332: Why are soil bacteria sensitive to SOC? Please explain further. Carbon serves as nutrient and energy sources.

Response: Thank you for your advice. We have explained this further in the revised manuscript (details in Line 433-435).

Line 348: Can Tax4Fun identify the microbial groups linked to the metabolic pathways listed?

Response: Thank you for your advice. We are also interested in functional characterization of microorganisms. However, linking soil microbial community structure to its functionality is still challenging. In the future, we will perform macro-genome sequencing to obtain more accurate functional information.

Conclusion:

The conclusion can be more detailed. State the contribution of your study to the body of knowledge. From your study, it is obvious that intercropping has several benefits from efficient land use, increasing soil nutrients, and promoting soil bacterial taxa involved in N cycling processes and plant promotion.

Response: Thank you very much for your valuable comments. We have revised the conclusions to make them more detailed.