22nd May 2026

Dear editor and reviewers,

We would like to resubmit our revised manuscript to Soil. The manuscript ID is: EGUSPHERE-2026-629

We thank you for reviewing our manuscript and for your valuable comments, which helped us improve it. We have revised the manuscript in accordance with your comments. The revised portions of the manuscript have been highlighted in red.

Our responses (in red font) to the comments are provided below.

We look forward to your response.

Sincerely,

Manuel Paneque (mpaneque@uchile.cl);

Reviewer #1 comments:

Fertilizer regimes reshape microbial interaction networks without altering sugarcane rhizosphere diversity

Overview:

In this effort, the authors work to address an important gap in current knowledge about microbial structure in sialitic soils and how monoculture practices with sugarcane in the Caribbean can influence the reactivity of soil microbial structure to varying fertilizer strategies. The team highlights how the longstanding agriculture practices have potentially stabilized the microbial community structure in a way where there are minimal shifts in microbial community members and their abundance in response to fertilizer but rather the interactions restructure based on what nutrient is abundantly applied. The work highlights the need for improved profiling of these soils which could act as a new source of microbial isolates for Ag biotechnology and serve to inform current and future Ag management.

Comments:

Major:

- This is not a concern but more of an intrigue. The rigor of this experimental design is substantial, but I am curious if there is any temporal data that is available. Sampling in the third cycle can certainly highlight what interactions or microbial structures have stably shifted compared to control plots, but I am curious how things changed over time. I would say more data is not necessary, but I am curious about the author's comments.

R. We sincerely appreciate the reviewer's encouraging feedback regarding the rigor of our experimental design. We completely agree with your assessment; capturing the temporal dynamics to observe how these microbial communities shift across different stages of the sugarcane growth cycle would be incredibly insightful. Unfortunately, we do not have temporal or longitudinal data available for this specific study. Our microbiome analysis collaboration was established specifically to evaluate the endpoint of this long-term trial (the third cycle). Our primary goal was to capture the stabilized, long-term shifts in the microbial structures and networks resulting from prolonged agricultural management. While we cannot retroactively track the successional dynamics of these specific plots, the robust stabilized networks we identified here provide an excellent baseline that strongly motivates future longitudinal studies. We thank the reviewer for highlighting this intriguing aspect, and we agree it is the logical next step for this line of research.

- Figure 6 and Supplemental 8S show some important data and I am curious what a breakdown would look like for those ASVs from the Venn diagrams. What are the classifications of the ASVs that overlap across the different fertilizer strategies? Are some consistently overlapping across all the fertilizer strategies? I think zooming in on this analysis more could provide more interesting results to strengthen the paper further similarly to how the network analysis data (Figure 5 and corresponding supplemental) was dissected. Also, the descriptions in the results and discussion around the Venn diagram data are inconsistent at times. In lines 345-350, the comparison of basal (N1—which is not labeled correctly in figure 6?) to nitrogen only (N0) seems inaccurate unless compartments are referring to the edges but there seems to be less overlap between the two controls and the treatment. Additionally, in the discussion in lines 500-505, it seems like the data suggests that at least phosphorus is another major driver besides just nitrogen?

R. We sincerely thank the reviewer for this highly constructive observation. We completely agree that a deep-dive taxonomic breakdown of the overlapping ASVs provides fascinating insights. Indeed, your comment prompted us to re-evaluate our approach, revealing a crucial trans-domain dynamic that significantly strengthens our manuscript.

First, to address the visual inconsistencies noted in lines 345-350, we have entirely recalculated the trans-domain Venn diagrams. Previously, overlaps were derived from sub-network topological nodes, which inadvertently masked true biological occurrences. The revised Venn diagrams (updated in Figure 6 and Supplemental Figure S8) are computed directly from the standardized abundance matrices (preserving unclassified taxa removal for taxonomic clarity). This corrects the visual discrepancies and explicitly maps true ASV overlaps among treatments.

Addressing your specific questions with our new trans-domain breakdown:

1. By extracting the ASVs consistently present across all nitrogen strategies (from N0 to N4), we identified a highly conserved trans-domain core of 76 ASVs. Remarkably, this core relies heavily on a dual-domain alliance: robust bacterial phyla like Bacillota (17 ASVs) and Pseudomonadota (13 ASVs), but also a massive persistence of fungal Ascomycota (26 ASVs, e.g., Fusarium and Cladophialophora). This confirms that long-term sugarcane cultivation establishes a stable basal microbiome across both domains that resists nitrogen fluctuations.

2. Your intuition regarding lines 500-505 was correct. We extracted the Phosphorus-related ASVs (present when P is applied but absent in the basal N-only plots). We discovered a robust recruitment of 59 unique ASVs. Crucially, this P-driven accessory microbiome is dominated by Fungi (34 fungal ASVs vs. 25 bacterial ASVs), primarily driven by Ascomycota (e.g., Talaromyces) and Basidiomycota. Potassium variations also showed a fungal-dominant recruitment, albeit smaller (19 unique ASVs).

This taxonomic breakdown provides direct molecular evidence supporting our discussion: While bacteria numerically dominate the interaction networks, Phosphorus is indeed a potent structural driver that specifically awakens and recruits the fungal accessory microbiome. To incorporate these insights, we have expanded our Discussion, updated the Venn visualizations, and added the complete trans-domain taxonomic lists of the N/P/K-Related ASVs as new Supplementary Tables.

- Given the manuscript relies heavily on the network analysis, were other calculation approaches considered (e.g., SparCC/SPIEC-EASI)? These approaches are robust with microbiome data and can help reduce false positives and more acutely pull out interactions. No method is perfect but these might be superior over standard Spearman—curious on authors thoughts here.

R. We highly appreciate the reviewer's thoughtful suggestion. We completely agree that tools like SparCC are very good for mitigating compositional biases. Following your advice, we computed a global trans-domain network using SparCC (filter_thres = 0.0001).

As expected, applying SparCC drastically reduced the network to an ultra-strict, highly conserved trans-domain core of only 24 nodes (17 Bacteria and 7 Fungi). While this perfectly highlights the few absolute, direct biological interactions, SparCC implicitly filters out co-occurrences driven by shared environmental preferences (environmental filtering). Given that the primary goal of our study is precisely to understand how an environmental driver (fertilization regimes) shapes microbial co-occurrence, removing environmental filtering masks the very treatment effects we aim to observe.

Therefore, to capture how N, P, and K fertilizers recruit specific modules and drive community restructuring, we retained the robust Spearman network for our main analysis (Figure 5). Crucially, to avoid the false positive risks associated with small sample sizes (a concern raised by Reviewer 2), we abandoned the n=3 per-treatment networks and computed a single, highly powered Global Spearman Network (n=39), which is an updated Figure 5 that includes: Domain, Module and Ecological Role, for comparison.

To provide the comprehensive perspective you suggested, we have now included the SparCC core network as Supplementary Figure, alongside a discussion acknowledging these strict, environment-independent trans-domain interactions. We believe this dual approach perfectly balances robustness with ecological relevance.

Minor:

- Line 149 could use some clarifying. These samples were collected after shaking the roots to remove excess soil?

R. Yes, that is correct. To ensure clarity, we have slightly modified the text in the Methods section (Line 149) to explicitly state: “The sugarcane roots were gently shaken to remove the loosely bound bulk soil, retaining only the firmly adhering soil, which is strictly considered the rhizosphere. These root-adhering soil samples were then carefully collected into 50 mL Falcon™ tubes…”.

- Looks like a typo regarding Walkley-Black in line 155

R. We thank the reviewer for catching this. The typographical error regarding the Walkley-Black method has been corrected in the revised manuscript.

- I would suggest moving the supplemental table (regarding yields) up to top of supplemental file to follow the chronological order of results—additionally believe this table is not referenced in the text especially in lines 235-239

R. We agree with this suggestion for better flow. We have moved the yields table to the top of the supplementary document (now labeled as Supplementary Table S1) to maintain chronological consistency. Furthermore, we have explicitly added the appropriate reference to this table in the main text (Line 235).

- I would say Line 240 needs clarity of breakdown of samples which could be added to methods. Based on beta diversity, it seems like all conditions including controls included three biological replicates so the total would be 39 when factoring in controls?

R. The reviewer is completely correct, and the previous phrasing was ambiguous. We had 11 specific treatments (33 samples) plus two types of controls (3 non-fertilized rhizosphere samples and 3 bulk soil samples), totaling 39 samples. We have rewritten this section (Line 240) to clearly state: 'Microbial DNA was successfully extracted from a total of 39 samples, comprising 33 fertilized rhizosphere samples (11 treatments × 3 replicates), 3 non-fertilized rhizosphere controls, and 3 bulk soil controls.

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

R. We completely agree with the reviewer's methodological insight. Since DADA2 algorithm inherently removes singletons during its error-correction and denoising process, Chao1 estimates become mathematically skewed. We have removed the Chao1 index from all analyses, figures, and text, retaining only the Observed Richness, Shannon and Simpson diversity metrics, which accurately and sufficiently represent the community structure.

- I am assuming weighted unifrac or other beta diversity calculations did not show anything interesting? These were mentioned in the methods but only Bray-Curtis shown. I would suspect the results to follow Bray-Curtis given the limited variance in the PCoA axes

R. Your assumption is correct. Weighted and unweighted UniFrac distance matrices showed highly similar clustering patterns to Bray-Curtis, with no additional significant differences or distinct separations among treatments. To keep the manuscript concise and focused on the most robust metric for our specific dataset, we elected to display only the Bray-Curtis PCoA. We have added a brief sentence in the Results section confirming that phylogenetic-based metrics (UniFrac) yielded parallel results. So, the following text will be changed to make this clearer: "Based on six indices (Bray–Curtis, Canberra, Jaccard, Robust Aitchison, Unifrac, and weighted Unifrac matrices), beta diversity analysis showed no clear clustering of samples according to fertilization treatment (PERMANOVA, $P > 0.05$; Figure 4 shows Bray–Curtis PCoA as representative).".

- I might prefer swapping supplemental figure 7 with the primary network analysis figure as the supplemental is more informative to me.

R. We appreciate your suggestion. Following your previous comments regarding the network methodology, we have generated an entirely new Figure 5 to represent the unified global trans-domain network. We strongly believe that this updated Figure 5 is now the most informative visualization for the main text, as it captures the structural topology and ecological roles of the microbiome. While the circular plot (now Supplementary Figure 7S) provides valuable taxonomic insights, it is limited to displaying pairwise correlations between phyla. Nevertheless, if the reviewer still feels that the manuscript would benefit from moving the circular plot to the main text, we are more than happy to make the swap or addition.

- I would consider adding a section break at line 340 especially if the authors decide to do more analysis on figure 6

R. This is a great suggestion for readability. We have added a new section break and sub-heading prior to the newly expanded Venn diagram and trans-domain Core analysis to properly highlight these findings.

- General comment on discussion, I personally enjoy long discussions to really dive into the data. Yet, this discussion section is a bit lengthy with some repetition in spots (the first two sections build on each other but tend to repeat). It isn't critical, but attempting to reduce length could help with ease of working through the discussion.

R. We appreciate this constructive criticism. We have carefully reviewed and edited the Discussion section, particularly the first two subsections, removing repetitive statements and consolidating our arguments to make the narrative more concise and impactful, while accommodating the new trans-domain findings requested in the previous comments.