Dear Referees,

We appreciate the time and effort you dedicated to providing feedback on our manuscript and are grateful for the insightful comments. In the revised version we have incorporated most of the suggestions made by reviewers. Please see below responses to the reviewers' comments.

Comments from Anonymous Referee #1:

The submitted paper is interesting. Although it's a typical paper on soil biodiversity and its relationship with soil properties, here, in my opinion, the main novelty is related to the study area and also the studied soil groups, once no several papers have been published about Chernozem, Solonchak, and Vertisols and due to this, the Pannonian region is one of the most fertile in Europe.

In the materials and methods section, the authors indicated that these fields are managed differently. However, how is the relationship between agricultural management and soil properties, and therefore, the microbiome? I can not see this in the Results. This is my major shortcoming.

Answer: We thank the reviewer for the positive assessment of our study and for recognizing the novelty of investigating microbial communities across the major soil groups of the Pannonian region. We agree that the relationship between agricultural management, soil properties, and the microbiome deserved further clarification.

The primary objective of this study was to evaluate the influence of soil group and associated physicochemical properties on microbial community composition. Agricultural and control plots were included primarily to capture the natural variability within each soil group rather than to compare management systems statistically. A formal statistical comparison of management effects was not appropriate because it was not possible to identify plots with comparable crop types and management practices across all three soil groups. This was particularly challenging for Solonchak soils, where the inherent soil properties substantially limit agricultural use and the range of available cropping systems and management practices. We have clarified this limitation in the revised manuscript and added a statement to the Discussion indicating that future studies employing a balanced sampling design with comparable management systems across soil groups are needed to disentangle the effects of soil type and agricultural management on the soil microbiome.

Other comments

L10. Indicate the soil groups (Chernozem, Solonchak, and Vertisol)

Answer: Thank you for the suggestion. We have revised the abstract to explicitly state that the analyzed soil groups were Chernozem, Solonchak, and Vertisol.

L11. Indicate which soil properties have been measured or almost, the number of soil health indicators.

Answer: We have revised the abstract to briefly specify the main categories of measured soil health indicators, including soil pH, texture, organic matter, nutrient status, cation exchange capacity, and selected macro- and micronutrients. This provides readers with a clearer overview of the evaluated soil properties.

L11. This sentence for me it's not clear. According to this, 26 field Plots were collected. However, according to material and methods, 15 fields (5*3) were collected. Is it right?

Answer: We thank the reviewer for identifying this ambiguity. The study included 26 field plots, comprising 8 Chernozem plots, 9 Solonchak plots, and 9 Vertisol plots. From each field plot, five composite soil samples were collected as biological replicates. We have revised the wording in Materials and Methods to clearly distinguish between the number of field plots and the number of replicate samples.

L26-113. The introduction section is a bit longer. I suggest to reduce. Try to reduce the soil health section or rewritte them,

Answer: We appreciate this suggestion. The Introduction has been revised and shortened by reducing background information on general soil health concepts and avoiding repetition while maintaining the context necessary to explain the study rationale.

L136. I suggest adding information about practices here, as a table or as SUPPLEMENTARY data.

Answer: We have added a supplementary table containing the description of each sampling plot, including geographic coordinates, land use, and crop type. The table is identified as adapted from Kuzmanović et al. (2024), where this information was previously published.

L168. "extracted using hot water" Please, indicate the method, water temperature, and extraction time.

Answer: Thank you for this helpful comment. We have clarified the procedure used for the determination of plant-available boron. Boron was extracted using the hot-water extraction procedure described by Ubavić and Bogdanović (2006), based on the original method of Berger and Truog (1939). Briefly, 20 g of air-dried soil (<2 mm) was extracted with 40 mL of deionized water (soil:water ratio 1:2). The suspension was heated under reflux to gentle boiling (approximately 100 °C) for 5 min, cooled, and filtered. The extract was subsequently analyzed for B by ICP-OES. These methodological details have now been added to the revised manuscript.

L171. Operation conditions can be moved to SUPPLEMENTARY data, although they are ok in this version.

Answer: We thank the reviewer for this suggestion. We have chosen to retain the operating conditions in the Materials and Methods section because they are concise and constitute essential methodological information that facilitates reproducibility. We believe that keeping these details in the main text improves the completeness of the analytical methodology without substantially affecting readability.

L246. Results previously published should be indicated here. This will help readers to understand your work. It's not easy to refer to previous work and see the info there. To avoid issues with auto-plagiarism, you can indicate a footnote that XXX were published in XX. In fact, the authors wrote about them, but they don't indicate them.

Answer: We thank the reviewer for this important suggestion. We have now included the results of the analysis of several physicochemical soil properties (pH, $CaCO_3$, organic matter, total nitrogen, organic carbon, available phosphorus, and available potassium) in Table 2 and indicated that these results were previously published in Kuzmanović et al. (2024). An explanatory note has been added underneath the table. The remaining soil physicochemical data, microbiome analyses, functional predictions, and nitrogen-cycling gene analyses are presented here for the first time.

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Comments from Anonymous Referee #2:

Overview:

In this effort, the authors work to characterize Serbian soil microbiome structure with a focus on agriculture soils and contextualize how different agriculture management practices and soil types influence the microbiome structure and function. The study was thorough in sequencing analyses performed and produced interesting results. Some comments below for consideration with ultimate consideration that more information is needed around Ag management practices etc to strengthen paper.

Comments:

Major:

If possible, in 2.1.2, a table might be good to outline the distinct agricultural practices and crop rotations for each location (maybe progressing back a few growing seasons or just general overview of standard practice in each area). This is quite intriguing on how it might vary between the soil types and location and thus leading to some of the outcomes—I read the cited paper and appreciated that detail but would appreciate the extra information here as well. Also, gives opportunity to expand a bit more based on the last sentence in this method.

Answer: We thank the reviewer for this valuable suggestion. We have added Table S1 to the Supplementary Material, providing detailed information on each sampling plot, including land use (agricultural/control), crop type, and coordinates. This table was adapted from Kuzmanović et al. (2024).

Figure 3/5, would consider combining figure 3 and 5 or move figure 5 up in front of figure 4 as it is referenced first and helps to contextualize figure 3. Figure 4 does help bring clarity but could come last. Also, if possible, would consider trying to lock in color patterns for the major phyla in Figure 3 and holding constant in the different subplots specifically maybe revamping subpanel A to match B and C more. Results are quite interesting and found the Verrucomicrobiota shift intriguing

Answer: We have reordered the figures so that the presentation follows the sequence in which they are discussed in the text, improving the overall flow of the Results section. We have also

revised the color scheme in Figure 3 to maintain consistent colors for the major bacterial phyla across all panels, improving visual comparison among subplots.

Figure 6/7 are quite insightful and interesting results

Answer: We thank the reviewer for this positive comment and are pleased that these results were found informative.

Table 4 and Figure 10 are interesting—going back to the first major point—a table highlighting Ag management strategies/crop rotations/fertilizer strategies could help tie into this more and add some context given lines 420-425. Also, this could help give some ideas about the plot variability

Answer: We thank the reviewer for this suggestion. To provide additional context for interpreting these results, we have added Table S1 summarizing land use, crop type, and geographic coordinates for each sampling plot.

Minor:

Line 187—guessing this is paired 300 bp?

Answer: The sequencing description has been revised to specify that paired-end 2 × 300 bp sequencing was performed.

In lines 216-217, were the reverse reads completely unusable or just lower quality? How much lower quality?

Answer: We thank the reviewer for this question. The reverse reads were not completely unusable; however, their quality began to decline after approximately 180 bp and deteriorated markedly beyond 200 bp, with considerably greater variability than the forward reads. This quality profile was considered insufficient for reliable DADA2 denoising and paired-end read merging. Consequently, only the forward reads were retained for downstream analyses. We have clarified this in the Materials and Methods section.

Lines 267-271/Table2, bringing the pH forward from the previous citation would help with doing comparisons—maybe as supplemental or addition to Table2. Agreed with the thoughts in this area though.

Answer: We thank the reviewer for this helpful suggestion. To facilitate interpretation of the results, the previously published soil physicochemical properties, including soil pH, have now been included in the revised manuscript (Table 2), with an appropriate indication that these data were previously reported by Kuzmanović et al. (2024).

Figure 1 and throughout, Chao1 richness is not readily compatible with ASVs generated from DADA2—would consider removing completely and just using observed richness as that is sufficient

Answer: We thank the reviewer for this suggestion. Chao1 richness has been removed from the manuscript.

Figure 2, Jaccard and Bray-Curtis are similar but in unweighted and weighted forms and only the weighted UniFrac is shown, was unweighted UniFrac calculated?

Answer: Unweighted UniFrac distances were also calculated. However, the resulting ordination showed patterns very similar to those obtained using the Jaccard distance metric and therefore did not provide additional biological insight. To avoid redundancy, we chose to present Jaccard, Bray-Curtis, and weighted UniFrac, which together capture complementary aspects of beta diversity based on taxon presence/absence, abundance, and abundance combined with phylogenetic relationships.

Line 369, P2O5 missing subscripts

Answer: We thank the reviewer for identifying this typographical error, we corrected this throughout the manuscript.

Line 478-479 reinforce the major comments made—interesting discussion point but having more context about the Ag management in each plot and soil type would strengthen these points

Lines 551-560 area thoughts are sensible under stable fertilizer regimes where micronutrients and other factors could become the major drivers—yet need to know more about this management practices to solidify these thoughts

Discussion on nif and nir genes follow the same ideas as comment immediately above and in conclusion. The more that can be known about Ag management and contextualization information, the stronger the conclusions become.

Answer to the last 3 comments: We thank the reviewer for these valuable comments. We agree that additional information on agricultural management would provide important context for interpreting these findings. Accordingly, we have clarified in the manuscript that the primary objective of this study was to evaluate the influence of soil group and associated physicochemical properties on microbial community composition rather than agricultural management. Because comparable crop types and management practices could not be identified across all three soil groups, particularly for Solonchak soils, the effects of agricultural management could not be evaluated independently. This limitation has now been acknowledged in the manuscript, and we have added a statement indicating that future studies employing

comparable management systems across different soil groups are needed to distinguish the effects of soil group from those of agricultural management.