

The submitted manuscript entitled “Metagenomic investigation of bacterial laccases in a straw-amended soil” studied the effects of long-term wheat and maize straw as soil amendment on soil and soil bacterial communities and genes related to bacterial laccases. The topic in this study is suitable for the aim of the journal of PeerJ and important subject for understanding the source on isolating bacterial laccases in the future for industrial used. This paper is well presented and discussed. However, there are several issues to understand the valuable data and discussion in this study. I recommend authors revise it after considering the following.

Introduction

The introduction is well-written, presenting clear and coherent ideas. However, it lacks information on whether most of the bacterial laccase are cultured or uncultured bacteria. To strengthen this section, the authors should include relevant literature in the final paragraph (Lines 109–119) to clarify this distinction. Highlighting this distinction will better support the choice of the metagenomic approach and reinforce the study's objectives.

Materials and Methods

1. The study does not clearly specify whether the straw used was fresh or composted in Line 126-129. Additionally, chemical properties of the straw, particularly the C-organic content, are not provided. To enhance clarity and reproducibility, the authors should specify the type of straw used and include its relevant chemical properties.
2. The authors clearly state that the soil sample was compositely collected from five cores in Line 131-133. However, it is unclear whether these cores were taken from a single plot or multiple plots. Additionally, the number of replicates used for the metagenomic analysis is not specified. To improve clarity, the authors should clarify and provide details the total number of samples analyzed.
3. Is the DNA concentration mentioned in Lines 140–142 referring to the extracted DNA? Please clarify this point for better understanding.
4. Regarding the metagenomic analysis mentioned in Lines 146–150, did the authors amplify the DNA prior to HiSeq sequencing? If so, please clarify this and provide details on the primer set used, as well as the targeted 16S rRNA regions in this study.
5. Please provide the reference(s) for the primer set used to amplify the ORF of *lacSI* (Lines 205–206).

Data and Results

1. The metagenomic data analysis Lines 262-265 showed that the classification values decrease from the order to species level. Could you clarify why this occurs and provide more context?
2. The authors mention that 14 putative bacterial laccase genes were randomly selected to validate the metagenomic sequencing in Lines 292-293. Is this sample size sufficient to represent the entire ecosystem? Please elaborate.
3. The results of described of authentic laccase genes in Lines 294-298 are not linked to any table or figure. Please indicate the corresponding table or figure to help readers locate the data.
4. When mentioning 17 orders of laccase genes in Line 322, please specify the relevant table or figure in parentheses for easier reference.
5. The values for laccase-coding genes in the text (Lines 322-325) differ from those in Figure 3. Please ensure consistency between the text and the figure.
6. The phyla-genera text in Figure 3 is difficult to read. Consider increasing the font size and providing a higher-resolution version.
7. The paragraph in Lines 314-325 lacks a clear statement highlighting the significance or novelty of the findings. Please add a sentence to emphasize this in the concluding part.
8. Remove "et al." in Line 325 and specify the table or figure containing the data in L.
9. The authors mention using 28 representative sequences in Lines 328-329, but Figure 4 shows 29. Please double-check and revise accordingly.
10. The references used for phylogenetic tree analysis are stated to belong to 20 genera in Lines 328-329. Have these genera been previously reported to produce bacterial laccases? Please clarify.
11. The authors separated bacterial laccase fragments into seven distinct clusters in Line 330. Could you explain the criteria or method used to distinguish these clusters?
12. Five representative bacterial laccase genes were identified using mTAIL-PCR. Please indicate the table or figure showing this result.
13. The text states a range of 450–630 amino acids in Lines 344-346, but Table 3 shows 445–629. Please verify the correct range and reference the table in the manuscript.
14. Specify the table or figure containing the relevant data in Lines 349-350 and 352.

15. After mentioning “across a broad pH range,” in Line 361, please include the specific pH range (pH 5–8) for clarity.
16. The authors mention that copper ions had no significant effect on enzyme activity in Line 366-367. Was statistical analysis conducted to confirm this? Please clarify.
17. Revise the Figure 7A and 7B to include error bars and indicate the results of the statistical analysis.
18. What do the “/” and “sum” in Table 2 represent? Please explain or revise for clarity.

Discussion:

1. Please provide the appropriate reference(s) to support the statements made in Lines 378-379.
2. The authors mention the role of the *Gemmatimonadetes* phylum in phosphorus removal in Lines 402-404. How does this relate to laccase bacterial activity? Please clarify this connection.
3. Replace “So” with “Therefore” for a more formal tone in Line 407.
4. Ensure there is a space between “laccases” and “have” in Line 413.
5. The authors discuss the predominant phyla and orders related to laccase genes in Lines 398–421 but do not emphasize which laccase gene is most abundant in the straw-amended soil. Concluding this paragraph with a clear statement highlighting the most abundant laccase gene would provide better clarity and strengthen the discussion.
6. The authors have not yet discussed the novel laccase genes identified in this study. Adding a dedicated paragraph to emphasize these novel findings would strengthen the manuscript’s originality and significance.