

December 24, 2025

Prof. Dr. Maria Jesus Gutierrez Gines
Editor,
Soil

Manuscript ID: EGUSPHERE-2025-4852

Subject: Manuscript resubmission — disclosure of related publication and revised manuscript (tracked changes attached)

Dear Prof. Maria,

On behalf of all authors, I am pleased to submit the revised version of our manuscript entitled: **“Comparative Impact of Bio-Organic and Inorganic Fertilizer Application on Soil Health, Grain Quality and Yield Stability in Nutrient Deficient Regions”**, Manuscript ID: EGUSPHERE-2025-4852.

We sincerely thank you and the reviewers for the constructive and insightful comments, which have greatly helped us to improve the quality, clarity, and scientific rigor of our manuscript.

In the revised version, we have carefully addressed all reviewers' comments point by point. The revisions include improvements in the Results and Discussion sections, clarification of the experimental rationale, enhanced interpretation of findings, and language polishing throughout the manuscript. All changes made in response to the reviewers' suggestions have been clearly highlighted in the revised manuscript, and a detailed response to reviewers' comments has been provided for your convenience.

Thank you very much for your time and consideration. We look forward to your favorable response.

Following is the point-by-point response to the reviewers' comments.

Reviewer 1:

We sincerely thank the reviewer for the careful evaluation of our manuscript and for the constructive comments that have helped us improve its scientific rigor, clarity, and presentation.

All suggestions have been carefully addressed, and the manuscript has been revised accordingly. Detailed responses are provided below.

Comment 1: In the Introduction, knowledge gaps and the scientific importance of this study should be clearly presented.

Response: Line 78 – 86; We appreciate this valuable suggestion. The Introduction has been revised to more clearly articulate the existing knowledge gaps and highlight the scientific novelty and importance of the present study. Specifically, we clarified that although Zn-enriched compost and microbial inoculants have been studied individually, field-scale validation of integrated bio-organic Zn fertilization under real, farmer-managed arid conditions remains limited. We also emphasized the lack of comparative assessments across multiple locations and the need to link soil biological indicators with yield stability and grain biofortification. These aspects establish the scientific importance of the study in advancing sustainable Zn management strategies for nutrient-deficient arid regions.

Comment 2: The results should be deeply discussed based on the published literature.

Response: Line 452 – 469; 499-511; We agree with the reviewer and have substantially strengthened the Discussion section. The revised Discussion now provides **mechanistic explanations** supported by **recent and relevant literature**, linking microbial activity, Zn solubilization, nutrient cycling, soil biochemical changes, and plant physiological responses. Redundancy with the Results section has been avoided, and emphasis has been placed on interpreting *why* integrated treatments performed better rather than restating numerical values.

Comment 3: The tables and figures should be carefully prepared; for example, in Figure 3, the bars are not clearly displayed.”

Response: Thank you for highlighting this issue. Figure 3 has been redrawn with improved graphical clarity. All figures and tables have been carefully rechecked to ensure readability, consistency, and proper labeling.

Comment 4: A key question is that a similar paper was published (Naeem et al., 2025), which should be fully explained and discussed in the manuscript.

Response: Line 87 - 99; We appreciate this important observation. A clear distinction between the previously published study (Naeem et al., 2025) and the present work has now been explicitly stated in the Introduction and reinforced in the Discussion. While the earlier study focused on development and validation of bio-activated Zn-enriched compost at a single research site, the present study advances this work by:

- Conducting multi-location field trials under farmer-managed conditions
- Integrating ZnO + ZSB + compost rather than compost alone
- Linking soil biological indicators (CFU, MBC, MBN) with yield stability and economic returns
- Demonstrating scalability and adaptability across variable arid environments

Comment 5: Please carefully check the reference format.”

Response: The reference list has been thoroughly revised to ensure consistency with the journal’s formatting guidelines. Author names, journal titles, volume/issue numbers, page ranges, and publication years have been standardized, and minor typographical errors have been corrected.

Comment 6: The manuscript still needs to be carefully checked to enhance its rigor, logic, and readability.

Response: We appreciate this general but important comment. The manuscript has undergone comprehensive language editing to improve clarity and rigor.

Reviewer 2:

Comment 1: Please elaborate on how PCA in this study predicts the relationship between crop performance and microbial population?

Response: We thank the reviewer for this suggestion. Principal Component Analysis (PCA) in our study is used as an exploratory multivariate tool to summarize covariation among many soil, microbial and plant variables and to visualize which variables move together (i.e., covary). PCA does not perform causal prediction in the strict sense, but it identifies the main axes (principal components) that explain the majority of variance in the dataset. In our results, PC-1 explained the large majority of variance (92.6% in Trial I, 91.5% in Trial II) and variables such as organic matter (OM), total organic carbon (TOC), nitrogen (N), phosphorus (P), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and colony forming unit (CFU) had strong positive loadings on PC-1. Treatments with high positive PC-1 scores (e.g., Compost + 2% ZnO + ZSB) therefore cluster together with higher microbial indicators and better crop performance, demonstrating a strong *co-variation* between microbial population/biomass and crop performance. In short, PCA shows that microbial population and crop performance co-vary along the same major gradient (PC-1), supporting the interpretation that improved microbial activity and soil fertility are associated with improved crop growth and yield in our trials.

Comment 2: Why significance level up to 0.0001 described?

Response: Thank you, The manuscript used conventional significance notation (asterisks) to indicate ranges of p-values; the table legend defined **** as $p < 0.0001$, while the global decision threshold for tests was $\alpha = 0.05$. The appearance of **** ($p < 0.0001$) simply reflects that some comparisons produced very small p-values (highly significant differences) when evaluated by ANOVA / post-hoc tests in Statistix / OriginPro. We will clarify the legend and methods, so readers are not confused: we will keep $\alpha = 0.05$ as the threshold for declaring significance but will report p-value ranges using the asterisk scheme.

Comment 3: How was LSD calculated? Is it for the overall treatment effect? Why is it significant to calculate here?

Response: Good point, we will clarify. The ANOVA F-test was used to evaluate the overall treatment effect (i.e., whether any treatment means differ). Where the ANOVA F-test was significant, we used the Least Significant Difference (LSD) procedure to perform pairwise

comparisons between treatment means. LSD was calculated from the ANOVA residual mean square (MSE) and the number of replicates according to the standard formula:

$$LSD_{\alpha} = t_{\alpha/2, df \text{ error}} \times \sqrt{2 \times MSE/r}$$

where $t_{\alpha/2, df \text{ error}}$ is the t-value at the chosen α (0.05) and the residual degrees of freedom, MSE is the mean square error from the ANOVA, and r is the number of replicates (here $r = 3$). The LSD values reported in Table 1 are the LSD at $p \leq 0.05$ computed from ANOVA outputs in Statistix 8.1. LSD is appropriate here because the experiment used an RCBD with balanced replications and our objective was to identify which specific treatments differed (pairwise) after a significant overall ANOVA.

With best regards

Dr. Azhar Hussain

Corresponding Author/ Associate Professor

Department of Soil Science

The Islamia University of Bahawalpur

Email: azharhaseen@gmail..com

Cell#: 0092 321 6814625