



International Journal of Research in Agronomy

E-ISSN: 2618-0618
P-ISSN: 2618-060X
© Agronomy
NAAS Rating (2026): 5.20
www.agronomyjournals.com
2026; SP-9(9): 283-290
Received: 15-07-2026
Accepted: 19-08-2026

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Rhizosphere microbial dynamics of contrasting rice genotypes under zinc nutrition across different growth stages

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DOI: <https://www.doi.org/10.33545/2618060X.2026.v9.i9Sd.6579>

Abstract

Rice grain zinc (Zn) enrichment is an important strategy for improving nutritional security, while rhizosphere microorganisms play a vital role in nutrient cycling and plant Zn acquisition. However, the influence of rice genotype and Zn nutrition on rhizosphere microbial dynamics across crop developmental stages remains poorly understood. The present study investigated the abundance of bacteria, archaea, fungi, and viruses in the rhizosphere of three rice genotypes differing in Zn accumulation potential, namely Karuppunel, CO 51, and ADT 39, under Zn-supplemented (+Zn) and Zn-deficient (−Zn) conditions at the vegetative, panicle initiation, and grain filling stages. Rhizosphere soil was analyzed using shotgun metagenomic sequencing, and microbial abundance was estimated using Kraken2. Marked variations were observed among genotypes, Zn treatments, and growth stages. Bacterial abundance ranged from 2.41×10^5 to 4.75×10^5 , with the highest population recorded in Karuppunel under +Zn at grain filling. Archaeal abundance varied from 2.76×10^3 to 5.28×10^3 , while fungal abundance ranged from 3.14×10^3 to 6.81×10^3 , both reaching their maximum values in Karuppunel under +Zn during grain filling. Viral abundance ranged from 5.29×10^2 to 1.14×10^3 , with the highest value also observed in Karuppunel at grain filling under Zn supplementation. In contrast, ADT 39 consistently exhibited reduced bacterial, archaeal, fungal, and viral abundance under +Zn, particularly during the reproductive stage. Overall, the results demonstrate that host genotype, Zn availability, and plant developmental stage strongly regulate rhizosphere microbial abundance. The consistently greater microbial populations associated with the Zn-efficient genotype Karuppunel suggest a close relationship between Zn accumulation capacity and rhizosphere microbiome assembly. These findings provide valuable insights for integrating genotype-specific plant-microbe interactions with Zn management to enhance nutrient-use efficiency and Zn biofortification in rice.

Keywords: Rice, zinc nutrition, rhizosphere microbiome, bacteria, archaea, fungi, viruses, genotype, growth stage, Zn biofortification

1. Introduction

Rice (*Oryza sativa* L.) is a major staple food for more than half of the world's population and is important for global food and nutritional security. Improving grain Zn concentration, along with yield, is therefore an important breeding objective, as Zn deficiency affects nearly one-third of the global population (WHO, 2002; Zubair *et al.*, 2024). Zinc is essential for plant growth, enzyme activation, protein synthesis, photosynthesis and reproductive development (Zeng *et al.*, 2021). However, Zn deficiency is widespread in paddy soils, particularly under flooded conditions, leading to reduced Zn availability, crop productivity and grain Zn accumulation (Saha *et al.*, 2017; Zubair *et al.*, 2024).

The rhizosphere is a dynamic soil environment where plant roots interact with diverse microbial communities that influence nutrient cycling and mineral transformation. Rhizosphere microorganisms contribute to nutrient acquisition through nitrogen fixation, phosphorus solubilization, organic acid production and siderophore-mediated mineral mobilization (Pantigoso *et al.*, 2022; Liu *et al.*, 2022). These microbial activities can modify Zn availability and facilitate its uptake by plants, highlighting the importance of the rhizosphere microbiome in crop nutrition and soil health (Northover *et al.*, 2022; Pantigoso *et al.*, 2022) [1, 2, 11, 16, 17, 18]

Plant genotype is an important factor shaping rhizosphere microbial communities because genetic differences can influence the quantity and composition of root exudates, including sugars, amino acids, phenolics and organic acids. These compounds serve as carbon sources and signaling molecules that influence the recruitment and establishment of specific microbial populations (Fitzpatrick *et al.*, 2020; Pantigoso *et al.*, 2022; Chen & Liu, 2024). Root exudation also varies with plant developmental stage, resulting in temporal changes in microbial abundance and community composition (Ma *et al.*, 2022; Afridi *et al.*, 2024). Thus, genotype and plant growth stage jointly influence the assembly and succession of rhizosphere microbiota, with important implications for nutrient acquisition and plant performance (Li *et al.*, 2024).

Recent advances in rhizosphere microbiome research have shown that micronutrient management can substantially alter microbial community structure and function. Zinc fertilization not only improves plant Zn nutrition but also stimulates microbial activity, enhances nutrient turnover, and promotes beneficial plant-microbe interactions. Changes in microbial abundance under Zn application have been associated with improved soil enzyme activities, enhanced phosphorus mineralization, and greater nutrient availability in the rice rhizosphere (Wu *et al.*, 2023; Ulbrich *et al.*, 2022) [6, 3, 15, 26, 23].

Despite growing evidence on Zn-microbiome interactions, limited information is available regarding the response of different microbial groups, particularly bacteria, archaea, fungi, and viruses, across contrasting rice genotypes and developmental stages. Understanding these microbial dynamics is essential for identifying microbiome-associated mechanisms that contribute to Zn accumulation in rice.

Therefore, the present study aimed to investigate the abundance of bacteria, archaea, fungi, and viruses in the rhizosphere of three rice genotypes differing in Zn accumulation potential (Karuppunel, CO 51, and ADT 39) under Zn-deficient (−Zn) and Zn-supplemented (+Zn) conditions at the vegetative, panicle initiation, and grain filling stages. We hypothesized that Zn-efficient genotypes would recruit a more abundant and functionally active rhizosphere microbiome, thereby enhancing nutrient cycling and Zn acquisition

2. Materials and Methods

2.1 Experimental site and plant materials

A pot culture experiment was conducted under wetland conditions using cement tanks at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University (TNAU), Coimbatore, India. Three rice (*Oryza sativa* L.) genotypes with varying grain zinc (Zn) accumulation potentials were utilized: Karuppunel (high), CO-51 (medium), and ADT-39 (low). Throughout the experimental period, all rice plants were maintained under uniform agronomic and environmental conditions.

2.2 Experimental design and zinc treatments

The experiment followed a completely randomized design (CRD) incorporating two zinc treatments across three biological replications. The treatment groups were defined as follows: (i) +Zn, receiving basal application of ZnSO₄ (24.7 kg ha^{−1}) followed by 0.5% foliar spray 30 days post-transplanting; and (ii) −Zn, receiving no supplemental zinc.

Rhizosphere soil samples were collected at three distinct physiological growth stages: vegetative (VG), panicle initiation (PI), and grain filling (GF). The experimental design comprised three rice genotypes, two Zn treatments and three growth stages.

2.3 Collection of rhizosphere soil

Rhizosphere soil was collected by standardized soil root sampling procedure. The rice plants have been carefully uprooted to minimise the root causing and loose bulk soil has been removed through gently shaking. The soil tightly attached soil was considered as rhizospheric soil and was gently removed with brushes and collected into sterile collection tubes. The samples were immediately stored at −80°C until metagenomic analysis. Three biological replicates were maintained for each treatment and growth stage.

2.4 Metagenomic DNA extraction

Total microbial DNA was extracted from 0.5 g of rhizosphere soil using the DNeasy PowerSoil Pro Kit (QIAGEN, Germany) following the manufacturer's protocol. DNA quality and concentration were assessed using a NanoDrop spectrophotometer and DNA integrity was confirmed through agarose gel electrophoresis. High-quality DNA samples were used for shotgun metagenomic sequencing (Su *et al.*, 2022) [21].

2.5 Shotgun metagenome sequencing

Metagenomic libraries were prepared using the Illumina DNA Prep library preparation kit according to the manufacturer's instructions. Libraries were sequenced on the Illumina NovaSeq 6000 platform using paired-end 2 × 150 bp (PE150) (Su *et al.*, 2022). Raw FASTQ reads were assessed using FastQC v0.11.9 and pre-processed using fastp v0.23.4. Adapter sequences, low-quality reads, short reads and reads containing ambiguous bases were removed, and the resulting high-quality reads were retained for downstream metagenomic analysis (Chen *et al.*, 2018; Su *et al.*, 2022) [21, 4].

2.6 Taxonomic annotation and microbial abundance analysis

Taxonomic classification of quality-filtered metagenomic reads was performed using Kraken2 v2.0.9, which assigns reads to taxa using exact *k*-mer matches and lowest common ancestor-based classification against reference genome sequences (Wood *et al.*, 2019; Lu *et al.*, 2022). The reference database comprised bacterial, archaeal, fungal and viral genome sequences. Taxonomic abundance was subsequently re-estimated using Bracken v2.6.0 (Lu *et al.*, 2017), which applies Bayesian re-estimation to redistribute Kraken2 assignments and improve abundance estimates at the selected taxonomic level (Lu *et al.*, 2022).

The Kraken2 classification report was converted to Biological Observation Matrix (BIOM) format using the Kraken2BIOM utility. Taxonomic assignments were summarized at phylum, class, order, family, genus and species levels. The classified taxa were partitioned according to their taxonomic lineage into Bacteria, Archaea, Fungi and Viruses (Su *et al.*, 2022; Su *et al.*, 2024) [20].

Taxonomic abundance tables generated from the Kraken2-Bracken output were used to estimate microbial abundance. Taxonomic units assigned to the domain Bacteria and Archaea, kingdom Fungi, and viral taxa were extracted separately. Abundance values of individual taxa within each microbial group were aggregated to obtain the corresponding total bacterial, archaeal, fungal and viral abundance for each rhizosphere sample. Low-abundance and poorly represented taxa were filtered before downstream analysis. The resulting taxonomic abundance matrix was used to compare microbial populations among rice genotypes, Zn treatments and crop growth stages (Lu *et al.*, 2022; Su *et al.*, 2024) [25, 13, 12, 20].

2.7 Statistical analysis

Statistical analyses were showed with R version 4.6.1 through RStudio version 2026.09.0+174. Data on the abundance of bacteria, archaea, fungi, and viruses were analyzed using a three-way analysis of variance (ANOVA) to evaluate the primary effects of rice genotype, Zn treatment, and growth stage, including their two-way and three-way interactions. The normality and homogeneity of variance assumptions were checked prior to analysis. Considerable differences between treatment means were assessed using Tukey's honestly significant difference (HSD) test at $P \leq 0.05$. Various lowercase letters placed above the bars signify statistically significant distinctions between treatment means. Principal component analysis (PCA) was conducted on the microbial abundance dataset to assess multivariate variation across rice genotypes, Zn treatments, and growth stages. All visual representations, such as microbial abundance graphs and PCA plots, were created in R employing the ggplot2 library.

3. Results

3.1 Rhizosphere microbial abundance under different zinc treatments and growth stages

The abundance of major rhizosphere microbial groups (Figures 1-4), including bacteria, archaea, fungi, and viruses, was quantified to determine the influence of zinc (Zn) fertilization across three rice genotypes (Karuppunel, CO 51, and ADT 39) at the vegetative, panicle initiation, and grain filling stages. Significant differences were observed among genotypes, growth stages, and Zn treatments, indicating that both host genotype and Zn availability influenced the composition and dynamics of the rhizosphere microbiome. In general, Karuppunel maintained higher microbial abundance than CO 51 and ADT 39, while Zn application enhanced bacterial, archaeal, fungal, and viral populations in Karuppunel and CO 51. In contrast, ADT 39 showed a reduction in microbial abundance under Zn supplementation, particularly during the grain filling stage. The

detailed responses of each microbial group are presented in Figures 1-4.

3.1.1 Bacterial abundance

Bacterial abundance varied significantly among rice genotypes, Zn treatments, and growth stages (Figure 1). In Karuppunel, bacterial abundance increased from the vegetative stage to grain filling under both Zn treatments, with the highest population recorded under +Zn at grain filling (4.75×10^5). Under -Zn, the population also peaked during grain filling (4.33×10^5). In CO 51, +Zn markedly enhanced bacterial abundance, particularly at the panicle initiation stage (4.17×10^5), whereas under -Zn the grain-filling stage showed the lowest population (2.51×10^5). In ADT 39, bacterial abundance was highest at grain filling under -Zn (4.09×10^5), while +Zn resulted in a gradual decline from vegetative to grain filling, with the lowest value observed during grain filling (2.41×10^5). Overall, Karuppunel exhibited the greatest bacterial abundance, and zinc application generally promoted bacterial populations except in ADT 39.

3.1.2 Archaeal abundance

Archaeal abundance ranged from approximately 2.76×10^3 to 5.28×10^3 and showed significant variation among treatments (Figure 2). Karuppunel recorded the highest archaeal abundance under +Zn at grain filling (5.28×10^3), followed by -Zn at grain filling (4.68×10^3). In CO 51, archaeal populations increased substantially under +Zn, reaching a maximum at panicle initiation (4.72×10^3), whereas -Zn maintained comparatively lower values throughout growth. In ADT 39, the highest archaeal abundance occurred under -Zn during grain filling (4.24×10^3), while +Zn reduced archaeal populations progressively, with the lowest value observed at grain filling (2.76×10^3). These results indicate that zinc supplementation favored archaeal proliferation in Karuppunel and CO 51, whereas ADT 39 responded negatively during the reproductive stage.

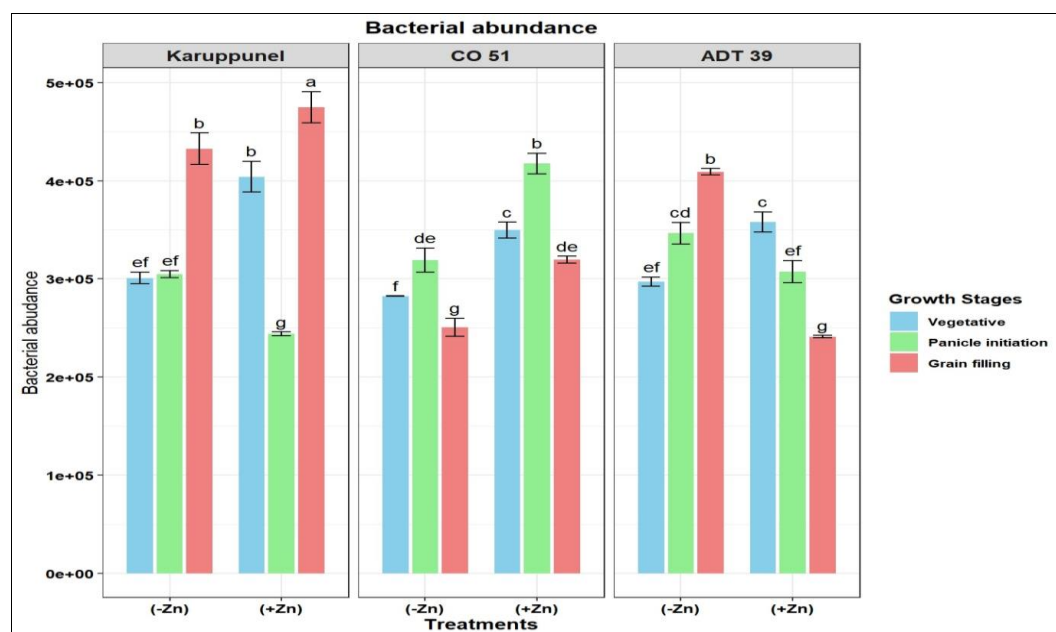


Fig 1: Rhizosphere bacterial abundance under zinc conditions

Figure 1. Rhizosphere bacterial abundance of three rice genotypes, Karuppunel, CO 51 and ADT 39, under zinc-deficient (-Zn) and zinc-supplemented (+Zn) conditions at the vegetative, panicle initiation and grain-filling stages. Bars

represent mean bacterial abundance and error bars indicate variability among three biological replicates. Different lowercase letters above the bars indicate significant differences among treatments at $P \leq 0.05$.

3.1.3 Fungal abundance

Fungal abundance differed significantly with genotype and Zn availability (Fig 3), ranging between 3.14×10^3 to 6.81×10^3 . Karuppunel consistently maintained the highest fungal population, with the maximum abundance observed under +Zn at grain filling (6.81×10^3), followed by -Zn at grain filling (6.01×10^3). In CO 51, fungal abundance increased markedly under +Zn, particularly at panicle initiation (5.99×10^3), while -

Zn exhibited relatively lower populations across all stages. Conversely, ADT 39 showed its highest fungal abundance under -Zn during grain filling (5.48×10^3), whereas +Zn caused a pronounced reduction, reaching the minimum value during grain filling (3.14×10^3). Overall, zinc application enhanced fungal abundance in Karuppunel and CO 51 but suppressed fungal populations in ADT 39 during grain development.

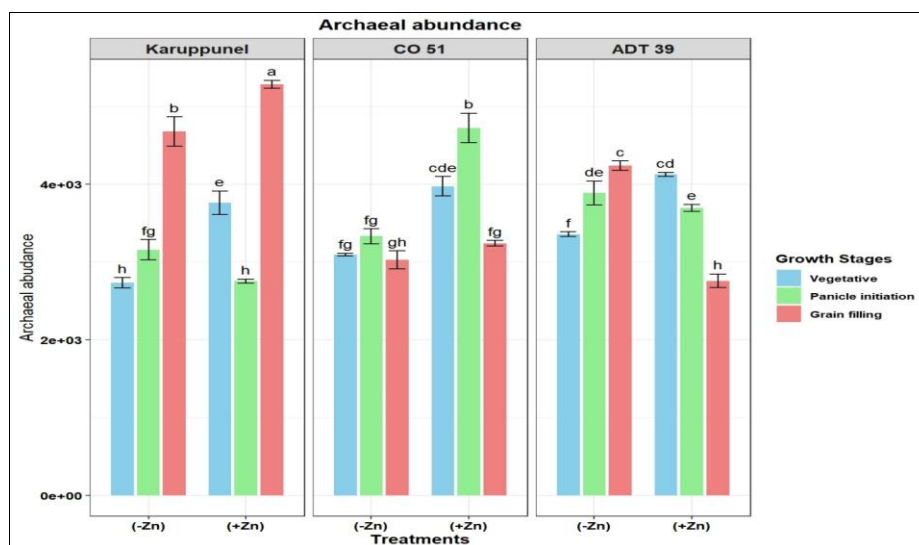


Fig 2: Rhizosphere archaeal abundance under zinc conditions

Figure 2. Rhizosphere archaeal abundance of three rice genotypes, Karuppunel, CO 51 and ADT 39, under zinc-deficient (-Zn) and zinc-supplemented (+Zn) conditions at the vegetative, panicle initiation and grain-filling stages. Bars represent mean archaeal abundance and error bars indicate variability among three biological replicates. Different lowercase letters above the bars indicate significant differences among treatments at $P \leq 0.05$.

3.1.4 Viral abundance

Viral abundance ranged from approximately 5.29×10^2 to 1.14×10^3 and exhibited significant differences among genotypes and treatments (Fig 4). In Karuppunel, viral abundance increased

sharply during grain filling under both Zn treatments, with the highest value recorded under +Zn at grain filling (1.14×10^3), indicating enhanced viral prevalence during the reproductive stage. In CO 51, zinc application promoted viral abundance, with the maximum population observed at panicle initiation under +Zn (9.72×10^2), while -Zn maintained comparatively lower viral counts. In contrast, ADT 39 displayed the highest viral abundance under -Zn at both panicle initiation and grain filling (9.18×10^2 and 9.11×10^2), whereas +Zn caused a substantial decline, reaching the lowest value at grain filling (5.29×10^2). Overall, viral abundance was greatest in Karuppunel under +Zn, while ADT 39 showed a strong reduction in viral populations following zinc application.

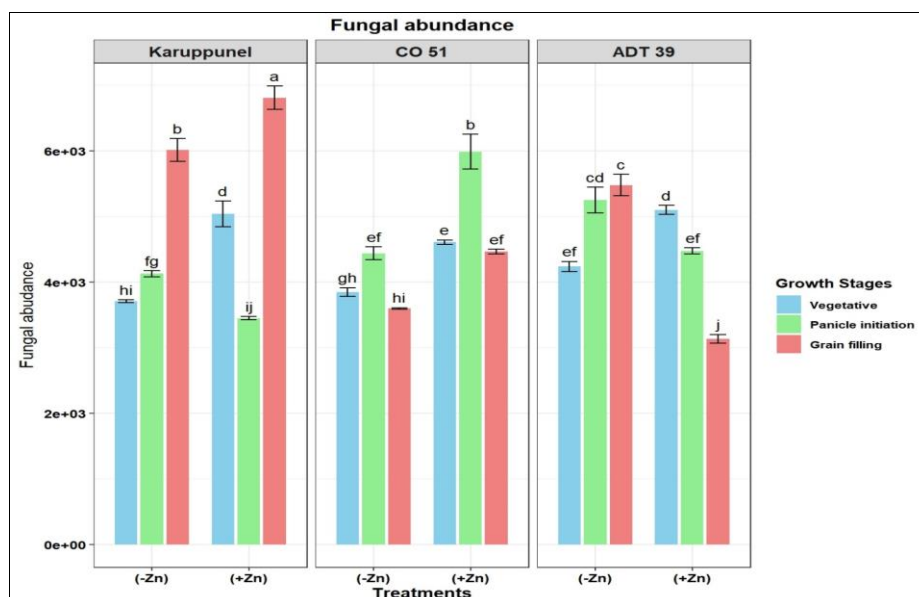


Fig 3: Rhizosphere fungal abundance under zinc conditions

Figure 3. Rhizosphere fungal abundance of three rice genotypes, Karuppunel, CO 51 and ADT 39, under zinc-deficient (-Zn) and zinc-supplemented (+Zn) conditions at the vegetative, panicle initiation and grain-filling stages. Bars represent mean fungal

abundance and error bars indicate variability among three biological replicates. Different lowercase letters above the bars indicate significant differences among treatments at $P \leq 0.05$.

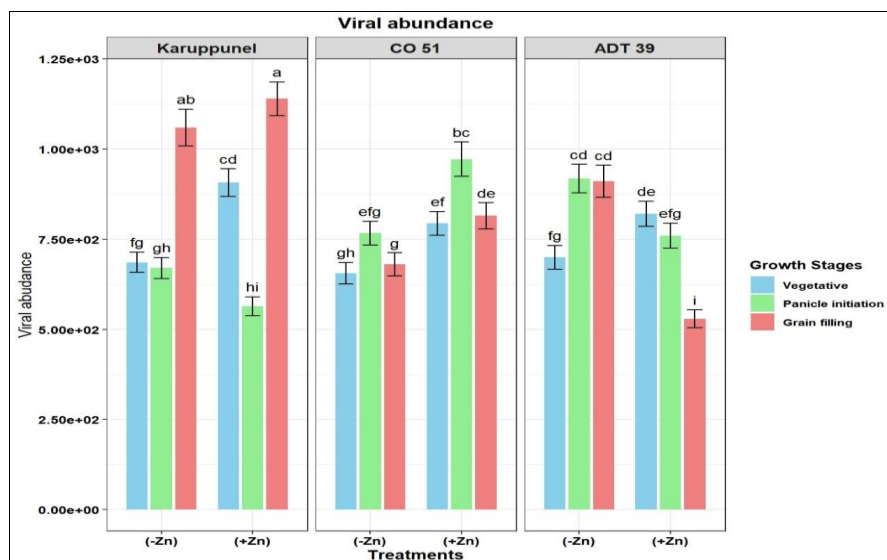


Fig 4: Rhizosphere viral abundance under zinc conditions

Figure 4. Rhizosphere viral abundance of three rice genotypes, Karuppunel, CO 51 and ADT 39, under zinc-deficient (-Zn) and zinc-supplemented (+Zn) conditions at the vegetative, panicle initiation and grain-filling stages. Bars represent mean viral abundance and error bars indicate variability among three biological replicates. Different lowercase letters above the bars indicate significant differences among treatments at $P \leq 0.05$.

3.2. Principal component analysis (PCA)

Principal component analysis (PCA) exhibited significantly variance in rhizosphere microbial communities associated with rice genotype, Zn application and crop growth stage (Fig. 5). PC1 accounted for 95.26% and PC2 for 2.59%, which together explained 97.85% of the total variation in microbial abundance patterns, showed that the microbial abundance patterns were largely structured along PC1. At the vegetative stage, Karuppunel was predominantly separated from CO 51 and ADT 39 along PC2, especially under -Zn conditions, and Zn-treated samples generally shifted towards positive PC1 scores, indicated

that a strong response of microbial abundance to Zn application. Under -Zn conditions, CO 51 and ADT 39 overlapped more, but under +Zn, their samples were displaced along PC1 and PC2. During the panicle initiation, the samples showed higher variance, and separation was driven by both genotype and Zn treatment. Karuppunel was predominantly negative PC1, while ADT 39 and CO 51 genotypes were revealed positive PC1. The use of Zn further altered the ordering positions within each genotype, suggested that a stage-dependent response of the microbial community in the rhizosphere. At the grain-filling stage, genotype-specific differentiation became particularly evident. Karuppunel was primarily located at positive PC1 scores; CO 51 occupied a predominantly negative PC1 score under -Zn but shifted to a positive PC2 score under +Zn, and ADT 39 showed a distinct pattern across the two genotypes. Overall, the PCA shows that microbial abundance is strongly differentiated between rice genotypes and stages of development, with Zn application further modifying these patterns depending on the genotype and stage.

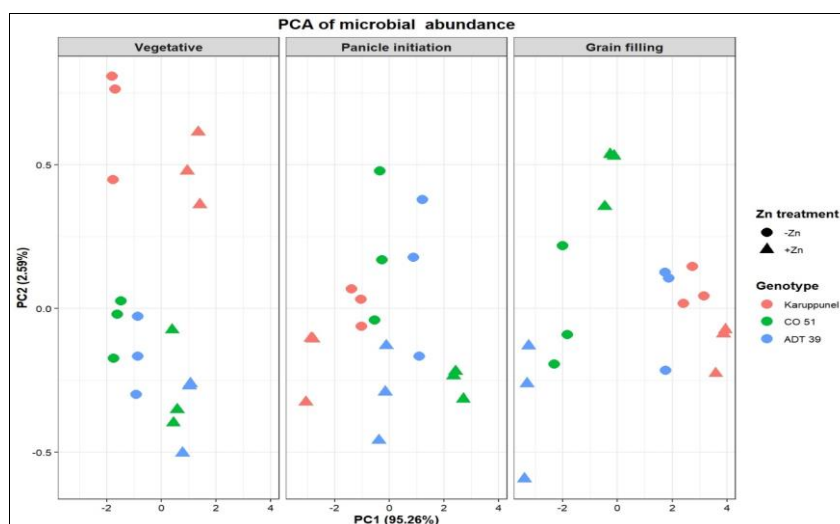


Fig 5: Principal component analysis of microbial abundance

Figure 5. Principal component analysis of microbial abundance in the rhizosphere of Karuppunel, CO 51 and ADT 39 under -Zn and +Zn treatments at vegetative, panicle initiation and grain-filling stages. PC1 and PC2 accounted for 95.26% and 2.59% of the total variance, respectively.

4. Discussion

The present study demonstrated that rhizosphere microbial abundance was significantly influenced by the interaction between rice genotype, zinc (Zn) nutrition, and plant developmental stage. Among the three genotypes, Karuppunel consistently maintained the highest abundance of bacteria, archaea, fungi, and viruses under Zn supplementation, whereas ADT 39 supported comparatively lower microbial populations, particularly during grain filling. These findings indicate that Zn-efficient rice genotypes possess a greater ability to recruit and sustain beneficial rhizosphere microorganisms, suggesting that host genetic variation is an important determinant of microbiome assembly. Similar genotype-dependent differences in rhizosphere microbial communities have been reported in rice and other crop species, where variations in root exudation patterns selectively shaped microbial diversity and functional composition (Xiong *et al.*, 2021; Afridi *et al.*, 2024) [28, 1, 2].

Further, PCA revealed that the combined effect of rice genotype, Zn nutrition, and developmental stages on microbial communities in rice rhizosphere, with the first two principal components explaining 97.85% of the total variation, with PC1 explaining 95.26% and PC2 explaining 2.59%. The separate clustering of Karuppunel, CO 51, and ADT 39 in the ordination space reflected a robust genotype-dependent differentiation of microbial profiles. Differences in host metabolic traits and the production of specific metabolites can exert selective effects on rice-associated microbiota and contribute to microbiome composition (Su *et al.*, 2024) [20]. The influence of host genetic background on microbiome composition has been shown in diverse rice germplasm, suggesting that host genetic variation is a factor in structuring rice-associated microbial communities (Su *et al.*, 2022) [20].

The higher microbial abundance observed in Karuppunel under Zn supplementation is likely associated with improved plant nutritional status and enhanced rhizosphere carbon deposition. Zinc functions as an essential cofactor for numerous enzymes involved in nucleic acid synthesis, protein metabolism, and antioxidant defense in both plants and microorganisms. Adequate Zn availability improves photosynthetic efficiency and root development, resulting in greater translocation of photosynthates belowground and increased secretion of sugars, amino acids, organic acids, and phenolic compounds into the rhizosphere. These root exudates serve not only as carbon and energy sources but also as signaling molecules that regulate microbial colonization and community succession (Huang *et al.*, 2014). Recent evidence further suggests that plant-derived metabolites actively recruit beneficial microbiota and strengthen plant-soil interactions by modifying the chemical environment surrounding roots (Afridi *et al.*, 2024) [1, 2].

The separation of +Zn and -Zn samples in the PCA further demonstrated that Zn availability modified rhizosphere microbial profiles, although the extent and direction of separation varied among the three rice genotypes. This genotypic response concurs with Xiao *et al.* (2022) [27], which showed significant changes in the bacterial community structure of the rice rhizosphere after fertilisation with Zn and showed that microbial response to Zn varies between rice cultivars. Similarly, contrasting levels of Zn were reported to produce

different distribution patterns in the bacterial and fungal communities of the rice rhizosphere, suggesting that changes in the availability of Zn may alter the rhizosphere microbiomes (Lv *et al.*, 2022) [14].

Bacterial abundance showed the most pronounced response to Zn fertilization, with Karuppunel exhibiting the highest population during grain filling. This increase indicates that Zn supplementation created a biologically favorable rhizosphere that supported rapid bacterial proliferation and metabolic activity. Similar results were reported by Xiong *et al.* (2021) [28], who observed that host genotype significantly influenced the recruitment of beneficial bacterial taxa and enhanced microbial network complexity in crop rhizospheres. The greater bacterial abundance in Karuppunel may therefore reflect its superior Zn uptake efficiency, which enhances carbon availability and stimulates microbial processes involved in nutrient mineralization and phosphorus transformation. Consequently, the enriched bacterial community is likely to contribute to improved nutrient cycling and greater nutrient acquisition by the plant.

Archaeal populations also responded positively to Zn nutrition, with Karuppunel and CO 51 maintaining higher abundance than ADT 39 throughout crop development. In flooded paddy ecosystems, ammonia-oxidizing archaea are major contributors to nitrogen cycling because they catalyze the first step of nitrification under low-oxygen conditions. The enhanced archaeal abundance observed under Zn supplementation suggests that improved root growth and rhizosphere nutrient availability generated favorable microsites for archaeal activity. Similar observations were reported by Li *et al.* (2021) [10], who concluded that rhizosphere microbial communities, including archaeal populations, are strongly regulated by root-derived compounds and play critical roles in maintaining nutrient transformation and soil fertility.

Fungal abundance followed a trend comparable to that of bacteria, with significantly greater populations in Karuppunel and CO 51 under Zn application, whereas ADT 39 exhibited a marked decline during grain filling. The higher fungal abundance indicates enhanced decomposition and nutrient mobilization within the rhizosphere because fungi produce extracellular enzymes capable of degrading complex organic substrates and releasing mineral nutrients. Similar results were reported by Afridi *et al.* (2024) [1, 2], who highlighted that qualitative and quantitative changes in root exudates selectively enrich beneficial fungal communities responsible for improving nutrient availability and rhizosphere resilience. The present findings therefore suggest that Zn-efficient genotypes create a metabolically active rhizosphere that simultaneously supports bacterial and fungal communities, resulting in complementary functions in organic matter decomposition and nutrient recycling.

The viral community exhibited a pattern closely resembling bacterial abundance, with the highest viral populations recorded in Karuppunel during grain filling under Zn supplementation. Because bacteriophages constitute the dominant component of soil viral communities, their abundance is largely dependent on the availability of bacterial hosts rather than direct nutrient effects. Similar observations were described by Trubl *et al.* (2018) [22], who demonstrated that bacteriophage-mediated lysis regulates microbial turnover through the release of dissolved organic carbon, nitrogen, and phosphorus into the soil, thereby sustaining the microbial loop and accelerating nutrient recycling. Thus, the simultaneous enrichment of bacterial and viral populations in Karuppunel reflects a highly dynamic rhizosphere

ecosystem with active microbial interactions rather than independent viral proliferation.

Plant developmental stage further influenced the succession of rhizosphere microorganisms throughout the crop life cycle. Although microbial abundance generally increased from the vegetative stage towards reproductive growth, the timing of peak populations differed among genotypes. Karuppunel reached maximum abundance during grain filling, whereas CO 51 showed comparatively higher populations at panicle initiation. These temporal differences correspond with developmental shifts in photosynthate allocation and changes in root exudate composition. Similar results were reported by Huang *et al.* (2014), who demonstrated that root exudate chemistry changes substantially across plant developmental stages, driving microbial succession and the establishment of distinct rhizosphere communities. More recently, integrated microbiome and metabolomic studies have confirmed that developmental changes in rice root exudates directly influence bacterial and fungal community assembly, emphasizing the close relationship between plant metabolism and rhizosphere microbial dynamics (Li *et al.*, 2023) [9].

This temporal change was consistent with the phase-wise PCA pattern, where the relative positions of the three genotypes and the Zn treatment shifted between the vegetative stage, the start of the panicle and the grain filling, as reported for the root-associated bacterial and archaeal communities in field-grown rice (Edwards *et al.*, 2018) [8]. Therefore, the PCA indicates that microbial response to Zn is not uniform across crop development but varies between genotypes and stages of development, and the sequence data together with the abundance data indicate a significant interaction between genotype $\times$ Zn and stage of development in the microbial profiles of the rhizosphere.

The consistently lower microbial abundance observed in ADT 39 indicates that Zn-inefficient genotypes possess a reduced capacity to establish beneficial plant-microbe interactions. Limited Zn accumulation may restrict root growth and decrease the secretion of carbon-rich metabolites, thereby reducing microbial recruitment and biological nutrient transformations. In contrast, the superior performance of Karuppunel demonstrates that Zn-efficient genotypes not only accumulate greater Zn within plant tissues but also foster a biologically active rhizosphere capable of sustaining diverse microbial communities involved in nutrient cycling. Similar conclusions were drawn by Afridi *et al.* (2024) [1, 2], who proposed that genotype-specific root exudation is a primary mechanism governing microbiome recruitment and enhancing plant nutrient-use efficiency.

5. Conclusion

The present study demonstrates that rice genotype, Zn availability and plant developmental stage collectively shape rhizosphere microbial abundance. Among the three genotypes, Karuppunel, which possesses high Zn accumulation potential, consistently supported greater bacterial, archaeal, fungal and viral abundance, particularly under Zn supplementation. CO 51 showed an intermediate response, whereas the low-Zn-accumulating genotype ADT 39 exhibited a contrasting response, with microbial abundance frequently declining under Zn application during grain filling. The stage-dependent variation further indicates that rhizosphere microbial communities undergo dynamic changes during plant development, probably in association with changes in root growth, carbon allocation and root-derived metabolites. Overall, the greater microbial abundance observed in Karuppunel under

Zn nutrition suggests a strong association between host Zn accumulation capacity and rhizosphere microbiome assembly. These findings provide a basis for further investigation of specific microbial taxa and functional pathways involved in Zn mobilization and acquisition, and support the integration of Zn-efficient rice genotypes with appropriate Zn management as a promising approach for sustainable rice production and nutritional biofortification.

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