

Reviewer 3: This study quantified soil fungal and bacterial communities, genes, and networks for both pure *Eucalyptus* (PP) and mixed *Eucalyptus-Acacia* (MP) plantations. The plantations have been growing for 17 years, allowing authors to report long-term differences caused by co-planting *Eucalyptus* with a nitrogen-fixing tree species. The results are interesting, consisting of many differences between the plantation types in the composition and function of the microbial communities. Although I cannot address many of molecular methods, as they are outside of the scope of my expertise, I hope my comments below help improve the manuscript. Once they are addressed, I believe it will be a good fit for *Biogeosciences*.

Response: Thanks for your good comments.

1. The hypotheses presented in the last paragraph of the introduction are unclear. For (1), it is stated that diversity and composition of soil microorganisms will change with mixed planting. How will they change? For (2), “mixed plantations intensify the response to the beneficial impacts of N-fixing tree” is unclear and should be reworded. For (3), this hypothesis seems to overlap with hypothesis (1) (both mention diversity), but is more specific, suggesting that there will be higher diversity in mixed plantations.

Response: As suggested, we rephrased the hypotheses, and made modification accordingly.

“We proposed the following hypotheses: (1) tree species mixing would alter the composition of soil microbial communities and increase microbial diversity and network complexity in the soil, and (2) the soil P transformation driven by tree species mixing may be positively regulated by microbial diversity and network complexity.”

The rationale for making measurements at the two depths (0-10 and 10-20cm) are unclear. Please provide an explanation for why these two depths were chosen.

Response: Thanks for your comment. According to our previous soil investigation, collecting soil samples from two layers can more systematically and comprehensively explore the influence mechanism of different factors on soil phosphorus conversion. Furthermore, this approach ensures that the resultant observational datasets exhibit enhanced representativeness by minimising vertical heterogeneity artefacts inherent to single-layer sampling protocols.

2. The rationale for the different alpha index analyses (ACE, Chao1, Shannon) should be mentioned. That is, why are all three used and in what ways do insights from them differ?

Response: Thank you for your comment. We have read numerous relevant references carefully. Chao 1 and ACE indexes were used to estimate the richness of the bacterial and fungal community, while Shannon index was

used to evaluate the diversity of bacterial and fungal community (Wang et al., 2018; Sun et al., 2021; Qiu et al., 2021; Malard et al., 2022). Therefore, these indices combined provide a more reliable and comprehensive view of microbial community structure and its potential links to soil nutrient cycling.

Relevant references are as follows:

Sun, Y., Ren, X., Rene, E. R., Wang, Z., Zhou, L., Zhang, Z., Wang, Q.: The degradation performance of different microplastics and their effect on microbial community during composting process. *Bioresource Technol.*, 332, 125133, <https://doi.org/10.1016/j.biortech.2021.125133>, 2021.

Qiu, L., Zhang, Q., Zhu, H., Reich, P. B., Banerjee, S., van der Heijden, M. G., Sadowsky M. J., Ishii S., Jia X., Shao M., Liu B., Jiao H., Li H., Wei, X.: Erosion reduces soil microbial diversity, network complexity and multifunctionality. *ISME J.*, 15(8), 2474-2489, <https://doi.org/10.1038/s41396-021-00913-1>, 2021.

Malard, L. A., Mod, H. K., Guex, N., Broennimann, O., Yashiro, E., Lara, E., Mitchell, A. D. E., Niculita-Hirzel, H., Guisan, A.: Comparative analysis of diversity and environmental niches of soil bacterial, archaeal, fungal and protist communities reveal niche divergences along environmental gradients in the Alps. *Soil Biol. Biochem.*, 169, 108674, <https://doi.org/10.1016/j.soilbio.2022.108674>, 2022.

Wang, C., Liu, D., Bai, E.: Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. *Soil Biol. Biochem.*, 120, 126-133, <https://doi.org/10.1016/j.soilbio.2018.02.003>, 2018.

3. It would be helpful to mention the perceived function of the different genes that were measured. For example, in the paragraph at L198 and in Figs. 5-7.

Response: Thank you for your suggestion. We have added some details about perceived function of the different genes in the methods and Figs. 5-7 sections to make it more readable.

4. I think that there should be a discussion of why there was higher TP in PPs than MPs and whether trees in MPs and PPs might differ in whether they are limited by N vs. P.

Response: Thanks for pointing this out. Detailed descriptions were added in the Discussion section.

5. The introduction and discussion would benefit from discussing mixed plantations between N-fixing and non-fixing trees in general. How representative are *Eucalyptus-Acacia* plantations of mixed plantations elsewhere?

Response: Thank you for your valuable suggestion. We have carefully re-checked the Introduction and Discussion sections and will add more relevant content of mixed plantations between N-fixing and non-fixing trees. In addition, we will add relevant supporting references about *Eucalyptus-Acacia* plantations of mixed plantations.

6. The direction of causality is unclear. Throughout the manuscript, the authors argue that microbial diversity, structure, complexity promote P transformation. However, sentences such as that on L68-70 suggest causality is in the other direction.

Response: Thank you for your suggestion. We have rephrased the sentence to avoid the confusion.

7. The manuscript should be checked for typos and grammar. There are many instances of minor mistakes.

Response: We will carefully check the entire manuscript and make appropriate about the organization and language of the content to make it more readable.

Specific comments:

1. Title: I would change to: "Soil microbial diversity and network complexity promote phosphorus transformation: A case of long-term mixed-species plantations of *Eucalyptus* with a nitrogen-fixing tree species"

Response: Changed.

2. L24-26: Clarify that the study was in both PPs and MPs. The sentence makes it sound like the study was just done in PPs.

Response: Corrected as follows:

"Therefore, we conducted a 17-year field experiment in pure *Eucalyptus* plantations (PPs) and mixed plantations (MPs) of *Eucalyptus* and N-fixing trees species to assess the effects of soil P transformation, with data collected from two soil layers: 0-10 cm and 10-20 cm depths."

3. L30: The two soil layers tested should probably be mentioned before reporting specific results for one of them.

Response: Specified.

4. L63: "soil health" is a vague statement. Be more specific.

Response: Specified.

5. L95: This sentence states that N content influences soil pH. Typically, the direction is one where an increase in N content lowers soil pH. The results show that pH however increased, which I found surprising. Although the discussion has a few lines on why, it may be good to address the hypothesized direction of change somewhere in the introduction.

Response: Thank you for your valuable suggestion. We will add some detailed description and make necessary modification.

6. L99: Change the part of the sentence that follows the comma to “thereby accelerating nutrient cycling and improving soil fertility”

Response: Corrected.

7. L106: It is unclear what is meant by “soil nutrient effectiveness”.

Response: Thanks for pointing this out. We have rephrased the sentence to avoid the confusion.

"However, monocultures and short-term rotation management of *Eucalyptus* plantation have led to soil degradation, reductions in soil nutrient effectiveness (i.e., the availability of nutrients such as nitrogen, phosphorus, and potassium in forms that can be absorbed and utilized by plants), and soil microbial function and diversity, as well as other adverse ecological effects."

8. L111: Replace “fewer” with “less or no”

Response: Changed.

9. L117: This might be a good time to mention the N-fixing tree species that is used in the MPs.

Response: We have revised it as suggested.

10. L125-126: I am unsure of what is meant by “along with genes associated with N and P cycling”.

Response: Accepted and it has been revised in the manuscript. Now read like: " along with genes involved in N and P cycling processes, regulate P transformation".

11. L262-263: Clarify that the increase was in going from PPs to MPs.

Response: Corrected.

12. L305: Can you explain by what metric pH is the most important regulator? It is not immediately clear from looking at Figure 3b.

Response: Thank you for your comment. The soil physicochemical properties influencing the variations of dominant microorganism phyla were identified by using redundancy analysis (RDA). The sequential selection process of RDA was used to identify the drastically distinguishing variables for soil physicochemical properties and specific microorganism phyla. Significant variables ($P < 0.05$) were employed in subsequent analysis. In our study, the value of pH ($F = 4.3$, $P = 0.003$) had the greatest impact compared to other factors ($P > 0.05$).

13. L376: Please provide a number for the “high goodness of fit.”

Response: Added.

14. L450-451: Having actinobacteria in this sentence is misleading. Actinorhizal plants form N-fixing symbioses with Frankia, which are actinobacteria. However, Acacia is not an actinorhizal N fixer. Instead, Acacia forms N-fixing symbioses with Rhizobia, which are Proteobacteria.

Response: Thank you for your suggestion. We have rephrased the sentence to avoid the confusion

15. Table 1: Clarify whether the +/- refers to the standard deviation or the standard error.

Response: Clarified.

16. Table 2: Bacteria is misspelled.

Response: Corrected.

17. Figure 1: In the caption mention the threshold p value (my guess is $p < 0.05$) that determines whether differences between treatments are significant or not.

Response: Added.

18. Figure 4: The Zi-Pi plots have the connectors (high among module connectivity) and module hubs (high within module connectivity) switched in the legend. Also, it is not clear what is meant by “node color node size” in the caption.

Response: Corrected.

19. Figure 9: The caption appears to explain 9a, but not 9b.

Response: Thanks for pointing this out. We added some detailed descriptions and made it clear and specific.

“Figure 9b presents the Standardized total effects (direct plus indirect effects) on P transformation derived from the PLS-PM.”

